Randomized Placebo-Controlled Clinical Trials Testing Gemfibrozil for Smoking Cessation and Melatonin for Alcohol-related Sleeping Problems

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy (PhD)
Department of Pharmacology and Toxicology
University of Toronto

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2019

Abstract

Substance use disorder (SUD) is not a temporary condition but it is a chronic and relapsing disorder that leads to serious health risks. SUD is considered a pathological behavior, affecting the reward circuit and stress system, and these impairments can last for a longer period even after detoxification and abstinence. The motivation behind exploring new therapeutic options for different kind of SUDs and their complications is the limited success of the available treatments. For instance, the Food and Drug Administration (FDA) approved medications for Tobacco use disorder (TUD) increase abstinence rates; however, relapse remains the most likely outcome, with success rates of only 20-30% at 1 year post-treatment follow up. The other motivation for exploring new therapeutics is the harmful side effects caused by current medications; for instance, the use of benzodiazepine receptor agonists, to treat alcohol use disorder (AUD)-sleep disorder, which are known to be addictive. This thesis focuses on two randomized clinical trials (RCTs) exploring the effect of two therapeutic options one for TUD using lab paradigms for nicotine craving and reinforcement and a brief quit attempt. The other RCT is exploring the effect of melatonin for sleep problems in AUD subjects for four weeks of treatments monitoring
sleep quality versus placebo. The result of the 1\textsuperscript{st} clinical trial showed no significant effect of gemfibrozil versus placebo on TUD lab indices. The 2\textsuperscript{nd} clinical trial showed a time effect on sleep scores that were significantly enhanced after 4 weeks of treatment. However, no drug effect was observed. In conclusion, it is highly recommended to conduct more RCTs in the fields of SUD in order to obtain better success rates with fewer side effects.
Acknowledgments

“I can do all things through Christ who strengthens me” Philippians 4:13

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<td>α</td>
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<td>β</td>
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<td>Delta</td>
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<td>Mu</td>
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<td>AE</td>
<td>Adverse Events</td>
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<td>AUD</td>
<td>Alcohol Use Disorder</td>
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<td>AUDIT</td>
<td>Alcohol Use Disorder Identification Test</td>
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<td>BAI</td>
<td>Beck Anxiety Inventory</td>
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<td>BDI</td>
<td>Beck Depression Inventory</td>
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<td>CAMH</td>
<td>Center for Addiction and Mental Health</td>
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<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
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<td>CBT</td>
<td>Cognitive Behavioral Therapy</td>
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<td>CI</td>
<td>Confidence Interval</td>
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<td>CO</td>
<td>Carbon Monoxide</td>
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<td>CPK</td>
<td>Creatinine Phosphokinase</td>
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<td>CRF</td>
<td>Corticotrophin Releasing Factor</td>
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<td>CYP450</td>
<td>Cytochrome P450</td>
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<td>Denic</td>
<td>Denicotinized Cigarette</td>
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<tr>
<td>DSM-5</td>
<td>Diagnostic and Statistical Manual of Mental Disorders fifth edition</td>
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<td>EMG</td>
<td>Electromyography</td>
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<td>FAAH</td>
<td>Fatty Acid Amide Hydrolase</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>Acronym</td>
<td>Full Form</td>
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<td>FTND</td>
<td>Fagerström Test for Nicotine Dependence</td>
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<td>GABA</td>
<td>Gamma Aminobutyric Acid</td>
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<td>Gamma-Hydroxybutyric Acid</td>
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<td>5HT</td>
<td>Hydroxytryptamine Receptors</td>
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<td>HDL</td>
<td>High Density Lipoprotein</td>
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<td>MMRM</td>
<td>Mixed Model for Repeated Measures</td>
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<td>MNWS</td>
<td>Minnesota Nicotine Withdrawal Scale</td>
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<td>MT</td>
<td>Melatonin Receptor</td>
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<tr>
<td>NAc</td>
<td>Nucleus Accumbens</td>
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<tr>
<td>nAChRs</td>
<td>nicotinic Acetyl Cholinergic Receptors</td>
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<tr>
<td>Nic</td>
<td>Nicotinized Cigarette</td>
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<td>NREM</td>
<td>Non–Rapid Eye Movement</td>
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<td>OEA</td>
<td>Oleoylethanolamide</td>
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<tr>
<td>Oz</td>
<td>Ounce</td>
</tr>
<tr>
<td>PEA</td>
<td>Palmitoylethanolamide</td>
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<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome Proliferator-Activated Receptors</td>
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<tr>
<td>PPRE</td>
<td>Peroxisome Proliferator Response Elements</td>
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<td>PSG</td>
<td>Polysomnography</td>
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<td>PSQI</td>
<td>Pittsburg Sleep Quality Index</td>
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<tr>
<td>REM</td>
<td>Rapid Eye Movement</td>
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<td>RCT</td>
<td>Randomized Clinical Trial</td>
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<tr>
<td>RXR</td>
<td>Retinoid X Receptor</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SKCond</td>
<td>Skin conductance</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<tr>
<td>SUD</td>
<td>Substance Use Disorder</td>
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<td>TCQ</td>
<td>Tobacco Craving Questionnaire</td>
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<tr>
<td>TUD</td>
<td>Tobacco Use Disorder</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scales</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral Tegmental Area</td>
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Chapter 1
Introduction

1 Introduction

Addiction is the most severe form of substance use disorder (SUD) that represents a global health problem characterized by a compulsive repetitive behavior to consume psychoactive substances despite their harmful effects. According to The Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5) the substances of abuse are alcohol, opioids, cannabis, hallucinogens, tobacco inhalants, sedatives/hypnotics, cocaine, and others (Mclellan 2017). SUD is not a temporary condition but it is a chronic and relapsing disorder that leads to serious health risks (NIH 2016; WHO 2014). Thousands of years ago, psychoactive substances have been used in some ancient medicinal and religious rituals and ceremonies (Saah 2005). Nowadays, SUD became a societal burden that affects the health and wellbeing of the whole society. Billions of dollars are spent each year on the health consequences, addiction-related crimes and the loss of productivity caused by substance use (Birnbaum et al. 2011).

The subsequent detrimental effects of chronic substance abuse leads to the deterioration of brain cognitive functions such as impaired memory, loss of impulse-control, and poor decision making (Rogers and Robbins 2001). Moreover, substance abuse which is considered a pathological behavior, affects the reward circuit and stress system, and these impairments can last for a longer period even after detoxification and abstinence from of the addictive substance (Goldstein and Volkow 2011; Hyman et al. 2006; Spiga et al. 2008). Upon the absence of the addictive substance craving or an intense urge to consume that substance is frequently experienced by all subjects developing SUD (Heishman et al. 2004). For instance, smokers who are trying to abstain from smoking experience daily craving to smoke and the more the intensity of the craving the more likely is the relapse (Hughes and Hatsukami 1986; Shiffman et al. 1997).

Repeated drug use, continuous drug seeking, use of higher doses and withdrawal symptoms are anticipated consequences for all substances of abuse due to the reinforcement effects of the substance. Hence, substance abuse becomes compulsive, uncontrollable and narrows the life scope of the abusers on obtaining and consuming the substance (Wise and Koob 2014). Subjects
that have developed SUD, do not necessarily experience the pleasurable effects of the substance, (positive reinforcement), after a period of time due to the brain adaptation changes; hence tolerance or the need of higher doses to feel high again. However, avoiding the negative withdrawal symptoms remains a main drive to seek more substances (Mitchell and Potenza 2014; Spiga et al. 2008).

There is an urgent need for a deeper understanding of the various brain mechanisms involved in the development and the maintenance of substances of abuse in order to formulate the appropriate therapeutics for each substance. Preclinical and clinical research showed that substances of abuse reinforcing effects are mainly mediated by an increase in the levels of dopamine in the nucleus accumbens projected from the ventral tegmental area (VTA) in the reward circuit upon the administration of the substance (Bressan and Crippa 2005; Di Chiara 1997; Justinova et al. 2005; Kelley and Berridge 2002). Apart from the dopaminergic system, there are other neurotransmitters involved in the drug seeking behavior that their release and/or reuptake are altered by substance of abuse. Those neurotransmitters include Gamma Amino Butyric Acid (GABA), Endogenous Opioids, Norepinephrine, Serotonin, Oxytocin, Corticotrophin-Releasing Factor (CRF), and Vasopressin (Bruchas et al. 2010; Edwards and Koob 2010; McGregor and Bowen 2012; Muller and Homberg 2015; Roth et al. 1998; Subiah et al. 2012; Zhou et al. 2005; Zhou et al. 2008).

For instance, it is suggested that alcohol administration induces the release of endogenous opioids (such as β-endorphin) (Marinelli et al. 2003) that subsequently activate the mu (µ) opioid receptor, located on the GABAergic interneurons which control the firing of the dopamine causing an elevation of dopamine levels in the striatal area (Gianoulakis 2009; Nestler 2005; Spanagel 2009; Walters et al. 2005; Wise and Rompre 1989). Regarding nicotine reinforcing effects, research showed that alpha-4 beta-2 (α4β2) nicotinic acetylcholine receptor located in hypothalamus, thalamus, midbrain, and mesolimbic dopaminergic neurons are the main receptors that mediate nicotine effects of dependence and pleasure. Upon binding to its nicotinic receptors, nicotine enhances the release of brain neurotransmitters such as acetylcholine, norepinephrine, dopamine serotonin, and others causing its potent stimulating and reinforcing effects on the reward pathway (Epping-Jordan et al. 1998; Jiloha 2010).
Therefore, all the studies aiming to find new therapeutics for substance dependence are targeting one or more of these systems and/or mediators. For years, researchers focused on replacing the harmful substance of abuse by another less harmful product inducing similar pharmacological effects but with different pharmacokinetics yielding a safer substitute, for instance, using nicotine in gums, patch, lozenge, or sprays as a replacement to the harmful effects of cigarettes during treatment (Malaiyandi et al. 2006; Rose et al. 2006). The use of the long-acting opioid (methadone) to replace opioids in opioid use disorder therapy is another example (Dole and Nyswander 1965; Freedman and Senay 1973). Another example is the use of substance inducing brain inhibition as gamma-hydroxybutyric acid (GHB) (Gallimberti et al. 1989) to mimic the inhibitory effects of alcohol and replacing alcohol during withdrawal period in alcohol use disorder (AUD) treatment. Although this strategy of harm reduction showed positive results, it takes a long time of therapy to achieve its therapeutic goals. More studies and other strategies are needed in order to find a variety of pharmaceuticals treating every form of SUD. For instance, to treat AUD and following the initial seminal studies of the teams from Drs. O’Brien and O’Malley (O’Malley 1995; O’Malley et al. 1992; Volpicelli et al. 1992) several studies have explored the utility of naltrexone, a μ receptor antagonist, as a suitable treatment for alcohol dependence. Naltrexone has demonstrated efficacy in many studies over the years in treating alcohol rewarding, consumption, and craving (Bouza et al. 2004; Heilig and Egli 2006; O’Malley et al. 1992; Rosner et al. 2010; Srisurapanont and Jarusuraisin 2005). In the 1st clinical trial of this thesis, a new therapeutic target was explored to treat TUD and in the 2nd clinical trial a new therapeutic alternative was explored to treat sleep problems present in subjects diagnosed with AUD.
1.1 Introduction to Clinical Trial I

Despite the decrease of the number of smokers in some countries during the past few years, it is estimated that there are over one billion smokers worldwide. Tobacco-related morbidity is thought to lead to 7 million deaths per year (WHO 2018) and this number is expected to increase to 8-10 million deaths per year by 2030 (Burki 2015). According to Statistics Canada, there are 16.2% of Canadians who are tobacco smokers (Statistics 2018). Smoking is responsible for nearly 17% of all deaths (Whiteford et al. 2013) and out of 70% of smokers who want to quit, only 4 to 7% would be successful (Fiore et al. 2008). Nicotine is the most addictive ingredient in cigarettes (Le Foll and Goldberg 2006). Thousands of chemicals are generated from the burning and smoke of tobacco leaves. Nicotine alkaloid (1-methyl-2-[3-pyrodyl] pyrrolidine), one of these chemicals, is the active ingredient responsible for tobacco dependence. It was extracted for the 1st time by Posselt and Reimann in 1828 (Atreya et al. 2003). Nicotine is known to be a highly toxic chemical that once inhaled enters in the lungs towards the blood stream, diffuses quickly to the brain within few seconds and to every organ in the body causing a number of patho-physiological changes. Once nicotine reaches the brain it causes an increase in blood pressure, respiration rate, heart rate, alertness, and causes arterial constriction. Nicotine binds to nicotinic cholinergic receptors (nAChRs), which are ligand-gated ion channels. The reinforcing effects of nicotine are mediated through the release of various neurotransmitters including dopamine, which plays a fundamental role in reward, as well as acetylcholine, vasopressin, serotonin, norepinephrine, glutamate, gamma-aminobutyric acid (GABA) and beta-endorphins (David et al. 2014; Le Foll 2013). Researchers are continuously searching for medications that target one or more of these neurotransmitter systems hoping to find new effective smoking cessation aids (Bozinoff and Le Foll 2018).

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1.1.1 Current Medications for TUD

There are currently three pharmacotherapies for smoking cessation that are approved by the Food and Drug Administration (FDA): nicotine replacement therapy (NRT), bupropion hydrochloride, and varenicline tartrate (Le Foll and George 2007; Prochaska and Benowitz 2016). NRT products include gum, patches, inhalers, nasal and oral sprays, and lozenges, and their main benefit is the reduction of cravings and withdrawal symptoms induced by tobacco cessation (Stead et al. 2012). Bupropion hydrochloride acts by blocking nAChRs, as well as inhibiting both norepinephrine and dopamine reuptake, which reduces smoking cessation-induced craving, withdrawal symptoms, and negative mood (Kotlyar et al. 2011; McCarthy et al. 2008). Varenicline tartrate, a partial agonist for selective heteromeric α4β2-containing receptors, referred to as α4β2* nicotinic receptor (Cahill et al. 2013), is thought to be the most effective medication for smoking cessation. Although FDA approved medications increase abstinence rates, relapse remains the most likely outcome, with abstinence rates of only 20-30% at 1 year post-treatment follow up (Cahill et al. 2007; Esterlis et al. 2013). New pharmacotherapies are needed for TUD in order to achieve higher rates of long-term abstinence.

1.1.2 PPAR receptors

Peroxisome proliferator-activated receptors (PPAR) are part of the nuclear receptors and considered as ligand-activated transcription factors that upon activation promote gene transcription by binding to peroxisome proliferator response elements (PPRE) within gene promoters and heterodimerize with retinoid X receptor (RXR) to enhance the rate of gene transcription (Miyata et al. 1994). These receptors are called PPAR as they are known to induce the peroxisome organelles in rodents’ hepatocytes but not in humans; therefore, they are considered as hepatic carcinogens in rodents. There are three types of PPAR receptors which are: PPAR-α, PPAR-β/δ, and PPAR-γ encoded by different genes; Chromosome 22, 6, and 3; respectively (Dreyer et al. 1992).

PPAR 1st cloning occurred in rodents as a part of research looking into targets for hepatic peroxisome proliferating agents. PPARs main function is to regulate lipid metabolism. However they were found to be involved in other physiological functions such as inflammation, atherosclerosis and energy balance, myelogenesis and fertility. The natural ligands for PPARs are mainly fatty acids, prostaglandins and eicosanoids. Every PPAR isoform consists mainly of
a DNA binding domain with two zinc fingers responsible for PPRE binding, and a ligand binding domain or pocket in the C-terminal half of the receptor and known to be larger in size than other nuclear receptors (Bright et al. 2008a; Bright et al. 2008b; Werman et al. 1997). PPAR-α is found to be expressed in many organs such as liver, heart, kidney, intestine, skeletal muscle and brain. Research showed that PPAR-α is involved in oxidative stress and neurotransmission besides its role in fatty acid metabolism. Fibrate medications that are used in hypercholesterolemia are synthetic ligands for PPAR-α (Murakami et al. 1998).

PPAR-β/δ is found to be expressed in all tissues and is involved in CNS myelination. PPAR-β/δ is involved also in the activation of the enzyme Acyl-CoA synthetase which regulates fatty acid utilization. The PPAR-β/δ agonist Prostaglandin I2 is the ligand involved in muscular fatty acid oxidation (Harrington et al. 2007; Polak et al. 2005). Finally, PPAR-γ was found to be expressed in liver, heart, intestine, retina, adipose tissue, hypothalamus and skeletal muscles. It was found to be involved in inflammatory process in the CNS as it is expressed in astrocytes and microglia. The natural ligands for PPAR-γ are eicosanoids and prostaglandin J2, whereas thiazolidinedione, an anti-diabetic medication, is a synthetic ligand for it (Barak et al. 1999; Bright et al. 2008a; Storer et al. 2005).

1.1.3 PPAR-α: A Promising Candidate for TUD

Preclinical Research

Animal studies showed that there is an increase in anandamide levels, the endogenous cannabinoids, mainly in the limbic forebrain in response to certain substances of abuse such as nicotine (Gonzalez et al. 2002). Previous studies have shown that fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of anandamide and other endogenous cannabinoids (Scherma et al. 2012), might be involved in nicotine dependence.\(^2\) For example, administration of a FAAH inhibitor leads to suppression of nicotine-induced conditioned place

preference, nicotine self-administration and nicotine-induced increase in dopamine level in the nucleus accumbens (Forget et al. 2009; Scherma et al. 2008). FAAH inhibition causes an increase in the levels of alpha-type of PPAR (PPAR-α) endogenous ligands, such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Fegley et al. 2005); thus PPARs might be involved in nicotine dependence. Studies showed that the binding of a PPAR-α ligands activate PPAR α receptors which lead to the phosphorylation of α4β2 nicotinic acetylcholine receptors in the dopaminergic neurons of VTA through the activation of tyrosine kinase. This mechanism leads to receptor internalization which decreases the ionic conduction and nicotine-induced dopamine firing in VTA (Melis et al. 2010; Melis et al. 2008; Zhu et al. 2000).

Researchers showed an interest in PPAR-α as being involved in substance use dependence as PPAR-α agonist administration decreased nicotine seeking behavior and nicotine self-administration in animals (Mascia et al. 2011). Further, PPAR-α agonist treated animals showed less nicotine-seeking behavior, and less nicotine induced relapse after a period of abstinence. Therefore, PPAR-α agonists were thought that they could interfere with the rewarding effects of nicotine. Interestingly, these effects disappeared in the presence of PPAR-α blocker (Mascia et al. 2011). The previous mentioned studies used PPAR-α chemicals in animals not available for humans. Therefore, Panlilio and colleagues examined the PPAR-α theory in animals using medications that could be examined in humans. They chose Clofibrate, one of the fibrates (PPAR-α agonist), as a candidate in their experiments on rats and squirrel monkeys. Clofibrate attenuated the nicotine-induced firing in VTA neurons; also, the nicotine-induced dopamine release in the nucleus accumbens was decreased. Clofibrate reduced nicotine acquisition in nicotine-naive animals and reduced reinstatement in experienced animals exposed to nicotine-associated cues after a period of abstinence the effects that were reversed by a PPAR-α antagonist (Panlilio et al. 2012). Together, these studies suggested that PPAR-α agonist medications could attenuate nicotine reward and reinforcement in humans, promote smoking cessation and could serve as a new potential treatment for nicotine addiction.

1.1.4 Gemfibrozil

In order to translate the previous research investigating the use of PPAR-α agonist for smoking cessation in human, a PPAR-α agonist medication, Gemfibrozil (Lopid®) which is one the fibrates, was selected for the present study. Gemfibrozil was introduced in the market in 1976
and it was approved by FDA and Health Canada to lower the elevated blood triglycerides and cholesterol levels (Betteridge et al. 1976; Ghosh and Pahan 2012; Robins et al. 2001). It is known that it decreases mainly serum triglycerides cholesterol by inhibiting peripheral lipolysis and reducing the hepatic free fatty acid extraction and triglyceride production. Also, gemfibrozil inhibits the very low density lipoprotein (VLDL) synthesis, VLDL carrier apolipoprotein B, and increases the beneficial cholesterol high density lipoprotein (HDL). Gemfibrozil is well absorbed orally, has a very short half-life of 1.5 hours, reaches its peak plasma level 1-2 hours after administration and shows a very high protein binding. Upon ingestion, gemfibrozil undergoes extensive hepatic metabolism mainly oxidation to form hydroxymethyl and a carboxyl metabolite and 70 % of it is excreted in urine mainly as glucuronide conjugate, 6% excreted in feces, and less than 2% of gemfibrozil is excreted unchanged (Busse et al. 2009; Spence 1998). Gemfibrozil is known to inhibit cytochrome P450 (CYP) 2C9, whereas its metabolites (gemfibrozil glucuronide) inhibit CYP2C8 (Ogilvie et al. 2006). Patients taking gemfibrozil are advised to take it 30 minutes before meals to enhance its absorption. Gemfibrozil is contraindicated to be used during pregnancy as its safety is not well documented in pregnant women (Parke-Davis 2013). Also, gemfibrozil is contraindicated to be used with the presence of liver or kidney diseases, gall bladder stones, known allergy to gemfibrozil and with the concomitant use with simvastatin or repaglinide. Serious drug-drug interactions could happen with the concomitant use of certain medications with gemfibrozil. For instance the concomitant use of both gemfibrozil and simvastatin could lead to increased risk of skeletal muscle toxicity (rhabdomyolysis), markedly elevated creatine phosphokinase (CPK) levels, and myoglobinuria, leading to acute renal failure and death (Parke-Davis 2013). Moreover, gemfibrozil and its metabolites inhibit the glucuronidation of repaglinide which causes an increase in the plasma concentration of repaglinide which is used to treat diabetes. This could lead to severe hypoglycemia upon the use of both medications (Gan et al. 2010). Bleeding could occur upon the use of gemfibrozil with anticoagulants, such as warfarin, as gemfibrozil displace them from plasma proteins and inhibit their metabolism by inhibiting CYP2C9 isoenzymes which potentiates their anticoagulation actions (Dixon and Williams 2009).

1.1.5 Brief Assessment for Smoking Cessation

The aims of the present study were to investigate the therapeutic potential of gemfibrozil in treating nicotine dependence. The classic way of conducting large clinical trials that could last
for years on a large number of tobacco smokers has been used for decades to test novel treatments for TUD. Such trials consume enormous amount of resources and effort. The results of such trials in most cases could yield negative results. Therefore, a more pragmatic, less expensive and short-term testing was needed to get a signal on the efficacy of a smoking cessation aid before going further steps of expanded testing (Perkins et al. 2006).

Studies for new smoking cessation candidates are directed to investigate the ability of this new medication to decrease the adverse effects of withdrawal and its ability to attenuate craving following a certain period of abstinence (Hatsukami et al. 1988a). The other alternative to test a new smoking cessation aid is to give the medication to the participants and then monitor their smoking behavior aspects such as smoking satisfaction and frequency changes under the effect of the medication (Perkins et al. 2006). Many studies that used the short-models screening procedures showed a lack of evidence to pursue large clinical trials. Some of them showed controversy of results even for the FDA-approved smoking cessation drugs that didn’t show a consistent reduction in withdrawal symptoms or ad libitum smoking in short-term lab studies (Benowitz et al. 1998; Perkins et al. 2010; Shiffman et al. 2000). This lack of effectiveness may be due to recruitment of participants not motivated to quit smoking or conducting the study within a short period of time, or studying the medication’s withdrawal relief rather than its effect on abstinence (Perkins et al. 2006). Therefore, a group of researchers proposed a pragmatic screening methodology: (a) recruitment of smokers planning to quit in the near future, (b) administration of drug for an extended duration, and (c) assessment of tobacco abstinence in participants’ natural environments. Such studies would also retain the advantages of the typical screening study, including brief duration and use of a within-subjects design, which allows an increased statistical power with a relatively small sample size. This screening methodology approach was tested in two studies one using varenicline and the other using nicotine patch (Perkins et al. 2010; Perkins et al. 2008). In both studies, smoking cessation aid was used versus placebo in a cross over design testing its ability to help motivated smokers quitting smoking in a short quit attempt for 1 week. Both Nicotine patch and varenicline increased the days of abstinence versus placebo for those motivated smokers. Therefore, in the present study all the previous mentioned measures were put into consideration using a validated screening methodology (Perkins et al. 2010; Perkins et al. 2008) to conduct a reliable and short-term clinical trial studying gemfibrozil. In order to increase the study statistical power a within-subject
design was implemented recruiting participants motivated to quit smoking in the near future. The number of abstinence days was the primary outcome of the present study investigating the therapeutic potential of gemfibrozil in treating nicotine dependence using lab paradigms to screen smoking behavior.

1.1.6 Lab Paradigms and Screening Models Used in TUD Research

Studies used different lab paradigms in translational research to screen the effect of different medication on different aspects of TUD such as smoking craving, nicotine reinforcement, lapse, relapse, and others. These human behavioral lab models represent a cost-effective and efficient way to obtain a signal for further clinical research based on data from preclinical research. However, sometimes, these models lack sensitivity to detect the effects of some medications even the FDA-proven ones. Therefore each study using these models should follow the technical steps accurately and interpret the results cautiously before taking any decisions for further costly investigations and large clinical trials phase II and III.

This section is presenting different lab paradigms used in the fields of smoking behaviors in humans. In this clinical trial the cue-elicited craving and the forced choice paradigms were used as explained in details in other sections.

1.1.6.1 Smoking Lapse Paradigm

Lapse or the 1st smoking incidence happening within an abstinence period is an important indicator for relapse (Shiffman et al. 1997). Lapse has been the focus of many researchers examining medication development for smoking cessation and their effects to maintain abstinence. For instance Mckee and colleagues have been using this lapse lab model for years studying many smoking cessation candidates. In this model, researchers observe mainly the ability of a smoker under treatment to resist smoking the 1st available cigarette and subsequent smoking pattern after a period of deprivation. After a period of treatment, all subjects are instructed to maintain abstinence for a certain period of time before coming to the lab. The model starts by using factors that could lead to relapse such as stress, nicotine deprivation, or alcohol. All subjects are then presented their preferred brand of cigarette, a lighter and ash tray and they are instructed that they could initiate smoking (tobacco self-administration session) or they could choose to postpone smoking for up to 50 minutes in exchange for money (monetary
reinforcement) and a fixed amount of money is exchanged for every 5 minutes of delaying smoking; this delay represents smoking resistance ability. If the participants decide to smoke they undergo a self-administration session where they can continue to smoke or receive money for each non-smoked cigarette (McKee 2009; McKee et al. 2015; McKee et al. 2011). The ability to resist smoking decreases significantly with longer period of nicotine deprivation and with the least amount of monetary reinforcement (McKee et al. 2012). One week of treatment with either bupropion or varenicline was used in order to validate the use of this lapse model after a period of nicotine deprivation. Both medications showed significant effects to resist smoking the 1st cigarette and subsequent smoking in smokers known to have their 1st cigarette within 5 minutes after waking up, (highly nicotine addictive), compared to placebo (McKee et al. 2012).

1.1.6.2 Forced Choice paradigm

Since the late 1970’s many studies have tested the reinforcing effects of addictive substances using the forced-choice paradigm in humans. Finally, this paradigm was validated after an enormous body of studies using either sedative or stimulant addictive substances (de Wit and Johanson 1987). Since its validation, the forced-choice paradigm has been used as a tool in the lab-based studies to predict the reinforcement and subsequent relapse of the addictive substance. In most of the studies the subjects are exposed to two identically designed types of drugs, A and B, where one is the active drug and the other is the placebo. After few trials of both drugs, the participants undergo a series of forced-choice trials where they have to make a choice between drugs A and drug B. The reinforcing effect of the drug is demonstrated whenever the active drug is chosen more often than the placebo (de Wit and Johanson 1987).

In case of testing nicotine reinforcement using the force choice paradigm, the subjects are exposed to two types of color-coded cigarettes with different amounts of nicotine: the nicotinized cigarette (Nic) and the denicotinized (Denic) cigarette, instead of drugs A and B. after testing both cigarettes, the subjects undergo a series of forced choice trials to choose puffs from either cigarette. Each trial is separated by 30 minutes of relaxation of reading and listening to music. The total session would take about 2 hours to perform a series of four forced choice trials. At the end of the session, the percentage of choice of Nic cigarette is calculated (Rukstalis et al. 2005). A group of researchers performed the forced choice paradigms using nicotine nasal spray and placebo spray where they presented both sprays to the participants during exposure trials. Then
the subjects had to choose one of the two nasal sprays in a series of forced choice tasks. The results showed that the percentage of choice of nicotine nasal spray was 74% of the total choice (Perkins et al. 1996). The reinforcing effects of both naltrexone and bupropion on nicotine smoking were investigated using the forced choice paradigm. The results of that study showed that naltrexone attenuated the reinforcing effect of smoking more than bupropion (Rukstalis et al. 2005).

In this thesis, the forced choice paradigm was used in the 1st clinical trial to test the ability of gemfibrozil to attenuate the reinforcing effect of nicotine after a period of treatment compared to placebo as explained in details in Chapter 2.

1.1.6.3 Cue-Elicited Craving

It is shown from studies that consuming substances of abuse and relapse could be related to the exposure to environmental cues related to that substance. Therefore, the cue elicited craving paradigm performed in the lab was used as a tool to test for the intensity of the craving for a specific substance using cues-related and non-related to the substance under investigation (Waters et al. 2003). For instance, when smokers were exposed to pictures of smokers or to another smoker with a cigarette they showed an increase in self-reported tobacco craving (Drobes and Tiffany 1997; Niaura et al. 1992; Sayette and Hufford 1994). A meta-analysis done on cue reactivity showed that smokers not only reported increased craving but also showed changes in autonomic functions such as an increase in heart rate and skin conductance, and a decrease in skin temperature in response to smoking cues but not to neutral cues (Carter and Tiffany 1999). Some researchers use what is called imagery scripts to elicit craving. In those imagery scripts, the participant while listening to an audiotaped scripted scene involving smoking, such as a party involving smoking, is asked to imagine themselves in that scenario. Then the participants are asked to imagine themselves in a neutral scene that doesn’t involve smoking and they have to rate their craving during each scene. The imagery scripted cues showed an increase in craving and physiological changes in response to the smoking scripts (Heishman et al. 2010; Heishman et al. 2004; Lee et al. 2007). Other studies use in vivo smoking cues such as asking the participant to light a cigarette without puffing and hold it for a certain period of time. As for the neutral cues, the participants would be handling non-smoking related objects such as glass of water, scissors or pencils. Meanwhile, the craving and the autonomic
responses are being recorded at the time of the cue presentation as well as before and after it. It was shown that using in vivo cues showed more robust results of increased craving and autonomic responses to the smoking cues than the imagery scripts describing smoking situations (Heishman et al. 2010).

1.1.6.4 Conditioned Cue Responding

As a continuation to the use of cue craving paradigm, facial electromyography (EMG) is another tool used in translational research to measure the facial reactions and the affective responses to smoking cues of certain muscles in the face. Miniature electrodes are put on the muscles which are mainly the smiling muscle or the zygomaticus major, the frowning muscle or corrugator supercilii muscle, and the orbicularis oris muscle or lip muscle (Berridge and Robinson 2003; Lang et al. 1993; Mueller et al. 2003). Researchers used this lab tool and found that when smokers are presented with smoking cues the results of EMG showed an increase in the positive affect and a decrease in the negative affect as a result of the incentive effects and rewards anticipated from smoking (Winkler et al. 2011). Also, it was found that the orbicularis muscle activity in the presence of smoking cues indicates the readiness for puffing (Mueller et al. 2003).

1.1.6.5 The Cue-Availability Paradigm

Carter and colleagues believed that when smokers have an access to consume cigarette during a cue-exposure session, their reactions and cravings in response to smoking cues might differ than during a conventional cue craving paradigms when the cigarettes are not consumed. Therefore, they created the cue-availability paradigm where the participants are presented with either a lit cigarette or a glass of water and they are instructed that they can consume the neutral or the addictive reinforcer for a certain percentage probability (0, 50, and 100 %). Consuming the cue means taking a puff from the cigarette or taking a sip of water, while the availability percentage at each cue presentation means whether the participant could not, might not, or could smoke or drink the water. Meanwhile, the physiological autonomic reactivity as well as self-reported craving and mood are recorded before each trial (Carter and Tiffany 2001). This type of cue manipulation is different than the cue-reactivity where the participants are totally deprived from smoking and from the instructed cue availability lead by other researchers where the participants are instructed at the end of cue presentation if they could smoke or no (Juliano and Brandon
Carter and colleagues believed that accessing the cigarettes during the cue-exposure session, not only after the session, might impact to a great extent the reactivity to the cue.

Their work shows that with increasing availability of consuming the cigarette, subjects showed an increase in craving, positive mood and a decrease in the negative mood with the smoking cue. These results are different from the cue reactivity paradigm where presentation of smoking cue increases the negative affect due to the long time of wait between cues and not being able to consume the cue i.e. smoking cigarette (Drobes and Tiffany 1997). However, the smokers included in their research were not treatment seekers or willing to quit in the near future. The authors concluded that this paradigm might work differently with abstinent smokers in a state to nicotine deprivation.

In this thesis, the forced choice and the cue-elicited craving paradigms were used in the 1st clinical trial to test the ability of gemfibrozil to attenuate the reinforcing effect of nicotine and smoking craving after a period of treatment compared to placebo as explained in details in Chapter 2.

1.2 Introduction to Clinical Trial II

Over 80% of Canadian population consumes alcohol among which 18% of Canadians meet criteria for alcohol use disorder (AUD) (Canada 2015).

According to DSM-5 AUD criteria are 11 criteria combining all criteria of abuse and dependence from the 4th edition, DSM-4, emphasizing craving and excluding legal issues. Symptom severity is divided into (mild = 2–3 criteria, moderate = 4–5 criteria, and severe ≥ 6 criteria). Any subject with at least 2 criteria is to be diagnosed with AUD (Hasin 2014; Takahashi et al. 2017).

Over 70% of AUD patients have alcohol-related sleeping problems and the available treatment have major health risks. According to the American Psychiatric Association, disturbance of

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sleep could be related to the timing, quality and amount of sleep. This disturbance can lead to distress and malfunctioning during the next morning (Kabel et al. 2018). Between 10 to 20% of the population suffer from sleep problems including insomnia. Insomnia or lack of sleep can be subdivided into initial insomnia or difficulties falling asleep, middle insomnia or difficulties to maintain a good sleep, and terminal insomnia or early waking up without the ability to fall asleep again (APA 2013). Sleep-Wake disorder could be for a short period ranging from few days to few weeks or could be more than that and become a chronic problem i.e. chronic insomnia. About 10% of adults suffer from chronic insomnia (Roehrs and Roth 2003). The consequences of insomnia could affect the general health of an individual both physically and emotionally and could lead to depression, cardiovascular complications and low quality of life (Pigeon et al. 2014).

Sleep-Wake disorder classification has changed over time in the different editions of DSM. In DSM-4 the sleep disorder was classified as primary insomnia if it happens as an independent disorder. The secondary insomnia was diagnosed when it is related to certain comorbidity or a medical condition (i.e. depression). Now, it is called Sleep-Wake disorder, in DSM-5, which highlights the presence of a medical or a mental condition co-existing and interacting with the sleep disturbances in a bidirectional relationship i.e. any condition could lead to the other. Sleep-Wake disorder is further subdivided to insomnia, hyper-somnolence, circadian rhythm sleep-wake disorder, restless legs syndrome narcolepsy, breathing-related sleep disorder, rapid eye movement (REM) disorder, non–rapid eye movement (NREM) sleep arousal disorders, nightmare disorder, and substance or medication-induced sleep disorder (Laudon and Frydman-Marom 2014; Reynolds and O’Hara 2013).

Some studies suggest that more than 54% of alcohol use disorder (AUD) patients suffer from substance-induced sleep problems (Brower 2015). In other studies the percentage is over 70% (Hartwell et al. 2015a) or higher (91%) (Zhabenko et al. 2012). The difference in percentage might be due to the difference in study design, severity of alcohol use, methods of intervention or assessment. Nevertheless, all studies assert that sleeping difficulties in AUD patients lead to serious health-deteriorating consequences. AUD associated insomnia and other sleep-wake problems are not only a very prevalent but also a very costly one. For instance billions of dollars are spent each year to the management of such problem (Bouchery et al. 2011; Pollack et al. 2009). It is also suggested that sleeping problems continue to be present even after early recovery.
in 69% of AUD subjects and even after long term recovery in 50% of the patients (Brower et al. 2001; Chaudhary et al. 2015; Kolla et al. 2014).

The reasons patients with AUD suffer from sleeping problems could be multifactorial: the genetic component could play a role, a comorbid or preexisting depression could be the reason, and of course a general disturbance in the physiological wake–sleep circadian rhythm is a main factor (Brower 2015). Studies done on AUD patients showed that their sleep problem is not related to a certain drinking phase, it is disturbed in all the drinking stages i.e. active use, early and prolonged abstinence, and even during withdrawal (Brower and Perron 2010; Foster and Peters 1999a). Ingestion of high doses of alcohol over long periods of time causes a decrease in the inhibitory response of GABA-A receptors accompanied with an increase in the excitatory activity of glutamate receptors (Knapp et al. 2014). Also, it was shown that when alcohol effects wear off, AUD subjects experience an increase in sleep latency or the time to fall asleep with a decrease of total sleep duration, (circadian rhythm sleep-wake disorder); the mechanisms by which tolerance to alcohol hypnotic effects could be explained (Angarita et al. 2016).

Moreover, The problem of not sleeping well or having difficulties falling asleep represents a special concern to AUD patients as it can lead them to relapse, vehicle accidents and suicidal ideation (Bernert et al. 2015; Brower 2015; Brower et al. 2001; Callaghan et al. 2013; Foster and Peters 1999b).

Polysomnography (PSG) studies done comparing sleep pattern between AUD subjects and healthy subjects found differences in different sleep stages. For instance, AUD sleep disruptions characterized by a persistent increase in light sleep or stage 1, a decrease in slow wave or deep sleep, and an increase in vivid dreaming as a result of disruption in REM sleep that could last for several months after sobriety (Colrain et al. 2009; Gann et al. 2001; Gillin et al. 1994). Moreover, between 5 to 9 months of continuous abstinence is needed to normalize sleep factors such as the time to fall asleep (sleep onset latency) and the percentage of sleeping time (sleep efficiency) (Drummond et al. 1998; Williams and Rundell 1981) and more than a year is needed to normalize the total sleep duration (Drummond et al. 1998). Studies showed that the relationship between alcohol and sleeping problems could be bidirectional which means failure to initiate and maintain good sleep could lead to the development and maintenance of substance use especially AUD in all age groups in one hand
(Miller et al. 2017b; Weissman et al. 1997; Wong et al. 2009) and on the other hand, AUD subjects tend to relapse to alcohol to sleep as a self-medication mechanism (Malcolm et al. 2007). Therefore no matter how successful the alcohol therapies are, persistent sleeping difficulties constitute a huge barrier to maintain long term abstinence (Miller et al. 2017a).

1.2.1 Treatment of AUD Induced Sleeping Problems

Sleep hygiene education is an important and frequent tool used by clinicians as a complementary strategy beside therapeutics in order to address sleeping problems in AUD subjects (Stepanski and Wyatt 2003). Practicing sleep hygiene techniques could only be successful if it becomes a habit or daily routine and that could take a long time to be achieved (Kaku et al. 2012; Taylor et al. 2010). Cognitive-behavioral therapy (CBT) is known to be a 1st line treatment for insomnia in general (Stepanski and Wyatt 2003) and thought to be effective in treating sleep difficulties in AUD with other pharmacotherapies. It usually takes several weeks and lots of sessions and commitment from the patients in order to observe improvement also highly trained professionals are needed in order to deliver the CBT properly, which sometimes limit CBT use in practice (Currie et al. 2004b; Stepanski and Wyatt 2003). CBT-I is the tool usually used in practice and it is divided into cognitive therapy, sleep hygiene, sleep restriction and stimulus control (Arnedt et al. 2007). Although published results from RCTs done on AUD subjects with insomnia treated with CBT-I are limited and with small sample size, the results were positive after 7-8 weeks of sessions versus placebo (Arnedt et al. 2011; Currie et al. 2004a).

Some Pharmacotherapies are also tried to treat AUD-induced sleeping problems as an Off-label use. For instance, Trazodone, a sedating antidepressant, is frequently used to treat sleeping difficulties in AUD subjects (Friedmann et al. 2003). There is controversy over its use since although it has proved superior efficiency in enhancing sleep quality compared to placebo, it is known to cause dizziness and hangover which could affect patient’s compliance (Le Bon et al. 2003). Also, in a RCT where trazodone were used for 12 weeks and showed self-reported sleep improvement in AUD participants, sleep worsening occurred in the trazodone group after stopping the medication and no difference in abstinence was observed between trazodone and placebo group (Friedmann et al. 2008). The same RCT showed drinking pattern worsening in the trazodone group that the authors tried to justify by the presence of an active trazodone metabolite that causes increased levels of cortisol, anxiety and cravings in AUD subjects (Umhau et al.
Therefore, it is advised that it is better to start with another medication to help the sleeping problems in early recovery or to use trazodone with an anti-craving medication for better results (Brower 2015). Another sedating antidepressant that was looked at for its use in AUD-induced sleeping problems was mirtazapine. PSG showed positive results for mirtazapine in AUD subjects diagnosed with sleeping problems and depression compared to placebo or other antidepressants. The results were in favor of its use in AUD subjects with anxiety or major depression (Liappas et al. 2005; Schmid et al. 2006; Shen et al. 2006). Moreover, it was shown that besides improving sleep quality it had some effects to decrease alcohol drinking and craving (Cornelius et al. 2012; Yoon et al. 2006). However, some treatment programs don’t favor the use of trazodone or mirtazapine especially in early recovery as their sedative property may act as an internal cue that could trigger alcohol drinking and relapse (Brower 2015).

Another drug with sedating properties is Quetiapine which is an antipsychotic medication. It was looked at for AUD with sleeping problems and showed improvement in sleep compared to placebo (Chakravorty et al. 2014; Litten et al. 2012). However, its use is limited by unwanted side effects mainly abuse properties and weight gain (Coe and Hong 2012).

Topiramate is a sedating anticonvulsant and used for migraine and some psychiatric disorders (Arnone 2005). In practice it is often prescribed to AUD subjects as it is thought to reduce relapse. A large RCT showed that topiramate improved sleeping and reduced heavy drinking days in AUD subjects (Johnson et al. 2008). Another sedating anticonvulsant is gabapentin where its use in AUD subjects showed a decrease in alcohol relapse, however, self-reported sleep and Polysomnography (PSG) didn’t show any sleep enhancement significance compared to placebo (Brower et al. 2008). Better drinking and sleep results were observed when gabapentin was administered alone in high dose and for long period (Mason et al. 2014) and when used with naltrexone for AUD subjects (Anton et al. 2011). It could be better to prescribe it for AUD with comorbidities as restless legs syndrome or anxiety (Brower 2015).

Acamprosate is a medication approved by FDA to treat AUD and it has been shown to improve related sleeping problems and enhancing the deep sleep stage in AUD subjects as per subjective reports as well as PSG (Perney et al. 2012; Staner et al. 2006).

Among the most classic treatment for sleep problems is prescribing benzodiazepine receptor agonist medications e.g. zopiclone, zolpidem, temazepam, triazolam, diazepam, oxazepam,
lorazepam and others. Although showing high efficiency, they also have high potential risks of impairing cognitive and psychomotor skills, increasing the risks of falling, dependence and abuse (Pigeon et al. 2014). These side effects including alcohol-induced risk for overdose and abuse potentials explain the reasoning of avoiding these medications by addiction medicine practitioners for long period after detoxification. Therefore, there is a true need for a safer sleep-aid treatment especially for patients suffering from SUD (Brower 2015).

1.2.2 Melatonin and Melatonin Receptors

Melatonin (N-acetyl-5-methoxytryptamine) is an important hormone secreted by the pineal gland in response to darkness. It binds to melatonin receptors MT1 and MT2, two G-protein coupled receptors, in the suprachiasmatic nucleus and plays a fundamental role in the sleep-wake cycle. MT1 and MT2 are G-inhibitory receptors, which mean that melatonin acts by decreasing cyclic adenosine monophosphate (c-AMP) levels thus modulating protein kinase A (PKA) activity (Jessica and Michael 2014; Liu et al. 2016). While MT1 is encoded by Chromosome 4, MT2 is encoded by Chromosome 11. Studies showed that MT1 is the receptor responsible for sleep onset, while MT2 receptor was shown to be responsible for shifting the timing of the circadian system. There is also MT3 which is a Quinone reductase 2 enzyme that belongs to the reductase group. It was shown that it inhibits the quinones electron transfer reactions thus preventing oxidative stress. MT3 receptor, a detoxification enzyme, is located in liver, intestine, kidney, heart, muscles, lung, and brown fat.

Melatonin levels are usually low during the day and can increase up to 80-90 pg/ml during night time with a high individual variability (Burgess and Fogg 2008). In normal individuals, the level of melatonin starts to increase before night-time sleep, reach its highest levels and peak sometime between 2:00 and 4:00 am then starts to decrease again after waking time. The melatonin supplement has a low oral bioavailability and very short half-life (20-30 minutes) and it is extensively metabolized by the liver enzymes to is metabolite 6-sulfatoxymelatonin (a6MTs), mainly by CYP 1A2 (Srinivasan et al. 2011).

In the market, melatonin is an over the counter supplement and it is not regulated by the FDA as a medicinal drug (NCCIH 2015). It is commonly used by people with sleep difficulties due to night shifts, jet lag, and restless leg syndrome or patients suffering from sleep-wake problems in general (Tajedin et al. 2015). According to Health Canada, melatonin is considered a natural
health product and is available as over the counter tablets, sublingual capsules and liquid form and is used as a sleep aid for adults. Regarding drug-drug interaction, the co-administration of melatonin and sedative medications as benzodiazepine receptor agonists can cause severe hypnosis. Also, it is found that some medications can cause an increase or a decrease in melatonin levels. For instance, oral contraceptive pills increases melatonin production in the body, while caffeine can decrease oral melatonin effectiveness. Concomitant use of fluvoxamine with melatonin increases the absorption of melatonin which could enhance its effect and increases its side effects (MayoClinic 2013). Moreover, melatonin is known to enhance the immune system, which explains why taking melatonin should be avoided with medications that suppress the immunity (Immunosuppressants) as melatonin inhibits their actions. Melatonin is found to reduce the blood coagulation activity; therefore taking melatonin with warfarin and other anticoagulants increases the risk of bleeding (Herxheimer and Petrie 2002). Regarding diabetic patients, melatonin decreases insulin secretion and interact with antidiabetic medications by decreasing their effects which leads to increased blood sugar (Dantas-Ferreira et al. 2018).

According to a systematic review studying the efficacy and safety of melatonin, it was concluded that melatonin can be considered as a safe drug. Nevertheless, there is a chance that patients taking it might have one or more of the following side effects: headache, short-term feelings of depression, daytime sleepiness, dizziness, stomach cramps, and irritability (Buscemi et al. 2005). Regarding pregnancy and breast-feeding: Melatonin is possibly unsafe when taken during pregnancy. Melatonin might also interfere with ovulation, making it more difficult to become pregnant. Not enough is known about the safety of using melatonin when breast-feeding. Therefore, according to Health Canada instructions it is contraindicated to take melatonin during pregnancy and breastfeeding. Also, driving and using machinery should be prohibited for four to five hours after taking it (HealthCanada 2018).

While melatonin is a natural product, Ramelteon and Tazimelteon, approved by FDA in 2005 and in 2014, respectively, are two synthetic melatonin agonists used for sleep problems. Both are characterized by a higher half-life compared to melatonin; Ramelteon T1/2 is 1–2.6 hours and Tazmelteon T1/2 is 1.3 to 3.7 hours. In vitro research for receptor binding assays showed that Ramelteon had a greater selectivity and affinity for both MT1 and MT2 receptors and a significantly higher dissociation constant from MT receptors binding sites. Also it showed lesser affinity than melatonin to MT3, the melatonin related enzyme Quinone reductase (Kato et al.
Clinically, Ramelteon proved to be 10 times more potent than melatonin to enhance sleep quality. For instance, it showed better results in shortening sleep onset latency and increasing sleep duration more than melatonin. Although studies on Tazimelteon showed that it is a melatonin-receptor agonist at the MT1 and MT2 receptors, it showed higher affinity for the MT2 receptor, which explains its use for the non-24 hour sleep-wake disorder in blind subjects. Nevertheless, both are much more expensive than melatonin (Johnsa and Neville 2014; Kato et al. 2005; Richardson et al. 2008).

Melatonin’s relation with sleep was first noticed in the late fifties by Lerner and his colleagues when they injected melatonin intravenously to volunteers who fell asleep after injection (Lerner et al. 1959; Lerner et al. 1958). Since that period many studies were conducted on melatonin to test its effectiveness on sleep. Clinical trials done on melatonin in patients with comorbid sleep problems showed that in most of the cases, melatonin had superior effects than placebo in improving the quality of sleep, reducing the sleep-onset latency period, reducing the number of night-awakening and improving morning activity assessed by validated sleep-wake questionnaires and/or PSG. Those studies included patients with Schizophrenia (Kumar et al. 2007), Alzheimer disease (Asayama et al. 2003), Parkinson’s disease (Dowling et al. 2005; Srinivasan et al. 2011), and hypertension (Scheer et al. 2012). The duration of treatment in those studies was 2-4 weeks and the dose of melatonin used was (2.5 mg-50 mg). Moreover, the trials studying melatonin were not merely looking into adults’ sleep problems; some studies were done on children too. Randomized clinical trial done on children with atopic dermatitis showed that melatonin shortened the sleep-onset latency by 21.4 minutes compared to placebo and the use of melatonin was safe in those children (Chang et al. 2016).

Controversy about an effect of a drug is not unusual in the science literature. Although all the previously mentioned studies concluded some positive results in favor of melatonin and its superiority over placebo use, there are other placebo-controlled trials showing no effect of melatonin as a sleep aid in comparison to placebo (Gehrman et al. 2009; Serfaty et al. 2002). Moreover, the results of three RCTs done on 209 patients with Alzheimer disease and sleep disturbances revealed no evidence of melatonin to be better than placebo (McCleery et al. 2014).
1.2.3 Melatonin and Alcohol

Preclinical and clinical studies showed that drinking alcohol for a long period alters melatonin production and function in circadian rhythm and that the peak melatonin surge is found to be blunted in AUD subjects (Kuhlwein et al. 2003; Peres et al. 2011; Zhou et al. 2003). These are the reasons that could explain the persistence of sleeping problems even after long term abstinence (Drummond et al. 1998). Interestingly, melatonin receptor MT1 protein was found to be expressed in prefrontal cortex, nucleus accumbens and amygdala, the well-known brain areas involved in addictive behaviors (Noori et al. 2012; Uz et al. 2005). In a preclinical study, melatonin and agomelatine (MT1 and MT2 agonist) were found to reduce the relapse-like alcohol consumption and decrease alcohol wanting in rats (Vengeliene et al. 2015).

In practice, it is not unusual to prescribe melatonin with or without other sleep medications for AUD in mental health institutes (Zhao 2016). However, there is currently no clear data showing efficacy of this approach with AUD although research shows that melatonin levels are reduced in subjects with AUD who are trying to be abstinent from alcohol (Conroy et al. 2012; Kuhlwein et al. 2003). Therefore, this double- blinded randomized placebo-controlled pilot study was conducted to fill the gap of knowledge of the effect of melatonin versus placebo on sleep quality in AUD patients with sleeping problems. To our knowledge, this is the first RCT using melatonin alone with AUD patients.

1.3 Overview of the Thesis

This thesis focuses on two randomized placebo-controlled clinical trials investigating the therapeutic effects of two potential novel treatments for subjects diagnosed with SUD. The first one aimed to investigate a novel therapeutic candidate for nicotine dependence in treatment-seeking smokers using lab paradigms modeling craving and relapse and two brief quit attempts in a crossover design. The second trial aimed to investigate a safe and affordable treatment, which is regulated as a natural health product, for sleeping problems that are prevalent in treatment-seeking AUD subjects using sleeping and mood scores for four weeks of treatment.
2 Methods of Clinical Trial I

2.1 Study Design

This study was a double-blind, placebo-controlled, crossover design comparing the effects of gemfibrozil and placebo on smoking in treatment-seeking smokers. As shown in Table 1, the study had two 2-week phases separated by a washout period of at least 1 week. At the assessment visit, the entire participants’ demographic information was collected as age, sex, ethnicity, education, and other inclusion criteria were assessed. Also, the Fagerstrom Test for Nicotine Dependence (FTND), (Appendix A), was assessed during the 1st visit. FTND, a reliable self-administered 6-item questionnaire, was used to identify severity of nicotine dependence. It has a total score ranging between 0 and 10, with higher scores indicating more dependence (Heatherton et al. 1991).

At the medication visit, each participant was randomized to receive either gemfibrozil or placebo for two weeks. At the end of the first week of medication, laboratory measures as cue-elicited craving and forced choice paradigms took place. The second medication week served as the quit attempt week in which the participants taking either treatment were asked to stop smoking for the whole week and report their abstinence daily by phone. A referral to smoking clinic in the Center for Addiction and Mental Health (CAMH) was provided to the participants during the last visit to help them quit smoking. The order of medication phases was randomized and counterbalanced. Participants were contacted by telephone 1 week after the last medication phase to ensure there were no side effects, and then were discharged from the study.

2.1.1 Recruitment

Participants were recruited through posters, newspaper ads and web-based advertisements such as Craigslist and Kijiji. Participants CONSORT flow chart is presented in Figure 1.
### Table 1 Overview of study design and visits

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<th>Study phase</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment</td>
<td>Screening for eligibility</td>
</tr>
<tr>
<td>Medication visit 1</td>
<td>Attend clinic to receive medication. Medication start day was selected to be at least 7 days before the laboratory test days.</td>
</tr>
<tr>
<td>1st week of medication</td>
<td>Smoke normally; take medication (gemfibrozil or placebo). Attend clinic for laboratory tests after taking medication for at least one week. Call in daily.</td>
</tr>
<tr>
<td>2nd week of medication</td>
<td>Quit attempt week while taking medication. Attend clinic at the end of the week to assess abstinence. Call in daily.</td>
</tr>
<tr>
<td>Washout week</td>
<td>No medication for at least a week.</td>
</tr>
<tr>
<td>Medication visit 2</td>
<td>Attend clinic to receive medication. Medication start day was selected to be at least 7 days before the laboratory test days.</td>
</tr>
<tr>
<td>3rd week of medication</td>
<td>Smoke normally; take medication (gemfibrozil or placebo). Attend clinic for laboratory tests after taking medication for at least one week. Call in daily.</td>
</tr>
<tr>
<td>4th week of medication</td>
<td>Quit attempt week while taking medication. Attend clinic at the end of the week to assess abstinence. Call in daily.</td>
</tr>
<tr>
<td>1 week follow-up</td>
<td>Calling the participants to rule out any side effects.</td>
</tr>
</tbody>
</table>
Participants were recruited through posters, newspaper ads and web-based advertisements. 498 participants were phone screened, 67 were eligible and came in for in person assessment, 28 participants were enrolled, and the results of 27 participants were analyzed.
Inclusion Criteria
1. 19-65 year old males and females
2. smoking at least 10 cigarettes per day for at least 2 years
3. intend to quit smoking within the next 3 months
4. medically and psychologically healthy as determined by screening criteria (Appendix B)
5. women capable of becoming pregnant must agree to use contraceptives during the study

Exclusion Criteria
1. currently attempting to quit smoking
2. treatment for tobacco addiction in the past 3 months
3. use of nicotine replacement products, bupropion, or varenicline in the past 3 months as an aid to quit or reduce smoking
4. use of any oral tobacco product in the past 3 months
5. history of drug or alcohol dependence within last 5 years
6. consumption of more than 15 alcoholic drinks per week on average during the past month
7. use of any illicit drug more than once per week on average during the past month
8. current use of gemfibrozil or other fibrate medication
9. current use of any medication that is contraindicated for gemfibrozil or that would interfere with the protocol in the opinion of the Qualified Investigator (QI). This includes, but is not limited to anticoagulants; statins; other fibrates; other lipid-lowering agents, such as colestipol, niacin, or herbal remedies; and any oral or injected medications for diabetes, such as rapaglinide.
10. any pre-existing gall-bladder disease or operation in the past 12 months
11. any history of or current cardiovascular, liver, hepatic or renal disease
12. diabetes
13. pregnant, nursing, or become pregnant during the study
14. use of psychoactive drugs or medications as revealed by urine toxicology

2.1.2 Medication

Gemfibrozil tablets were supplied by Pfizer; Inc. CAMH Research Pharmacy formulated the gemfibrozil and the placebo into capsules and dispensed them to the participants in blister packs. The gemfibrozil dose used in this study was as clinically indicated for hypercholesterolemia, 600
mg twice daily taken orally 30 minutes before the morning and evening meals. Although gemfibrozil is considered a safe drug, side effects were monitored on a scale of 0-3 (none, mild, moderate, and severe). This scale included nausea, agitation, nervousness, constipation, dry mouth, fatigue, insomnia, headache, increased appetite.

2.1.3 Cigarettes

During the forced-choice procedure, Quest® 3 (Vector Tobacco Inc.) and commercially-available cigarettes were used. Genetically engineered tobacco was used to manufacture the Quest® 3 cigarettes, such that a (Denic) cigarette delivers less than 0.05 mg nicotine. Quest® 3 cigarettes were used in previous studies during the forced-choice procedure. The Nic cigarette used was a regular nicotine-containing cigarette that delivers 1.2 mg nicotine and for the cue-reactivity procedure, participant’s preferred brand of cigarette was used.

2.1.4 Medication Compliance

Medication compliance was monitored by requiring participants to call into a dedicated study phone line once a day after taking their second capsule. During the call, they also indicated the total number of cigarettes they have smoked that day. Also, the participants were asked to return the blister packs to verify the number of doses taken and breath carbon monoxide (CO) was measured in each visit. Abstinence was assessed by self-reports of no smoking and by breath CO < 5 ppm on clinic visits.

2.1.5 Lab Paradigms: Forced Choice and Cue Craving

Forced Choice paradigm: In this study, the forced choice session started with the participant having four puffs of the preferred brand of cigarettes and then relaxed for 30 mins listening to music or reading. Then, four exposure trials started that were separated by 30 minutes of relaxation time. In each exposure trial, the participant took four puffs of a Nicotinized (A) or Denicotinized (B) cigarette in the order of ABAB or BABA. Cigarettes were color-coded and rated with the modified cigarette evaluation questionnaire (Cappelleri et al. 2009). Then the participant started the four choice trials separated by 30 minutes of relaxation. In each trial, the participant chose any combination of 4 puffs from the two cigarettes. The Nic cigarettes, commercially-available cigarettes were used (Players Rich). The Denicotinized cigarettes were
Quest® 3 (Vector Tobacco Inc.) cigarettes such that a cigarette delivers less than 0.05 mg nicotine.

Cue-elicited craving: In this study, after 1 week of medication, the participant came for the cue reactivity session where two types of cues, smoking and neutral, were presented to the participants. Upon arrival the participant was seated on a comfortable chair, smoked 4 puffs from the preferred-brand cigarette and completed the baseline questionnaires: Mood Form (Diener and Emmons 1984) (Appendix A), Tobacco Craving Questionnaire-Short Form (TCQ-SF) (Heishman et al. 2008) (Appendix B), Visual Analog Scale (VAS) (Heishman et al. 2010) (Appendix C) and Minnesota Nicotine Withdrawal Scale (MNWS) (Patten and Martin 1996) (Appendix D). The details of the questionnaire were as follows: in the mood form the participant has to answer this question regarding 9 different moods: “Please indicate how much you are experiencing each of the following moods right now”: happy, depressed/blue, joyful, unhappy, pleased, enjoyment/fun, frustrated, worried/anxious, and angry/hostile. Each mood is rated from 0 to 6 where 0 is not at all and 6 is extremely. TCQ-SF factor 1= Emotionality, which is the relief from withdrawal symptoms or negative mood where lower scores are less relief. Factor 2= Expectancy, which is the anticipation of positive outcomes from smoking where lower scores are less positive outcomes. Factor 3= Compulsivity reflecting a lack of control over tobacco use where a decrease is less compulsive. Factor 4= Purposefulness reflecting intention and planning to smoke for positive outcomes where a decrease is less intention. The VAS is a horizontal line scale from 0-100 mm where 0 is not at all and 100 is extremely. The participant has to mark the answer on that scale. It has 4 components: negative mood: “How negative is your mood now?” positive mood: “How positive is your mood now?” craving to a cigarette: “How much do you crave a cigarette now?” and urge to smoke: “How much is your urge for a cigarette now? The MNWS is composed of 7 questions related to withdrawal and the participant had to rate each one from 0 to 4 where 0 is not at all and 4 is severe.

The participant was then connected to the Biopac device electrodes to record the physiological responses before, at and after (15 and 30 minutes) each cue presentation. The smoking cue was a covered box inside which there was a pack of cigarettes and a lighter. The participant had to light the cigarette without puffing and hold it for 30 seconds while the physiological recordings were measured. Then the participant was asked to extinguish the cigarette. The neutral cue box contained an unsharpened pencil, a notepad and a sharpener. The participant had to sharpen the
pencil and hold it as if writing for 30 seconds while the Biopac is recording the physiological responses. Each participant completed the TCQ-SF, Mood Form, and VAS forms just before, at the cue and after each cue presentation.

2.1.6 Study outcomes

The main outcomes of the present study were days of abstinence, smoking cue reactivity and Nicotine reinforcement as shown in Figure 2.

![Outcome Measures Diagram]

Figure 2 Main outcomes of the study

2.2 Data Analysis

Cue reactivity subjective and physiological measures data were analyzed using the Linear Mixed Model for Repeated Measures (MMRM). Subjects were defined as clusters and then random intercept was fitted for every subject. Treatment (gemfibrozil; placebo), cue type (smoking; neutral), and period (before, during, after cue) were fixed effects. Medication sequence was also controlled for. Forced choice data were analyzed using generalized estimating equation with a binary logistic model that uses logit link. There were measures to control for the medication sequence, the cigarette type randomization, and for the period of choice (1st and 2nd study phases). The mean number of choice was multiplied by 100 to calculate the percentage of Nic
puffs choice. The mean (±SEM) number of days of abstinence was calculated for each participant during quit attempts for both treatments. The effects of gemfibrozil or placebo on the number of cigarettes smoked each day for 7 days during the quit attempt week was analyzed with MMRM where the sequence of medication and the period of abstinence were controlled for baseline, 1st study phase, 2nd study phase. For all analyses, results were considered significant at p < 0.05 (SPSS version 21.0).
Chapter 3  
Results of Clinical Trial I

3  Results of Clinical Trial I

Participants (n = 27) were 10 females and 17 males; 52% were White, 15% were Black, 7.5% were Asian, and the remainder participants were of mixed race. Mean (±SD) age of the participants was 43 (±12) years, and mean years of education after high school was 2.4 (±2.2) years. Mean (±SD) cigarettes/day was 18.8 (±7) and participants smoked for 21.2 (±11.6) years at baseline. FTND was measured at screening; mean (±SD) FTND was 5.4 (±1.7). Mean (±SD) baseline smoking contemplation ladder score, as an indicator for motivation to quit (Biener and Abrams 1991) was 7.2 (±2.9). During the study, gemfibrozil was well tolerated, with mild to moderate side effects reported as follows: participants taking gemfibrozil in the 1st phase then placebo (n = 14) reported headache (n = 3), change in appetite (n = 2) and stomach upset (n = 3). Participants taking placebo in the first phase then gemfibrozil (n = 13), reported change in appetite (n = 4) and stomach upset (n = 2). All side effects were resolved at following up. No participants reported serious side effects.

3.1  Days of Abstinence

No significant difference was found between both treatments with respect to number of days of self-reported abstinence during both quit attempt weeks: mean (±SEM) = 0.2 (±0.1) day for gemfibrozil versus 0 day for placebo as shown in Figure 3. The average number of smoked cigarettes for 1 week at baseline mean (±SEM) was significantly higher than the average number of smoked cigarettes during either gemfibrozil or placebo quit attempt week: mean (±SEM) at baseline: 16.8 (±1.1); F (2,54) = 31.8; p = (< 0.001); 95% CI [14.5, 19]; (gemfibrozil 9.8 (±1.1); 95% CI [7.5, 12] and placebo 9.6 (±1.1); 95% CI [7.3, 11.9]); respectively. Bonferroni adjustment for multiple comparisons revealed no significance between both treatments (mean diff. = 0.2 (±1.03); 95% CI [-2.3, 2.7]; p = 1) as shown in Figure 4.
Figure 3 The Number of days of abstinence for both treatments.

The X axis represents both treatment conditions and the Y axis represents the number of days of abstinence. No significant change in the number of days of abstinence was observed using either gemfibrozil or placebo. Mean days of abstinence during the quit attempt weeks for both gemfibrozil and placebo: mean days $\pm$ SEM were $0.15 \pm 0.09$ for gemfibrozil versus 0 days for placebo.
Figure 4 Number of smoked cigarettes/day during quit attempt week.

This figure is showing the Self-reported number of smoked cigarettes per day (on the Y axis) for 7 days (on the X axis) at baseline (black bars), during the quit attempt week taking gemfibrozil (pattern bars) and during the quit attempt week taking placebo (grey bars). After comparing the effects of both treatments using a linear mixed model, the number of smoked cigarettes/day was significantly higher during baseline compared to both treatments (< 0.001). No significant difference was detected between gemfibrozil and placebo groups with means ± SEM of (0.77 + 0.04 vs 0.79 + 0.04 respectively) p=0.7.
3.2 Forced Choice

The effect of both treatments on Nic puff choice was calculated using generalized estimating equation. No significant difference was found between treatments [estimated marginal means (±SEM): 0.77 (±0.04); 95% CI [0.68, 0.84]; and 0.79 (±0.04); 95% CI [0.7, 0.86] for gemfibrozil and placebo; respectively p = 0.7, as shown in Figure 5.

![Figure 5 The percentage of choice for Nicotinized cigarettes.](image)

This figure is showing the percentage of choice of nicotinized (Nic) cigarette puffs during the forced choice paradigm (Y axis) for both Treatment conditions (X axis). The results showed no significant difference between gemfibrozil (77% of Nic choice) versus placebo (79% of Nic choice).
3.3 Smoking Cue Reactivity (Physiological Measures)

Linear mixed model for repeated measures (Drug x Time x Cue) revealed no drug effect. However, a significant effect of cue was observed for skin conductance, (Figure 6), where values were higher in response to smoking cue with mean difference (mean diff.) = 0.26 (±0.12), F (1,390) = 4.7; p = 0.031; 95% CI [0.02, 0.5]. For heart rate, (Figure 7), there was a significant decrease over time for both treatments, F (3,390) = 5.9; p = 0.001. Bonferroni adjustment for multiple comparisons revealed a significant decrease after 30 minutes of cue presentation than at cue, with mean diff. = -2.6 (±0.8); F (3,390) = 5.9; p = 0.006; 95% CI [-4.7, -0.5] and from before cue, with mean diff. = -3.1 (±0.8); F (3,390) = 5.9; p = 0.001; 95% CI [-5.1, -0.9]. Skin temperature (Figure 8), showed a cue effect where temperature was lower in smoking cue versus neutral cue, mean diff. = -0.2(±0.1); F (1,390) = 5.6; p = 0.02; 95% CI [-0.4, -0.04]. Blood pressure data was hard to be interpreted with many missing periods of recording due to technical issues.
Figure 6 Smoking cue reactivity –Physiological measures- Skin Conductance.

This figure is showing the changes in the sweat gland activity in response to both neutral and smoking cues with both treatment conditions. The X axis is presenting the timing of the skin conductance recording; before cue, at cue, after 15 minutes and after 30 minutes of the cue presentation. The Y axis is presenting the measurement of the skin conductance in micosiemens. A cue effect was observed where skin conductance significantly increased with the presentation of the smoking cue. No drug effect was observed. SkCond = Skin conductance, gem= gemfibrozil. Scores are shown as mean ± SEM.
Figure 7 Smoking cue reactivity – Physiological measures - Heart Rate (H.R).

This figure is showing the changes in heart rate in response to both neutral and smoking cues under both conditions. The X axis is presenting the timing of the heart rate recording; before cue, at cue, after 15 minutes and after 30 minutes of the cue presentation. The Y axis is presenting the measurement of the heart rate measured by the number of beats/minute. A significant decrease over time was detected. No drug effect was observed. Gem = gemfibrozil, smok = smoking cue. Scores are shown as mean ± SEM.
Figure 8 Smoking cue reactivity – Physiological measure - Skin Temperature.

This figure is showing the skin temperature (SKT) changes to both neutral and smoking cues under both conditions. The X axis is presenting the timing of the skin temperature recording: before cue, at cue, after 15 minutes and after 30 minutes of the cue presentation. The Y axis is presenting the measurement of the skin temperature measured by degree Celsius. A significant decrease over time was detected. No drug effect was observed. Gem = gemfibrozil, smok = smoking cue. Scores are shown as mean ± SEM.
3.4 Smoking Cue Reactivity (Subjective Measures)

Linear mixed model for repeated measures (Drug x Time x Cue) revealed no drug effect. However, a significant decrease of VAS positive mood over time, $F(2, 286) = 5.8; p = 0.003$ was observed. Bonferroni adjustment for multiple comparisons showed that VAS positive at cue and after cue were lower than before cue for both treatments (with mean diff. = -3.3 (±1.2); 95% CI [-6.2, -0.4]; $p = 0.02$) and (-3.8 (±1.2); 95% CI [-6.7, -0.9]; $p = 0.005$); respectively, (Figure 9).

VAS urge showed a significant increase over time, $F(2, 286) = 19.3; p < 0.001$. Bonferroni adjustment for multiple comparisons showed that VAS urge was higher at and after cue than before for both treatments (with mean diff. = 8.9 (±2.4); 95% CI [3.2, 14.8]; $p = 0.001$) and (14.9 (±2.4); 95% CI [9.1, 20.7]; $p < 0.001$); respectively, (Figure 10). There was a significant increase in VAS craving over time for both treatments, $F(2, 286) = 10.3; p < 0.001$. Bonferroni adjustment for multiple comparisons showed that VAS craving at and after cue were higher than before (with mean diff. = 7.2 (±2.7); 95% CI [0.7, 13.7]; $p = 0.023$) and (12.2 (±2.7); 95% CI [5.7, 18.7]; $p < 0.001$); respectively, (Figure 11). VAS negative mood didn’t show any significant effect ($p = 0.56$) as shown in Figure 12. Mood positive questionnaire showed a significant decrease over time where after cue was lower than before cue (with mean diff. = -0.9 (±0.3); 95% CI [-1.7, -0.1]; $F(2, 286) = 4.1; p = 0.025$), (Figure 13). Mood negative didn’t have any drug effect ($p = 0.75$) as shown in Figure 14. Also, TCQ factor 1 for emotionality, (Figure 15), and TCQ factor 2 for expectancy, (Figure 16), showed a significant increase over time for both treatments, where at and after cue scores were higher than before cue in both questionnaires [Bonferroni adjustment for multiple comparisons: (TCQ1 at cue mean diff. = 0.8 (±0.3); 95% CI [0.1, 1.5]; $F(2, 286) = 6.5; p = 0.028$); (TCQ1 after cue mean diff. = 1 (±0.3); 95% CI [0.3, 1.7]; $F(2, 286) = 6.5; p = 0.002$); (TCQ2 at cue mean diff. = 1.5 (±0.4); 95% CI [0.4, 2.5]; $F(2, 286) = 10.4; p = 0.002$); (TCQ2 after cue mean diff. = 1.9 (±0.4); 95% CI [0.8, 2.9]; $F(2, 286) = 10.4; p < 0.001$). TCQ factor 3 for compulsivity and TCQ factor 4 for emotionality didn’t show any significant effect as shown in Figures 17 and 18, respectively.
This figure is showing the results of the question: “How positive is your mood now?” in the visual analogue scale (VAS). The X axis is presenting the timing of the self-reported VAS; before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the positive mood measured by a scale 0-100 mm. VAS Positive (pos) mood decreased significantly from baseline to at and after cue for both treatments (p < 0.001). No drug effect was detected. gem = gemfibrozil, smok = smoking cue. Scores are shown as mean ± SEM.
Figure 10 Smoking cue reactivity (subjective measures – VAS Urge).

This figure is showing the results of the question: “How much is your urge for a cigarette now?” in the visual analogue scale (VAS). The X axis is presenting the timing of the self-reported VAS; before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the urge measured by a scale 0-100 mm. Urge to smoke increased significantly from baseline to at and after cue for both treatments (p < 0.001). No drug effect was detected. Gem = gemfibrozil. Scores are shown as mean ± SEM.
Figure 11 Smoking cue reactivity (subjective measures – VAS Craving).

This figure is showing the results of the question: “How much do you crave a cigarette now?” in the visual analogue scale (VAS). The X axis is presenting the timing of the self-reported VAS; before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the craving measured by a scale 0-100 mm. Craving increased significantly from baseline to at and after cue for both treatments (p < 0.001). No drug effect was detected. Scores are shown as mean ± SEM.
**Figure 12 Smoking cue reactivity (subjective measures – VAS Negative Mood).**

This figure is showing the results of the question: “How negative is your mood now?” in the visual analogue scale (VAS). The X axis is presenting the timing of the self-reported VAS; before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the negative mood (neg) measured by a scale 0-100 mm. No drug effect was detected. Gem = gemfibrozil. Scores are shown as mean ± SEM.
Figure 13 Smoking cue reactivity (subjective measures –Positive Mood).

This figure is showing the results of positive mood under both treatments. The X axis is presenting the timing of the self-reported mood: before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the mood measured by a sum of all the positive mood scores: “Please indicate how much you are experiencing each of the following moods right now”: The positive mood questions were happy, joyful, pleased, enjoyment/fun. Each mood is rated from 0 to 6 where 0 is not at all and 6 is extremely. Positive mood (pos) decreased significantly over time. No significant drug effect was observed. gem = gemfibrozil, smok = smoking cue. Scores are shown as mean ± SEM.
Figure 14 Smoking cue reactivity (subjective measures – Negative Mood).

This figure is showing the negative mood under both treatments. The X axis is presenting the timing of the self-reported mood; before cue, at cue, after the cue presentation. The Y axis is presenting the measurement of the mood measured by a sum of the negative mood scores: “Please indicate how much you are experiencing each of the following moods right now”: depressed/blue, unhappy, frustrated, worried/anxious, and angry/hostile. Each mood is rated from 0 to 6 where 0 is not at all and 6 is extremely. No significant drug effect was observed. Neg = negative; gem = gemfibrozil; smok = smoking cue. Scores are shown as mean ± SEM.
Smoking cue reactivity (subjective measures – Tobacco Craving Questionnaire - TCQ1).

This figure is showing the results of TCQ factor 1= Emotionality, which is the anticipation of a relief from withdrawal symptoms or negative mood with smoking. The X axis is presenting the timing of the self-reported TCQ: before cue, at cue, after the cue presentation. The Y axis is presenting the scoring measurement. A significant increase over time for both treatments was observed, where at and after cue scores were higher than before cue presentation. gem= gemfibrozil; smok= smoking cue. Scores are shown as mean ± SEM.
Figure 16 Smoking cue reactivity (subjective measures – Tobacco Craving Questionnaire - TCQ2).

This figure is showing the results of TCQ factor 2= Expectancy, which is the anticipation of positive outcomes from smoking where lower scores are less positive outcomes. The X axis is presenting the timing of the self-reported TCQ; before cue, at cue, after the cue presentation. The Y axis is presenting the scoring measurement. A significant increase over time for both treatments was observed, where at and after cue scores were higher than before cue presentation. gem= gemfibrozil; smok= smoking cue. Scores are shown as mean ± SEM.
Figure 17 Smoking cue reactivity (subjective measures –Tobacco Craving Questionnaire - TCQ3).

This figure is showing the results of TCQ factor 3=Compulsivity, which is inability to control tobacco use. The X axis is presenting the timing of the self-reported TCQ; before cue, at cue, after the cue presentation. The Y axis is presenting the scoring measurement. No drug effect was observed. gem= gemfibrozil; smok= smoking cue. Scores are shown as mean ± SEM.
Figure 18 Smoking cue reactivity (subjective measures – Tobacco Craving Questionnaire - TCQ4).

This figure is showing the results of TCQ Factor 4= Purposefulness, reflecting intention and planning to smoke for positive outcomes. No drug effect was observed. The X axis is presenting the timing of the self-reported TCQ: before cue, at cue, after the cue presentation. The Y axis is presenting the scoring measurement. No drug effect was observed. gem= gemfibrozil. Scores are shown as mean ± SEM.
Chapter 4  
Discussion of Clinical Trial I

4 Discussion of Clinical Trial I

According to The United Nations, drug addiction is considered the most complex and dominating neuropsychiatric disorder of the modern era. The patho-psycho-physiological pathway of addiction is still not clear. Therefore, it is very challenging to “treat” such disorder (Fattore and Diana 2016).

Based on preclinical findings showing promising results regarding the effect of PPAR-α agonists on nicotine seeking behaviors in animals and nicotine-induced dopamine firing in VTA, this trial was looking into the effects of gemfibrozil, a PPAR-α agonist, on smoking cessation in treatment seeking smokers. Gemfibrozil did not increase the number of days of smoking abstinence during the quit attempt week versus placebo. Percent choice of Nic cigarettes during the forced-choice test was almost the same while taking gemfibrozil or placebo. Similarly, the effects of gemfibrozil were not significantly different from placebo on physiological measures as heart rate, sweating, and skin temperature as well as on the subjective measures as craving a cigarette, urge to smoke and positive/negative moods during smoking cue reactivity paradigm.

4.1 Study Outcomes

The primary outcome was the number of smoking abstinence days. In this study, participants tried to quit smoking during both quit attempt weeks as shown by the decrease in the average number of cigarettes smoked per day for 7 days at baseline and during the quit attempt week using either gemfibrozil or placebo. However, it is surprising that the subjects could not abstain longer than one day. In previous studies the mean quit days in placebo groups could reach 1.9 days (Perkins et al. 2016). The explanation of this difference could be that the participants knew that they could try to quit smoking but there will be a second week of trial (another chance) in the second phase of the study according to the cross-over design. Also, the participants knew that they wouldn’t be excluded from the trial if they do smoke. Although most of smokers who want to quit relapse within their first week of trial (Hughes et al. 2004), an enforcing quitting design could have resulted in more robust commitment (Perkins et al. 2006). In previous studies, the
participants visited the lab every day to monitor their CO during their abstinence. In the present study, the abstinence visit occurred 7 days after the lab day and the participants were instructed to call the designate phone number to record the number of cigarettes smoked during the past 24 hours. It is possible that difference in the design of monitoring every single day of abstinence resulted in that difference in the abstinence days.

During the cue reactivity paradigm, changes in physiological parameters such as heart rate, skin temperature skin conductance and self-reported mood and craving were measured in the presence of a neutral and a smoking-related cues. In general, the interpretation of physiological changes is difficult as these parameters are influenced by factors other than smoking cues, such as stress, hunger and fatigue. In this study, there was no effect of gemfibrozil over placebo with respect to physiological changes or the self-reported responses that might demonstrate its potential use as smoking cessation aid. Significant decreases in heart rate occurred after the presentation of neutral and smoking cues for both gemfibrozil and placebo. Previous studies showed increased heart rate and decreased skin temperature in response to smoking cues (Erblich et al. 2011; Niaura et al. 1992). Other studies showed contradictions in physiological responses to different cues (Niaura et al. 1988). In this study, skin conductance response was greater and skin temperature was significantly lower in response to smoking cue versus neutral cue which agrees with other studies (Carter and Tiffany 1999; Niaura et al. 1992). Self-reported craving and urge to smoke increased over time concomitant with a significant decrease in positive mood. These findings might be explained by the wait between each cue and the frustration of not being able to smoke (Perkins and Grobe 1992).

During the forced choice paradigm, the number of puffs from Nic cigarettes was calculated for both medications. Previous studies showed that medications reducing nicotine reinforcement effects result in choosing less puffs from Nic cigarettes (Rukstalis et al. 2005). In the present study, gemfibrozil didn’t show a significant effect on nicotine reinforcement as the percentage of choice of Nic puffs was very similar between gemfibrozil and placebo.

### 4.2 Safety Measures

In this study, the number of puffs taken during the Forced-choice paradigm was accurately calculated in order not to exceed the ad libitum smoking. For instance, participants started the choice trials after 30 minutes from the last nicotine exposure trial. In this paradigm, there were 4
forced-choice trials occurring at 30-minute intervals. Every subject had to choose any combination of 4 puffs from the two cigarettes at each trial. The smoking rate during the entire session was as follows: 8 puffs every 60 minutes which is less than the rate of the typical ad libitum smoking pattern of a daily smoker of one cigarette which is equivalent to 10 puffs every 30-40 minutes (Hatsukami et al. 1988b).

4.3 Considerations for the Crossover Design

This study had an advantage of using a crossover or within-subjects design as it is known by its efficiency. A small sample size can yield a greater statistical power compared to other study designs using between-subjects or parallel-groups, as they require more subjects in order to overcome large inter-subject variability. Moreover, in a crossover study, each participant serves as his/her own control with matched smoking history and sociodemographic variables that could act as confounders on the outcome measures. Crossover designs are best suited to be used in short-term outcome studies of chronic relapsing disorders such as TUD (Sibbald and Roberts 1998). However, due to the high smoking relapse rate even with the use of medications (Fiore et al. 2008), in this study, participants were told that they do not have to resume smoking or continue the study if they achieve abstinence in the first study phase.

When this study was conducted in 2014-15, no other clinical studies were published on the use of PPAR-α agonists as aids in smoking cessation. However, Perkins and colleagues published a lab-based study on the effect of fenofibrate, another fibrate medication, on smoking cessation in treatment-seeking smokers. They used cue reactivity paradigm, brief quit attempt and comparing smoking topography for both fenofibrate and placebo. Consistent with the current findings, they reported no significant effect of fenofibrate versus placebo with respect to laboratory measures of nicotine dependence, nicotine craving, days of abstinence during a brief quit attempt or smoking topography (Perkins et al. 2016).

4.4 Study Limitations

Regarding the limitations of this study, the study was limited by the dose of gemfibrozil prescribed clinically for lipid control (600 mg twice daily) which might explain the difference between clinical and preclinical studies. Also, in the current study, the mean (±SD) cigarettes/day was 18.8 (±7) for 21.2 (±11.6) years at baseline. Different smoking pattern would
have shown a different response to gemfibrozil. Finally, gemfibrozil was given for two weeks of treatment. This relatively short period could have masked potential effects of the medication.

4.5 PPAR-α Receptor Response in Preclinical and Human Studies

Translational research aims to export preclinical findings towards human applications. Unfortunately, preclinical data do not always predict the human response to medications. Studies comparing the effect of PPAR-α ligands, including fibrates, in rodents versus humans showed a species difference in the response of the nuclear receptor. These studies were done on liver hepatocytes and suggested some genetic structural differences between species (Foreman et al. 2009; Nakamura et al. 2009; Tateno et al. 2015). This species variation may explain a potential difference in the PPAR-α receptor response in the brain. Although the three types of the receptor are distributed throughout the body, PPAR-α expression in the brain is lower than in other organs (Cullingford et al. 1998; Moreno et al. 2004) and only a small fraction (0.4-0.7%) of PPAR-α agonist can reach the brain (Blednov et al. 2015; Weil et al. 1988). Therefore, animal studies typically use high drug doses to achieve effects. In humans, the dose fraction of PPAR-α agonists that reach the brain is not yet known, which could explain the negative results of studies using the dose of medication indicated for lipid control. Recently, Jackson and colleagues showed that the selective experimental PPAR-α agonist WY14643 had better results than fenofibrate for nicotine seeking behavior in a conditioned place preference and nicotine self-administration tests in mice (Jackson et al. 2017). It is known that fibrates as ligands have low affinity to PPAR-α receptors (Duez et al. 2005) and that the clinical available fibrates show moderate selectivity on PPAR receptors (Willson et al. 2000). Therefore, a more potent and selective PPAR-α full agonist, once clinically available, might be a potential treatment for smoking cessation.

Fibrate medications are not the only anti-lipid medications studied for their smoking cessation potentials. Statins, which are HMG CoA reductase inhibitors and used to treat hypercholesterolemia, were also looked at for their potential use as smoking cessation agents (Law and Rudnicka 2006). Preclinical studies showed that statins reduce nicotine-induced reinstatement in animals, although the mechanism for this remains unclear (Chauvet et al. 2016). However, a placebo-controlled RCT has tested a statin for smoking cessation in humans and found no effect of 40 mg of simvastatin on craving, number of cigarettes smoked per day, or
sustained abstinence. In their explanation, the study authors suggest that these negative results could be due to differences in simvastatin brain penetration between animals and humans (Ingrand et al. 2018).

Failure to show positive results after promising preclinical studies is not surprising. For instance, in the last decade, many drugs were looked at after promising results based on animal and lab studies aiming to find new candidates for smoking cessation. Nevertheless, most of them didn’t survive further RCTs. These candidates were N-methyl-D-aspartate (NMDA) receptors modulators (Elrashidi and Ebbert 2014; Evins et al. 2011; Kamboj et al. 2012; Kenny et al. 2009; Santa Ana et al. 2009; Trujillo 1995; Yoon et al. 2013), antidepressant herbal supplement (St. John’s wort) (Catania et al. 2003; Sood et al. 2010) and Dianicline (a partial agonist of the α4β2* nicotinic acetylcholine receptor subtype) (Tonstad et al. 2011). Researchers are still looking for new therapeutic options for TUD.

4.6 Conclusions and Future Direction

Although preclinical studies demonstrated that PPAR-α agonists might be an aid to smoking cessation, the results of current two clinical trials, the present study and the one by Perkins et al (Perkins et al. 2016), showed no effect of gemfibrozil or fenofibrate, respectively, on laboratory measures of nicotine dependence, cue reactivity, and smoking abstinence during a brief quit attempt.

Every year, billions of dollars are spent treating smoking and related comorbidities (Goodchild et al. 2018). Despite these efforts, abstinence rates following pharmacotherapy remain low. A deeper understanding of the complex relationship between the cholinergic system and other neurotransmitter systems will be necessary in order to discover novel treatment targets for TUD. Among the pharmacotherapies investigated in the past ten years, some candidates showed promising results such as cytisine and endocannabinoid modulators, whereas others failed to produce significant effects. However, many trials have been limited by small sample sizes and short duration of follow up. Larger trials that monitor long-term abstinence rates are necessary.

There is no magical treatment that can fit all smokers due to the wide variation in smokers’ behavior and neurochemistry. More research will be needed to determine how to tailor specific
pharmacotherapies to smoker subpopulations such as smokers with obesity, mental illnesses, and other comorbidities or comorbid SUD in order to find various options to treat nicotine addiction.

Regarding future steps and recommendations, it is highly recommended using different doses of gemfibrozil or any drug under investigation to precisely determine the appropriate dose that could be used in TUD. Also, a larger sample size is an asset in order to have enough statistical power for more robust results. Compliance to the study steps should be more meticulously observed, for instance daily visits to the clinic to record CO and urine samples in order to verify abstinence objectively. A longer period of treatment is also recommended as gemfibrozil was used for two weeks only which is a relatively short period to observe the drug effects if any. Also, it is recommended performing brain imaging studies in order to determine the percentage of drug penetration in the human brain which could advance the knowledge of PPAR different dosing and effects.
Chapter 5
Methods of Clinical Trial II

5 Methods of Clinical Trial II

5.1 Study Design

This trial is a double blind Randomized Controlled Trial with two arms. Subjects (n=60) with Alcohol use disorder and sleep problems were recruited and assigned randomly to the active treatment Melatonin or Placebo arms. All subjects were assessed at baseline for their demographics. Sleeping problems was the primary outcome of the study, and was measured by the Pittsburg sleep quality index (PSQI) scale. A follow-up measures at the end of 4 weeks of treatment were followed for all subjects and their PSQI score was measured.

5.1.1 Recruitment

Participants were recruited from Addiction Medicine Service clinic and other clinics in CAMH, Toronto, using study posters and staff referrals. In this study, phone screens were done in order to verify primary criteria for inclusion. 60 treatment-seeking subjects with AUD criteria and sleep problems were recruited and randomized to either melatonin or placebo group (30 in each treatment arm) as shown in Figure 19.
Assessed for eligibility (n=174) →

- Not eligible after phone screen (n=89)

Elflegible after phone screen (n=85) →

- Not interested/no show (n=21)

- Not meeting inclusion criteria (n=4)

Enrolled (n=60) →

Analysed (n=60)

Figure 19 Study flow chart
**Inclusion criteria:**

1. Age: 19 or older
2. AUD in any stage
3. Sleep problems in the past month
4. PSQI score > 5 at baseline
5. Participants must agree not to use other sleep aid during the study
6. Women capable of becoming pregnant must agree to use contraceptives during study

**Exclusion criteria:**

1. Pregnancy, lactation or plans to become pregnant during the study timeline.
2. Use of other sleep aid in the past month (either prescribed or over the counter remedies)
3. Use of benzodiazepines and/or Z- drugs: (zaleplon, zolpidem and zopiclone) in the past month
4. Known allergy to melatonin
5. Participants taking immunosuppressive drugs.

### 5.1.2 Study Steps

Visit 1: participants signed the consent form, REDCap brief assessment including: contact information sheet, demographic data, FTND, Beck anxiety, Beck depression, concomitant medication TLFB, PSQI, AUDIT form, AUD criteria, and Adverse events (A.E) log. Pregnancy test was done to female participants. Participants received $20 compensation for their time. The participants completed the medical conditions and treatments list which needs consultation with a physician before taking melatonin according to Health Canada melatonin monograph. If any of these conditions or medications was checked, a physician verified the participant eligibility for the study at the same day or after. In this case, an extra $15 was paid to the participants as a compensation for their time.

Visit 2: participants took the medication blister and a sleep hygiene instructions form.

An E-mail after 2 weeks of medication: participants had to complete TLFB, first two weeks of the Study medication log and respond to two questions “Is there any new medical issue since the last visit?” And “Did you take any new medication since the last visit?” if any of the previous
questions were answered (yes), the participant had to complete the AE log and the concomitant medication log respectively by phone.

Visit 3: after 4 weeks, the participants came in to complete PSQI, AE log, Beck anxiety, Beck depression, TLFB, concomitant medication and Study medication log. They had to return back the blister to ensure the intake of pills (pill count). They got their compensation for participation in the study which is $50.

Visit 3b: in case participants preferred to do the questionnaires of the 3rd visit online, they received the questionnaires via email on day 28 of treatment. The email also included two questions “Is there any new medical issue since the last visit?” And “Did you take any new medication since the last visit?” if any of the previous questions was answered (yes), the participant had to respond to a phone call to complete the AE log and the concomitant medication log respectively. After that, they had to return the blister pack to do the pill count to get their compensation for participation in the study which is $50.

In case participants preferred to do the questionnaires of the 3rd visit online, 2 reminders (day 29, day 30) have been sent to the participants to complete the questionnaire if they haven’t completed it.

5.1.3 Medication

The melatonin tablets used were Nature’s bounty Melatonin (5mg), NPN: 80033974, fast dissolving tablets. All study medications (melatonin and placebo) were dispensed by the CAMH pharmacy. Also blinding procedures and randomization were handled by the same pharmacy. All subjects were instructed to take 1 pill 1 hour before bedtime.

5.1.4 Forms Used in the Study

PSQI: (Appendix F): is a validated and reliable tool to evaluate sleep quality. The PSQI is widely used by sleep specialists to evaluate sleep disturbance as a diagnostic tool for sleep problems and used in research for assessment of treatment outcomes (Grosshans et al. 2014; Medeiros et al. 2007). It is formed of 19 self-rated questions and another 5 questions answered by the bed partner or roommate if present. The scoring of the PSQI is dependent on the 19 self-rated questions, the 5 other questions are used for clinical evaluation only not for scoring. The scoring
of the questionnaire consists of 7 components each on a 0-3 scale. The sum of all 7 components forms the global PSQI score which is arrange from 0-21. The higher the score the worse is the sleep quality. For this project, component # 6 which is the number of times of using a sleeping aid during the past month was scored as zero because it is one of the exclusion criteria. Usually, a score higher than 5 indicates a sleeping problem (Buysse et al. 1989). During this study, PSQI score was collected at two time points: at baseline and after 4 weeks of treatment.

Alcohol Use Disorders Identification Test (AUDIT): (Appendix G): it is a questionnaire developed by the World Health Organization as a simple screening tool to identify the signs of hazardous, harmful drinking and alcohol dependence. A score of 8 or more is associated with harmful or hazardous drinking, a score of 13 or more in women, and 15 or more in men, is likely to indicate alcohol dependence (Babor et al.; Saunders et al. 1993)

The Fagerstrom Test for Nicotine Dependence (FTND): this highly reliable, self-administered 6-item questionnaire was used to identify severity of nicotine dependence. It has a total score ranging between 0 and 10, and higher scores indicating more dependence (Heatherton et al. 1991).

The Time-Line Follow-Back (TLFB): is a reliable and valid, interviewer-administered calendar to evaluate daily patterns and frequency of alcohol consumption. (Sobell and Sobell 1992). In this study, it was used to assess the frequency and quantity of alcohol, caffeine, nicotine, cannabis intake and other substances.

For daily alcohol use the units were considered according to the National Institute of Alcohol Abuse and Alcoholism. One alcohol drink contains about 14 grams of ethanol or “pure” alcohol: 12 ounce (Oz) of beer (about 5% alcohol), 8-9 Oz of malt liquor (about 7% alcohol), 1.5 Oz of hard liquor (about 40% alcohol), or 5 Oz of wine (about 12% alcohol) (NIAAA 2018).

The Beck Depression Inventory-II (BDI): (Appendix H): a 21-item questionnaire used to assess the severity of depressive symptomatology over the preceding 2 weeks. Total scores range from 0 to 63, where mild, moderate and severe depression is indicated by scores of 14–20, 21–30 and ≥31, respectively. (Beck et al. 1996)
Beck Anxiety Inventory (BAI): (Appendix I): A 21-item measure, assessed severity of anxious symptomatology over the preceding 2 weeks. Total scores range from 0 to 63, where mild, moderate and severe anxiety is indicated by scores of 8–15, 16–25 and ≥26, respectively. (Beck and Steer 1993)

AUD Criteria: (Appendix J): Those are the criteria adapted from DSM-5. To be diagnosed with an AUD, individuals must meet any two of the 11 criteria during the past year according to DSM-5. The severity of an AUD—mild, moderate, or severe—is based on the number of criteria met.

Medical conditions and treatments: The participants completed the medical conditions and treatments list which needs consultation with a physician before taking melatonin according to Health Canada. If any of these conditions or medications is checked, a physician verified the participant eligibility for the study.

Sleep Hygiene (Center for Clinical Interventions 2007): (Appendix K): a form with specific instructions used to help the participants to have better sleep. Sleep hygiene education is an important and frequent tool used by clinicians as a complementary strategy beside therapeutics in order to address sleeping problems in AUD subjects (Stepanski and Wyatt 2003).

Study medication log: This form tracks the participant’s status with regard to the study drug intake. It was completed in the middle of the study (email sent on week 2) and at the end of the study, at week 4, during the last visit.

5.1.5 Compliance Measures

In this study, a pill count procedure was used after the return of the medication package at the end of the study and a self-reported study medication log was used at 2 weeks and 4 weeks emails/visits to ensure the participant’s compliance of taking their assigned medication daily.

5.1.6 Sample Size and Study Population

The sample size was calculated depending on Mixed Effect Models and significance tests, where all tests were two-sided, using a confidence level of 0.05 with a power level fixed at 80%. Additionally, a 20% attrition rate was assumed for the post assessment, which was assumed to be random. With these specifications, it was found that a sample size of 30 subjects per group
would allow us to detect a significant drop in the treatment group in comparison to placebo group.

5.2 Data Analysis

The primary outcome measure was sleeping problems measured using the PSQI global score at baseline and after 4 weeks of treatment. Secondary outcome measures included the subscales of PSQI which are 1) subjective quality of sleep; 2) sleep onset latency; 3) sleep duration; 4) sleep efficiency; 5) presence of sleep disturbances; and 7) presence of daytime disturbances, as an indication of daytime alertness. Subscale 6 is the use of hypnotic sedative medication; it was considered zero as it was one of the exclusion criteria. Also, BDI and BAI scores before and after treatment were considered as secondary outcomes. Linear mixed models that use subjects as random effects were used to analyze the outcomes. An interaction between time (pre/post) and treatment groups (melatonin/placebo) was assessed since it tests the difference in change in PSQI global score as well as sub-scores, BDI and BAI scores between study groups. All analyses were done using SPSS v.24. Associations with p-values of less than 0.05 were considered statistically significant, and all tests were 2-sided. Sex and age have been controlled for in the analysis.
Chapter 6
Results of Clinical Trial II

6 Results of Clinical Trial II

6.1 Main Demographics

This study sample included 60 treatment-seeking participants diagnosed with AUD as shown in Figure 1. All subjects were randomly distributed into the melatonin group (n=30) or placebo group (n=30). There were 46 males (76.7%) and 14 females (23.3%). 75% of the sample were of Caucasian race, 3.3% were from Black/African race, 1.7% Asians, and the rest of the sample were from mixed races. The mean PSQI score (+ SD) collected at baseline was 12.33 (2.93). Mean BDI and BAI (+ SD) were 17.38 (8.44) and 15.43 (11.13) at baseline, respectively. The AUDIT score at baseline was 25.83 (8.37). Detailed demographics for each group are shown in Table 2.

Table 2 Melatonin/Placebo groups’ demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Melatonin (N=30)</th>
<th>Placebo (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (76.7%)</td>
<td>23 (76.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (23.3%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td><strong>Age range</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-25</td>
<td>0 (0.0%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>26-40</td>
<td>10 (33.3%)</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>41-60</td>
<td>16 (53.3%)</td>
<td>17 (56.7%)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>4 (13.3%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>14 (46.7%)</td>
<td>17 (56.7%)</td>
</tr>
<tr>
<td>Married</td>
<td>3 (10.0%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>12 (40.0%)</td>
<td>6 (20.0%)</td>
</tr>
<tr>
<td>Widowed</td>
<td>1 (3.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Employment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full time (&gt; 35 hr/wk)</td>
<td>4 (13.3%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>Category</td>
<td>Short-term disability</td>
<td>Self-employed</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td>3 (10.0%)</td>
<td>3 (10.0%)</td>
</tr>
</tbody>
</table>

| Highest completed education     | Part of high school   | 0 (0.0%)      | 2 (6.7%)|         |                       |                      |              |         |
|                                 | High school           | 10 (33.3%)    | 6 (20.0%)|         |                       |                      |              |         |
|                                 | College               | 12 (40.0%)    | 7 (23.3%)|         |                       |                      |              |         |
|                                 | University            | 7 (23.3%)     | 14 (46.7%)|         |                       |                      |              |         |
|                                 | Graduate degree       | 1 (3.3%)      | 1 (3.3%) |         |                       |                      |              |         |

| Ethnicity                       | Black or African American | 1 (3.3%) | 1 (3.3%) |         |                       |                      |              |         |
|                                 | Asian                  | 0 (0.0%)    | 1 (3.3%) |         |                       |                      |              |         |
|                                 | Caucasian              | 24 (80.0%)  | 21 (70.0%)|         |                       |                      |              |         |
|                                 | Mixed races            | 5 (16.7%)   | 7 (23.3%)|         |                       |                      |              |         |

| Smoking                         | Smokers                | 16 (53.3%)  | 14 (46.7%)|         |                       |                      |              |         |
|                                 | Non smokers            | 14 (46.7%)  | 16 (53.3%)|         |                       |                      |              |         |

| AUD severity                    | Mild (2-3)             | 1 (3.3%)    | 1 (3.3%) |         |                       |                      |              |         |
|                                 | Moderate (4-5)         | 2 (6.7%)    | 1 (3.3%) |         |                       |                      |              |         |
|                                 | Severe (>6)            | 27 (90.0%)  | 28 (93.3%)|         |                       |                      |              |         |

| Mean scores at baseline (+ SD)  | BDI                    | 16.30 (7.05) | 18.47 (9.64) |         |                       |                      |              |         |
|                                 | BAI                    | 14.53 (10.08)| 16.33 (12.20)|         |                       |                      |              |         |
|                                 | AUDIT                  | 24.53 (8.67)| 27.13 (7.99) |         |                       |                      |              |         |
|                                 | Global PSQI            | 12.97 (2.28)| 11.70 (3.37) |         |                       |                      |              |         |

Three participants dropped out the study and 1 participant was excluded in the middle of the study due to not following the study procedures. The results of doing pill count and self-reported study medication logs (n=56) showed 75% compliance (took all the pills). Also, the results of
self-reported daily use of alcohol (n=56) subjects showed that 78.6% (n=44) were successful in completely abstaining from alcohol during the 4 weeks of the study while 12 subjects (21.4%) consumed alcohol during the study among which some subjects (n=5) had less than 10 drinks during the 4 weeks and the rest (n=7) had more than 10 drinks, although it was highly recommended not to drink alcohol during the study according to Health Canada recommendations. Melatonin was overall well tolerated over the course of the study where mild to moderate side effects were reported and resolved over the course of the treatment. Irritability (n=1), weakness and dizziness (n=1) were reported in the melatonin group, while daytime sleepiness (n=1), rash (n=1) and vomiting (n=1) were reported in the placebo group. No severe side effects were reported at all.

6.2 PSQI Score

Linear mixed model done to analyze PSQI global score from before and after treatment for both groups revealed a significant decrease over the period of the study for both treatments. Nevertheless, there was no significant drug effect for that decrease (Table 3; Figure 20).
## Table 3 Summary of PSQI Global scores, PSQI subscales, BDI and BAI

<table>
<thead>
<tr>
<th>Variable measure mean (±SEM)</th>
<th>Melatonin group (N=30)</th>
<th>Placebo group (N=30)</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Treatment</td>
<td>Baseline</td>
<td>Treatment</td>
</tr>
<tr>
<td><strong>PSQI global score</strong></td>
<td>12.967 (0.594)</td>
<td>9.242 (0.611)</td>
<td>11.700 (0.594)</td>
<td>8.199 (0.611)</td>
</tr>
<tr>
<td><strong>Comp 1</strong></td>
<td>2.167 (0.133)</td>
<td>1.321 (0.138)</td>
<td>2.000 (0.133)</td>
<td>1.250 (0.138)</td>
</tr>
<tr>
<td><strong>Comp 2</strong></td>
<td>2.300 (0.165)</td>
<td>1.551 (0.170)</td>
<td>2.233 (0.165)</td>
<td>1.387 (0.170)</td>
</tr>
<tr>
<td><strong>Comp 3</strong></td>
<td>2.300 (0.150)</td>
<td>1.464 (0.155)</td>
<td>2.133 (0.150)</td>
<td>1.500 (0.155)</td>
</tr>
<tr>
<td><strong>Comp 4</strong></td>
<td>2.600 (0.217)</td>
<td>2.143 (0.224)</td>
<td>2.167 (0.217)</td>
<td>1.464 (0.224)</td>
</tr>
<tr>
<td><strong>Comp 5</strong></td>
<td>1.900 (0.116)</td>
<td>1.464 (0.120)</td>
<td>1.633 (0.116)</td>
<td>1.393 (0.120)</td>
</tr>
<tr>
<td><strong>Comp 7</strong></td>
<td>1.400 (0.146)</td>
<td>1.071 (0.151)</td>
<td>1.533 (0.146)</td>
<td>1.214 (0.151)</td>
</tr>
<tr>
<td><strong>BDI</strong></td>
<td>16.300 (1.653)</td>
<td>10.042 (1.690)</td>
<td>18.467 (1.653)</td>
<td>13.222 (1.690)</td>
</tr>
<tr>
<td><strong>BAI</strong></td>
<td>14.533 (2.050)</td>
<td>11.074 (2.097)</td>
<td>16.333 (2.050)</td>
<td>13.295 (2.097)</td>
</tr>
</tbody>
</table>
Figure 20 PSQI, BDI, BAI scores before and after treatment.

This figure is showing the main outcomes results: PSQI, BDI, BAI scores (Y axis) before and after treatment (X axis). All scores significantly decreased over time for the placebo group from baseline (black bars) to after treatment (2nd pattern bars). Also, the same scores decreased over time for the melatonin group from baseline (grey bars) to after treatment (4th pattern bars). No significant difference between groups was detected ($p > 0.05$). Scores are shown as mean ± SEM.
6.3 PSQI Subscales, BDI, BAI Scores

PSQI subscales showed a significant time effect that was observed for both groups but no significant drug effect of melatonin was shown, Figure 21. Anxiety and depression scores were collected before and after treatment using BAI and BDI, respectively. A significant decrease over time was shown but melatonin didn’t show any significant changes over placebo as shown in Table 3.

Figure 21 PSQI subscales.

This figure is showing the scores (Y axis) of PSQI subscales (X axis) that showed a significant decrease over time for the placebo group from baseline (black bars) to after treatment (2nd pattern bars). Also the scores showed a significant decrease over time for the melatonin group from baseline (grey bars) to after treatment (4th pattern bars). No significant difference between groups was detected ($p >0.05$). Subscale (6) which is the use of hypnotic sedative medication is not shown in this figure as it was considered zero for this study. Comp = component. Scores are shown as mean ± SEM.
6.4 Other Results and Secondary Analysis

A list of the concomitant medications and concurrent medical conditions assessed at baseline for all the subjects by a physician is shown in Table 4. 56 subjects reported their use of medications at 2 weeks and 4 weeks of the study. Only 10 (17.9%) participants were not using anti-craving and/or antidepressant medications that would affect sleep. Further analysis for their PSQI excluding all subjects using anti-craving and or antidepressant medications was conducted. PSQI score showed a significant decrease over time with no significant melatonin effect; [F (1, 16 = 0.629); p = 0.439]. Also, secondary analysis for the subjects who maintained complete abstinence from alcohol (n=44) during the 4 weeks of the study showed a significant time effect where PSQI decreased over time with no drug effect for melatonin [F (1, 86 = 0.031); p = 0.861].

Secondary analysis of the results was conducted to explore a potential correlation between scores collected during the study. The results showed a positive correlation between PSQI and BDI score means before treatment \([r = 0.375, p = 0.003, N = 60]\); and a positive correlation between PSQI and BAI score means before treatment \([r = 0.363, p = 0.004, N = 60]\). Also, a positive correlation was found after treatment between PSQI and BDI score means \([r = 0.073, p = 0.005, N = 56]\) as well as between PSQI and BAI score means \([r = 0.547, p < 0.001, N = 56]\).
Table 4 Concomitant medications and medical conditions at baseline.

<table>
<thead>
<tr>
<th>Medications/Conditions</th>
<th>Melatonin n (%)</th>
<th>Placebo n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medications:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>2 (6.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>4 (13.3%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Blood pressure medications</td>
<td>5 (16.7%)</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Sedatives or hypnotics</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Psychotropic medications</td>
<td>20 (66.7%)</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>Steroids</td>
<td>0 (0.0%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Others</td>
<td>12 (40.0%)</td>
<td>16 (53.3%)</td>
</tr>
<tr>
<td>None</td>
<td>5 (16.7%)</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td><strong>Medical condition:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>4 (13.3%)</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>6 (20.0%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Depression</td>
<td>20 (66.7%)</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>0 (0.0%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Migraine</td>
<td>1 (3.3%)</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Seizure disorders</td>
<td>1 (3.3%)</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>Hormonal disease</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>None</td>
<td>9 (30.0%)</td>
<td>7 (23.3%)</td>
</tr>
</tbody>
</table>
Chapter 7
Discussion of Clinical Trial II

7  Discussion of Clinical Trial II

Serious sleeping problems are prevalent in AUD subjects with rates as high as 74% during active drinking phase, nearly 69% during early recovery phase, and up to 50% even after a long period of abstinence (Brower et al. 2001; Chaudhary et al. 2015). It is not unusual to prescribe melatonin with or without other sleep medications for AUD in medical institutes (Zhao 2016) as it was shown that melatonin levels are reduced in subjects with AUD who are trying to be abstinent from alcohol (Conroy et al. 2012; Kuhlwein et al. 2003). However, there is currently no clear data showing evidence-based trials supporting this approach with AUD subjects. Therefore, this double-blinded randomized placebo-controlled pilot study was conducted to fill the gap of knowledge of the effect of oral melatonin versus placebo on sleep quality in AUD patients with sleeping problems. To our knowledge, this is the first RCT using melatonin alone with AUD patients with sleeping problems.

7.1  Study Outcomes

The current RCT studied the effect of oral melatonin, 5 mg, on sleep problems in 60 treatment-seeking AUD subjects versus placebo for 4 weeks of treatment. PSQI was the main tool used in the study to compare sleeping components before and after treatment for both treatments.

PSQI global score and subscales were significantly decreased at the end of the treatment period. Nevertheless, there was no significant difference between treatment groups. Further analyses on mood showed a significant time effect where anxiety and depression scores decreased significantly after 4 weeks. However, no drug effect was observed as well.

Controversy about the effect of a drug is not unusual in the science literature. Although studies concluded some positive results in favor of melatonin and its superiority over placebo use, there are other placebo-controlled trials showing no effect of melatonin as a sleep aid in comparison to placebo. For instance, the present results are consistent with a study done on Alzheimer’s disease (AD) patients where melatonin (8.5 mg immediate release and 1.5 mg sustained release) (n = 24)
didn’t improve sleep in AD patients vs placebo (n = 17) (Gehrman et al. 2009). Another study done with Dementia patients (n = 25) didn’t show a significant effect of melatonin (6 mg) on sleep for 2 weeks of treatment (Serfaty et al. 2002). Also, the results of three RCTs done on 209 AD patients and sleep disturbances revealed no evidence of melatonin to be better than placebo (McCleery et al. 2014). These results could be interpreted by the presence of a mental illness that would interfere with the circadian rhythm and the actions of melatonin as it is known that sleeping problems are not uncommon in mental disorders (Jansson-Frojmark and Lindblom 2008; Sivertsen et al. 2012).

On the other hand, PSQI index improved significantly, (p = 0.03), after four weeks of treatment in a study done with 18 patients diagnosed with Parkinson’s disease and sleeping disturbances using melatonin (3mg) or placebo. Nevertheless, PSG changes were not improved in the same study and the authors suggested that this difference between PSQI and PSG changes is due to the small sample size and the complexity and variations of the polysomnography observations (Medeiros et al. 2007).

In this study, all subjects were asked to maintain 2-3 weeks of abstinence before the start of the medication to ensure their adherence of 4 weeks of abstinence during the study. Their abstinence was verified by TLFB for the last 2 weeks at baseline and every 2 weeks during the study. Most of the sample was in early recovery from alcohol (2 to 8 weeks) at the start of the study. Our findings of sleep disturbances are consistent with other studies showing that the persistence of sleeping disturbance after acute withdrawal (the 1st 1-2 weeks) including sleep onset latency, decreased sleep duration, and low sleep efficiency, is a component of the late withdrawal symptoms or the protracted abstinence and the CNS hyper-excitability found in AUD subjects after few weeks of abstinence i.e. during early recovery (Alling et al. 1982; Begleiter 1981; Heilig et al. 2010; Kolla et al. 2014).

It was shown that AUD subjects are known to suffer mainly from initial insomnia or difficulty to initiate sleep (PSQI component #2) and middle insomnia or frequent interrupted sleep or difficulty to maintain continuous sleep (Curry et al. 2008; Zhabenko et al. 2012). Therefore, in the current study, it was hypothesized that this sample of AUD subjects would benefit from the use of melatonin as it demonstrated in a meta-analysis of 15 studies done on healthy subjects that melatonin specifically reduced the sleep onset latency by 3.9 minutes and enhanced the overall
sleep duration by 13.7 minutes (Brzezinski et al. 2005). However, in this study there was a decrease in component # 2 (sleep onset latency) and the other PSQI subscales over time but that decrease wasn’t accompanied by a drug effect. The interpretation of the discrepancy in the results of the current project and the previous mentioned studies could be that participants in the other studies didn’t have any medical or psychological comorbidity that could interfere with the action of melatonin on sleep, and that the damage of alcohol on wake-sleep cycle was difficult to restore within four weeks of treatment.

7.2 Melatonin Agonists and AUD

To our knowledge, this is the first RCT using melatonin (5 mg) taken orally 1 hour before bedtime for 4 weeks in AUD patients suffering from sleeping problems during the past month period prior to the start of the study. However, melatonin agonists were looked at in other smaller non-controlled studies. For instance, the current results were not consistent with the results of open-label studies that explored MT1 and MT2 agonists in AUD subjects. Ramelteon (8 mg) was used 30 minutes before bedtime in an open-label study with 5 AUD participants for 4 weeks. This study showed that Ramelteon decreased sleep problems according to the Insomnia Severity Index and sleep diary (Brower et al. 2011). Another open-label study showed that Agomelatine (25-50 mg) led to a significant improvement in PSQI score in 9 AUD subjects after 6 weeks of treatment (Grosshans et al. 2014). Nevertheless, none of these trials had a placebo control group which results in weak statistical evidence compared to the randomized placebo control design of the current study.

7.3 Alcohol and Sleeping Problems

In this sample of patients the average AUDIT score at baseline was 25.83 (± 8.37) with a majority of severe dependence according to DSM-5 criteria and a PSQI global score of more than 11 in both groups. These criteria were also showed in previous studies, for instance, a correlation study done by Hartwell and colleagues showed a positive correlation between AUD severity as measured by DSM-4 and sleep disturbance as measured by PSQI, perceived sleep quality factor, and daily disturbance factor in non-treatment seeking AUD subjects, (N = 295) where more severe AUD criteria were associated with higher PSQI and other sleep factors scores (Hartwell et al. 2015b). Also, research suggests that heavy alcohol consumed over a long period of time causes severe damage to sleep efficiency and quality and that it could
take more than 5 to 9 months of sobriety to enhance the sleep onset latency (Brower et al. 2011; Cohn et al. 2003) and up to 14 months to restore normal sleep time (Drummond et al. 1998). PSG also showed persistence in sleep abnormalities after several months of abstinence (Brower 2001). This could partially explain the negative results in this study taking into consideration the short period of abstinence (4 weeks).

Because alcohol drinking could have affected melatonin sleep effects, secondary analysis was done to examine if compliance could have affected the results. The results for the subjects who maintained complete abstinence from alcohol, as per self-reported zero alcohol in the TLFB in both 2 weeks and 4 weeks points of the study, \( n = 44, 78.6\% \) showed a significant time effect where PSQI decreased over time with no drug effect for melatonin \( (P > 0.05) \).

### 7.4 Melatonin Effect on Anxiety and Depression

A secondary analysis of the results revealed a positive correlation between PSQI scores and BDI, BAI scores before and after treatment. Higher sleep scores which indicate worsening of the sleep quality were positively correlated to higher scores in both anxiety and depression indices. The finding that is in accordance with other studies showing a strong association between sleep disturbance and depression or anxiety symptoms. Moreover, the persistence of sleep disturbance was found to be an indicator for high recurrence of depression even after treatment and one of the factors that could lead to suicide (Nutt et al. 2008). Sleep architecture in depressed individuals was found different compared to healthy controls with an increase in sleep onset latency, interrupted sleep and a decrease in total sleep duration (Benca et al. 1992); the criteria that are similar to the findings of our present study.

Although anxiety and depression scores decreased significantly over the 4 weeks of treatment melatonin didn’t show a significant effect compared to placebo. It was hypothesized that melatonin would enhance the mood and anxiety based on research done in this context showing that melatonin has significant anxiolytic effects besides its effect in enhancing sleep quality (Acil et al. 2004). For instance, agomelatine which is melatonin MT1 and MT2 receptors agonist, is used as anti-depressant in some countries; however, researchers explain its anxiolytic property to be mediated through its antagonism to hydroxytryptamine (5-HT) 2C receptors not through its action on MT1 and MT2 (Laudon and Frydman-Marom 2014). On the other hand, the current results are consistent with the pooled data form eight clinical trials done on patients diagnosed
with mood disorders including depression and bipolar showing no significant mood improvement using melatonin (De Crescenzo et al. 2017).

Therefore, the interpretation of the current data could be that a time factor or a placebo effect with the persistence of being abstinent from alcohol lead to the enhancement of sleep quality which in turn had a positive effect on decreasing anxiety and depression indices.

7.5 Another Supplement for Sleep in AUD

Magnesium is another supplement that was looked at for AUD-related sleeping problems as its levels are decreased in AUD subjects. An open label trial done on 11 AUD subjects using magnesium every day for 4 weeks showed an improvement in sleep onset latency and sleep efficiency as measured by PSQI. Nevertheless the authors concluded that their results need careful interpretation as the exact relationship between magnesium and alcohol is still not clear. Further, the very small sample size and the absence of a placebo control group limit the quality of the results (Hornyak et al. 2004).

7.6 Study Limitations

This work had some limitation; for instance, only one dose (5 mg) of fast dissolving melatonin tablets was used. It would be worthwhile to explore higher doses and an extended release formulation of melatonin or melatonin agonists that could have led to greater improvements in subjective sleep quality (PSQI).

Also, the compliance of participants was verified by a self-reported diary collected after 2-week and 4-week time points of the study, also a pill count was done at the end of the study. Meticulous follow-up is also recommended in addition to the self-reporting of sleep and mood scores in order to find a safe strategy for alcohol-related sleeping problems to prevent relapse and to limit recall bias. Also, weekly verification of sleep components could have been followed instead of the comparison of sleep after 4 weeks of medication.

In this study, melatonin was used alone as the use of any other sleeping pills was considered as an exclusion criterion in order to detect the unique effects of melatonin on sleep changes, whereas in practice it is observed that melatonin is sometimes, prescribed as an adjuvant drug with other sleeping pills. It is worth exploring the different effects on sleep quality when
melatonin is used alone versus in conjunction with other sleep aids. Another limitation is that participants were notified not to drink during the treatment period but they knew that they wouldn’t be excluded from the trial if they lapsed to drinking. Finally, sleep was not objectively assessed and future research could use wrist actigraphy to see if melatonin improves objectively measured sleep in AUD subjects during the course of treatment, or do PSG pre and post treatment.

7.7 Conclusions and Future Directions

It is quite common among AUD patients to relapse to drinking alcohol to self-medicate their sleeping problems (Brower et al. 2001). The results of the current study didn’t show a significant difference in using oral melatonin (5 mg) in AUD subjects versus placebo for four weeks of treatment in the reduction of sleeping problems.

Regarding future directions, it is worth conducting further studies with melatonin using higher doses as the damage to the circadian cycle caused by chronic alcohol use might need larger doses of the medication and using sustained release formulation instead of using the fast dissolving tablets. Also, it is recommended to use melatonin synthetic agonists for further research as they show more potency and greater affinity to MT receptors than the natural melatonin.

This study duration was only 4 weeks long; a longer period of the medication with a follow-up period following the treatment period is worth exploring in order to monitor the long term effects of the drug on sleep. A larger sample size is also recommended in order to detect a small effect size of the medication and to have more robust results.

Also, the timing of the melatonin administration was not objectively assessed, although all subjects were instructed to take their medication 1 hour before bedtime every night. Future research could use Smart pill bottles to objectively record the timing of the pill taken each night. Meticulous follow-up on a weekly basis is also recommended instead of waiting for 4 weeks to record the new scores for sleep and mood after treatment in parallel to the self-reporting sleep and mood scores in order to find a safe strategy for alcohol-related sleeping problems to prevent relapse.
Finally, future research could use sleep diaries and actigraphy to see if melatonin improves objectively measured sleep in AUD subjects during the course of treatment, or with polysomnography in the lab pre and post treatment.

29.5 million subjects are diagnosed with SUD among which 1 in 6 have access to evidence-based treatment (UNODC 2017). Serious consequences follow substance use. For instance, in 2016 more than 63,000 people died in the US from drug overdose (CDC 2018). Moreover, billions of dollars are spent each year on the health consequences, addiction-related crimes and the loss of productivity caused by substance use (Birnbaum et al. 2011). It is unlikely that one medication will benefit all SUD subjects due to individual variability in neurochemistry and behavior. Therefore, more research will be needed to determine how to tailor specific pharmacotherapies to each individual. Hopefully such research will provide clinicians with an improved pharmacological arsenal which can be used to curb the growing burden of addiction.
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Appendix A
Fagerström Test for Nicotine Dependence (FTND)

1. How soon after you wake up do you have your first cigarette?
   A. Within 5 minutes (3)
   B. 6-30 minutes (2)
   C. 31-60 minutes (1)
   D. After 60 minutes (0)

2. Do you find it difficult to refrain from smoking in places where it is forbidden, e.g., in church, at the library, in a cinema, etc.?
   A. Yes (1)
   B. No (0)

3. Which cigarette would you hate to give up most?
   A. The first one in the morning (1)
   B. All others (0)

4. How many cigarettes per day do you smoke?
   A. 10 or fewer (0)
   B. 11-20 (1)
   C. 21-30 (2)
   D. 31 or more (3)

5. Do you smoke more frequently during the first hours after waking than the rest of the day?
   A. Yes (1)
   B. No (0)

6. Do you smoke if you are so ill that you are in bed most of the day?
   A. Yes (1)
   B. No (0)
Please indicate how much you are experiencing each of the following moods right now. Use a number from 0 to 6 to indicate how you are feeling.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed/Blue</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joyful</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unhappy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enjoyment/fun</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frustrated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worried/Anxious</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angry/Hostile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C
Tobacco Craving Questionnaire (TCQ)

Circle the horizontal bar that represents your level of craving right now for each of the items below.

1. I would enjoy a cigarette right now.
   Strongly Disagree ____ : ____ : ____ : ____ : ____ : ____ : ____ Strongly Agree

2. If I smoked right now, I would not be able to stop.
   Strongly Disagree ____ : ____ : ____ : ____ : ____ : ____ : ____ Strongly Agree

3. If I had a lit cigarette in my hand, I probably would smoke it.
   Strongly Disagree ____ : ____ : ____ : ____ : ____ : ____ : ____ Strongly Agree

4. A cigarette would taste good right now.
   Strongly Disagree ____ : ____ : ____ : ____ : ____ : ____ : ____ Strongly Agree

5. I would be less irritable now if I could smoke.
   Strongly Disagree ____ : ____ : ____ : ____ : ____ : ____ : ____ Strongly Agree
6. It would be hard to pass up the chance to smoke.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

7. I could not stop myself from smoking if I had some cigarettes here.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

8. Smoking a cigarette would be pleasant.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

9. If I were smoking now I could think more clearly.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

10. I would not be able to control how much I smoked if I had some cigarettes here.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

11. I could not easily limit how much I smoked right now.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**

12. I could control things better right now if I could smoke.

**Strongly Disagree** : _____ : _____ : _____ : _____ : _____ : _____ **Strongly Agree**
Appendix D
Visual Analog Scales (VAS) (0-100 mm line)

Place a tick on the line to represent your answer to each of these questions. Ticks to the left are low in score, whereas ticks to the right are high in score.

1. How positive is your mood now?

   
   Not at all          Extremely

2. How much do you crave a cigarette now?

   
   Not at all          Extremely

3. How negative is your mood now?

   
   Not at all          Extremely

4. How much is your urge for a cigarette now?

   
   Not at all          Extremely
Appendix E
Minnesota Nicotine Withdrawal Scale (MNWS)

Rate yourself based on how you feel right now.

0 = not at all, 1 = slight, 2 = mild, 3 = moderate, 4 = severe

- Depressed mood, sad  0 1 2 3 4
- Insomnia, sleep problems 0 1 2 3 4
- Angry, irritable, frustrated 0 1 2 3 4
- Anxious, nervous 0 1 2 3 4
- Difficulty concentrating 0 1 2 3 4
- Restless, impatient 0 1 2 3 4
- Increased appetite, hungry 0 1 2 3 4
Appendix F
Pittsburg Sleep Quality Index (PSQI)

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions. During the past month,

1. When have you usually gone to bed? ____________________________

2. How long (in minutes) has it taken you to fall asleep each night? __________

3. What time have you usually gotten up in the morning? ________________

4. How many hours of actual sleep did you get at night? _________________

5. During the past month, how often have you had trouble sleeping because you …
(0) Not during the past month (1) Less than once a week (2) Once or twice a week (3) Three or more times a week.

   A. Cannot get to sleep within 30 minutes ______
   B. Wake up in the middle of the night or early morning ______
   C. Have to get up to use the bathroom ______
   D. Cannot breathe comfortably ______
   E. Cough or snore loudly ______
   F. Feel too cold ______
   G. Feel too hot ______
   H. Have bad dreams ______
   I. Have pain ______
   J. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s): ____________________________

6. During the past month, how would you rate your sleep quality overall? (Very good - Fairly good - Fairly bad - Very bad)

7. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep? (Not during the past month - Less than once a week - Once or twice a week - Three or more times a week)
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity? (Not during the past month - Less than once a week - Once or twice a week - Three or more times a week)

9. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done? (Not a problem – Only a very slight problem – Somewhat of a problem – A very big problem)

10. Do you have a bed partner or roommate? (No bed partner or roommate - Partner/roommate in other room - Partner in same room, but not same bed - Partner in same bed)

If yes, How often in the past month you had ... (0) Not during the past month (1) Less than once a week (2) Once or twice a week (3) Three or more times a week.

A. ...loud snoring? __________
B. ...long pauses between breathes while asleep __________
C. ...legs twitching or jerking while you sleep __________
D. ...episodes of disorientation or confusion during sleep __________
E. …other restlessness while you sleep __________

Other restlessness while you sleep, please describe: _________________________________
SCORING INSTRUCTIONS FOR THE PITTSBURGH SLEEP QUALITY INDEX:

The Pittsburgh Sleep Quality Index (PSQI) contains 19 self-rated questions and 5 questions rated by the bed partner or roommate (if one is available). Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points, "0" indicating no difficulty and "21 " indicating severe difficulties in all areas. Scoring proceeds as follows:

Component 1: Subjective sleep quality

Examine question #6, and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Very good&quot;</td>
<td>0</td>
</tr>
<tr>
<td>&quot;Fairly good&quot;</td>
<td>1</td>
</tr>
<tr>
<td>&quot;Fairly bad&quot;</td>
<td>2</td>
</tr>
<tr>
<td>&quot;Very bad&quot;</td>
<td>3</td>
</tr>
</tbody>
</table>

Component 1 score: ________

Component 2: Sleep latency

1. Examine question #2, and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
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<tbody>
<tr>
<td>&lt;15 minutes</td>
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<tr>
<td>16-30 minutes</td>
<td>1</td>
</tr>
<tr>
<td>31-60 minutes</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 60 minutes</td>
<td>3</td>
</tr>
</tbody>
</table>

Question #2 score: ________
2. Examine question #5a, and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td>Not during the past month</td>
<td>0</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>1</td>
</tr>
<tr>
<td>Once or twice a week</td>
<td>2</td>
</tr>
<tr>
<td>Three or more times a week</td>
<td>3</td>
</tr>
</tbody>
</table>

*Question #5a score: _______

3. Add #2 score and #5a score

*Sum of #2 and #5a:_______

4. Assign component 2 score as follows:

<table>
<thead>
<tr>
<th>Sum of #2 and #5a</th>
<th>Component 2 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-2</td>
<td>1</td>
</tr>
<tr>
<td>3-4</td>
<td>2</td>
</tr>
<tr>
<td>5-6</td>
<td>3</td>
</tr>
</tbody>
</table>

*Component 2 score: _______

**Component 3: Sleep duration**

Examine question #4, and assign scores as follows:

<table>
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<tr>
<th>Response</th>
<th>Component 3</th>
</tr>
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<tbody>
<tr>
<td>&gt; 7 hours</td>
<td>0</td>
</tr>
<tr>
<td>6-7 hours</td>
<td>1</td>
</tr>
<tr>
<td>5-6 hours</td>
<td>2</td>
</tr>
<tr>
<td>&lt; 5 hours</td>
<td>3</td>
</tr>
</tbody>
</table>

*Component 3 score: _______

**Component 4: Habitual sleep efficiency**

1. Write the number of hours slept (question #4) here: ______
2. Calculate the number of hours spent in bed:
   Getting up time (question #3): _____
   Bedtime (question #1): _____

   Number of hours spent in bed: _____

3. Calculate habitual sleep efficiency as follows:

   Number of hours slept / Number of hours spent in bed x 100 = Habitual sleep efficiency (%)

   ( _____ / _____ ) x 100 = _____%

4. Assign component 4 score as follows:

<table>
<thead>
<tr>
<th>Habitual sleep efficiency %</th>
<th>Component 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;85%</td>
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<td>75-84%</td>
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</tr>
<tr>
<td>65-74%</td>
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<tr>
<td>&lt; 65%</td>
<td>3</td>
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   Component 4 score: _____

Component 5: Step disturbances

1. Examine questions #5b-5j, and assign scores for each question as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not during the past month</td>
<td>0</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>1</td>
</tr>
<tr>
<td>Once or twice a week</td>
<td>2</td>
</tr>
<tr>
<td>Three or more times a week</td>
<td>3</td>
</tr>
</tbody>
</table>

   5b score: _____  5c score: _____  5d score: _____
   5e score: _____  5f score: _____  5g score: _____
   5h score: _____  5i score: _____  5j score: _____

2. Add the scores for questions #5b-5j:

   Sum of #5b-5j: _____

3. Assign component 5 score as follows:

<table>
<thead>
<tr>
<th>Sum of #5b-5j</th>
<th>Component 5 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-9</td>
<td>1</td>
</tr>
</tbody>
</table>
Component 5 score: _____

Component 6: Use of sleeping medication

Examine question #7 and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Component 6 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not during the past month</td>
<td>0</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>1</td>
</tr>
<tr>
<td>Once or twice a week</td>
<td>2</td>
</tr>
<tr>
<td>Three or more times a week</td>
<td>3</td>
</tr>
</tbody>
</table>

Component 6 score: ------

Component 7: Daytime dysfunction

1. Examine question #8, and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>0</td>
</tr>
<tr>
<td>Once or twice</td>
<td>1</td>
</tr>
<tr>
<td>Once or twice each week</td>
<td>2</td>
</tr>
<tr>
<td>Three or more times each week</td>
<td>3</td>
</tr>
</tbody>
</table>

Question #8 score: _______

2. Examine question #9, and assign scores as follows:

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No problem at all</td>
<td>0</td>
</tr>
<tr>
<td>Only a very slight problem</td>
<td>1</td>
</tr>
<tr>
<td>Somewhat of a problem</td>
<td>2</td>
</tr>
<tr>
<td>A very big problem</td>
<td>3</td>
</tr>
</tbody>
</table>

Question #9 score: _______

3. Add the scores for question #8 and #9:

Sum of #8 and #9: _______
4. Assign component 7 score as follows:

<table>
<thead>
<tr>
<th>Sum of #8 and #9</th>
<th>Component 7 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-2</td>
<td>1</td>
</tr>
<tr>
<td>3-4</td>
<td>2</td>
</tr>
<tr>
<td>5-6</td>
<td>3</td>
</tr>
</tbody>
</table>

Component 7 score: _____

Global PSQI Score

*Add the seven component scores together*

Global PSQI Score: _____
Appendix G
Alcohol Use Disorder Identification Test (AUDIT)

1. How often do you have a drink containing alcohol?
   - Never 0
   - Monthly or less 1
   - 2 to 4 times a month 2
   - 2 to 3 times a week 3
   - 4 or more times a week 4

2. How many drinks containing alcohol do you have on a typical day when you are drinking?
   - 1 or 2 0
   - 3 or 4 1
   - 5 or 6 2
   - 7 to 9 3
   - 10 or more 4

3. How often do you have 5 or more drinks on one occasion?
   - Never 0
   - Less than monthly 1
   - Monthly 2
   - Weekly 3
   - Daily or almost daily 4

4. How often during the last year have you found that you were not able to stop drinking once you had started?
   - Never 0
   - Less than monthly 1
   - Monthly 2
   - Weekly 3
   - Daily or almost daily 4
5. How often during the last year have you failed to do what was normally expected of you because of drinking?

- Never 0
- Less than monthly 1
- Monthly 2
- Weekly 3
- Daily or almost daily 4

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?

- Never 0
- Less than monthly 1
- Monthly 2
- Weekly 3
- Daily or almost daily 4

7. How often during the last year have you had a feeling of guilt or remorse after drinking?

- Never 0
- Less than monthly 1
- Monthly 2
- Weekly 3
- Daily or almost daily 4

8. How often during the last year have you been unable to remember what happened the night before because of your drinking?

- Never 0
- Less than monthly 1
- Monthly 2
- Weekly 3
- Daily or almost daily 4

9. Have you or someone else been injured because of your drinking?

- No 0
10. Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?

- No 0
- Yes, but not in the last year 2
- Yes, during the last year 4

Total: ____________
Appendix H
Beck Depression Inventory (BDI)

Please read each question carefully and pick out the one statement in each category that best describes how you are feeling for the past 2 weeks. If several statements in the group seem to apply equally well, select the one with the highest number.

<table>
<thead>
<tr>
<th>Question</th>
<th>Score</th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>I do not feel sad</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>I feel sad</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>I am sad all the time and I can't snap out of it</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>I am so sad or unhappy that I can't stand it</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>I am not particularly discouraged about the future</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>I feel discouraged about the future</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>I feel I have nothing to look forward to</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>I feel that the future is hopeless and that things cannot improve</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>I do not feel like a failure</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>I feel I have failed more than the average person</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>As I look back on my life, all I can see if a lot of failure</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>I feel I am a complete failure as a person</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>I get as much satisfaction out of things as I used to</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>I don't enjoy things the way I used to</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>I don't get real satisfaction out of anything anymore</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>I am dissatisfied or bored with everything</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>I don't feel particularly guilty</td>
</tr>
<tr>
<td></td>
<td>I feel guilty a good part of the time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I feel quite guilty most of the time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I feel guilty all of the time</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I don't feel I am being punished</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I feel I may be punished</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I expect to be punished</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I feel I am being punished</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I don't feel disappointed in myself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I am disappointed with myself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I am disgusted with myself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I hate myself</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I don't feel I am any worse than anybody else</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I am critical of myself for weaknesses or mistakes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I blame myself all the time for my faults</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I blame myself for everything bad that happens</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I don't have any thoughts of killing myself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I have thoughts of killing myself, but I would not carry them out</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I would like to kill myself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I would kill myself if I had the chance</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>I don't cry more than usual</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I cry more now than I used to</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I cry all the time now</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I used to be able to cry, but now I can't cry even though I want to</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>I am no more irritated now than I ever am</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Score</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>I get annoyed or irritated more easily than I used to</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I feel irritated all the time now</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I do not get irritated at all by things that used to irritate me</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I have not lost interest in other people</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>I am less interested in other people than I used to</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I have lost most of my interest in other people</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I have lost all my interest in other people</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>I make decisions about as well as I ever could</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>I put off making decisions more than I used to</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I have greater difficulty in making decisions than before</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I can't make any decision at all anymore</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>I don't feel that I look any worse than I used to</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>I am worried that I am looking old or unattractive</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I feel that there are permanent changes in my appearance that make me look unattractive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I believe that I look ugly</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>I can work about as well as before</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>It takes an extra effort to get started at doing something</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I have to push myself very hard to do anything</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I can't do any work at all</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>I can sleep as well as usual</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>I don't sleep as well as I used to</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I wake up 1-2 hours earlier than usual and find it hard to get back to sleep</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I wake up several hours earlier than I used to and cannot get back to sleep</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>I don't get more tired than usual</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>----</td>
<td>---</td>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
<td>I get tired more easily than I used to</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I get tired from doing almost anything</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I am too tired to do anything</td>
<td></td>
</tr>
</tbody>
</table>

| 18 | 0 | My appetite is no worse than usual |
|    | 1 | My appetite is not as good as it used to be |
|    | 2 | My appetite is much worse now |
|    | 3 | I have no appetite at all anymore |

| 19 | 0 | I haven't lost much weight, if any |
|    | 1 | I have lost more than five pounds |
|    | 2 | I have lost more than ten pounds |
|    | 3 | I have lost more than fifteen pounds |
|    |     | (Score 0 if you have been trying to lose weight) |

| 20 | 0 | I am no more worried about my health than usual |
|    | 1 | I am worried about physical problems such as aches and pains, or upset stomach or constipation |
|    | 2 | I am very worried about physical problems, and its hard to think of much else |
|    | 3 | I am so worried about my physical problems that I cannot think about anything else |

| 21 | 0 | I have not noticed any recent changes in my interest in sex |
|    | 1 | I am less interested in sex than I used to be |
|    | 2 | I am much less interested in sex now |
|    | 3 | I have lost interest in sex completely |

**Total BDI Score:_____________________**
Appendix I
Beck Anxiety Inventory (BAI)

<table>
<thead>
<tr>
<th>Scoring: Not At All</th>
<th>Mildly but it didn’t bother me much</th>
<th>Moderately - it wasn’t pleasant at times</th>
<th>Severely – it bothered me a lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today.

1) Numbness or tingling  
2) Feeling hot  
3) Wobbliness in legs  
4) Unable to relax  
5) Fear of worst happening  
6) Dizzy or lightheaded  
7) Heart pounding/racing  
8) Unsteady  
9) Terrified or afraid  
10) Nervous  
11) Feeling of choking  
12) Hands trembling  
13) Shaky / unsteady  
14) Fear of losing control  
15) Difficulty in breathing  
16) Fear of dying  
17) Scared  
18) Indigestion  
19) Faint / lightheaded  
20) Face flushed  
21) Hot/cold sweats

The total score is calculated by finding the sum of the 21 items.

Score of 0 – 21 = low anxiety  
Score of 22 – 35 = moderate anxiety  
Score of 36 and above = potentially concerning levels of anxiety
Appendix J
Alcohol Use Disorder (AUD) Criteria

To be diagnosed with an AUD, individuals must meet any two of the 11 criteria during the past year according to DSM-5. The severity of an AUD—mild, moderate, or severe—is based on the number of criteria met. Mild = (2-3 criteria), Moderate = (4-5 criteria), Severe (6 or more)

In the past year, have you:

- Had times when you ended up drinking more, or longer than you intended?
- More than once wanted to cut down or stop drinking, or tried to, but couldn’t?
- Spent a lot of time drinking? Or being sick or getting over the aftereffects?
- Experienced craving — a strong need, or urge, to drink?
- Found that drinking — or being sick from drinking — often interfered with taking care of your home or family? Or caused job troubles? Or school problems?
- Continued to drink even though it was causing trouble with your family or friends?
- Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?
- More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?
- Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?
- Had to drink much more than you once did to get the effect you want? Or found that your usual number of drinks had much less effect than before?
- Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, irritability, anxiety, depression, restlessness, nausea, or sweating? Or sensed things that were not there?
Appendix K
Sleep Hygiene

1) **Get regular.** One of the best ways to train your body to sleep well is to go to bed and get up at more or less the same time every day, even on weekends and days off! This regular rhythm will make you feel better and will give your body something to work from.

2) **Sleep when sleepy.** Only try to sleep when you actually feel tired or sleepy, rather than spending too much time awake in bed.

3) **Get up & try again.** If you haven’t been able to get to sleep after about 20 minutes or more, get up and do something calming or boring until you feel sleepy, then return to bed and try again. Sit quietly on the couch with the lights off (bright light will tell your brain that it is time to wake up), or read something boring like the phone book. Avoid doing anything that is too stimulating or interesting, as this will wake you up even more.

4) **Avoid caffeine & nicotine.** It is best to avoid consuming any caffeine (in coffee, tea, cola drinks, chocolate, and some medications) or nicotine (cigarettes) for at least 4-6 hours before going to bed. These substances act as stimulants and interfere with the ability to fall asleep.

5) **Avoid alcohol.** It is also best to avoid alcohol for at least 4-6 hours before going to bed. Many people believe that alcohol is relaxing and helps them to get to sleep at first, but it actually interrupts the quality of sleep.

6) **Bed is for sleeping.** Try not to use your bed for anything other than sleeping and sex, so that your body comes to associate bed with sleep. If you use bed as a place to watch TV, eat, read, work on your laptop, pay bills, and other things, your body will not learn this connection.

7) **No naps.** It is best to avoid taking naps during the day, to make sure that you are tired at bedtime. If you can’t make it through the day without a nap, make sure it is for less than an hour and before 3pm.
8) **Sleep rituals.** You can develop your own rituals of things to remind your body that it is time to sleep - some people find it useful to do relaxing stretches or breathing exercises for 15 minutes before bed each night, or sit calmly with a cup of caffeine-free tea.

9) **Bath time.** Having a hot bath 1-2 hours before bedtime can be useful, as it will raise your body temperature, causing you to feel sleepy as your body temperature drops again. Research shows that sleepiness is associated with a drop in body temperature.

10) **No clock-watching.** Many people who struggle with sleep tend to watch the clock too much. Frequently checking the clock during the night can wake you up (especially if you turn on the light to read the time) and reinforces negative thoughts such as “Oh no, look how late it is, I’ll never get to sleep” or “it’s so early, I have only slept for 5 hours, this is terrible.”

11) **Use a sleep diary.** This worksheet can be useful ways of making sure you have the right facts about your sleep, rather than making assumptions. Because a diary involves watching the clock (see point 10) it is a good idea to only use it for two weeks to get an idea of what is going and then perhaps two months down the track to see how you are progressing.

12) **Exercise.** Regular exercise is a good idea to help with good sleep, but try not to do strenuous exercise in the 4 hours before bedtime. Morning walks are a great way to start the day feeling refreshed!

13) **Eat right.** A healthy, balanced diet will help you to sleep well, but timing is important. Some people find that a very empty stomach at bedtime is distracting, so it can be useful to have a light snack, but a heavy meal soon before bed can also interrupt sleep. Some people recommend a warm glass of milk, which contains tryptophan, which acts as a natural sleep inducer.

14) **The right space.** It is very important that your bed and bedroom are quiet and comfortable for sleeping. A cooler room with enough blankets to stay warm is best, and make sure you have curtains or an eye mask to block out early morning light and earplugs if there is noise outside your room.

15) **Keep daytime routine the same.** Even if you have a bad night sleep and are tired it is important that you try to keep your daytime activities the same as you had planned. That is, don’t avoid activities because you feel tired. This can reinforce the insomnia.