Chamomile and Oregano extracts synergistically exhibits anti hyperglycemic, anti hyperlipidaemic and renal protective effects in Alloxan induced Diabetic rats

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Chamomile and Oregano extracts synergistically exhibits anti hyperglycemic, anti hyperlipidaemic and renal protective effects in Alloxan induced Diabetic rats

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Running Title: Chamomile and Oregano synergistic action in diabetic rat models

Novelty & Impact Statements: The data of this study show for the first time that treatment with a combined mixture of aqueous extracts of Chamomile and Oregano induce synergistic ameliorative actions on certain diabetic symptoms in the Alloxan-rat model. The current observations may provide support to the development and formulation of new natural dietary supplements and pharmaceutical agents for the control and management of diabetes mellitus complications.

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Abstract

The bio-activities of separate Matricaria chamomilla (chamomile) and Origanum vulgare (oregano) are well studied; however, the combined effects of both natural products in animal diabetic models are not well characterized. In this study, Alloxan induced male albino rats were treated with single dose aqueous suspension of chamomile or oregano at dose level of either 150 mg/kg or 300 mg/kg body weight or as equal parts as combination by stomach tube for 6 weeks. Post treatment, blood samples were assessed for diabetic, renal and lipid profiles. Insulin, amylase activity and diabetic renal apoptosis were further evaluated. Treatment with higher dose of the extracts (300 mg/kg) as individual or as mixture of low doses (150 mg/kg of both the extracts) had significant weight gain, hypoglycemic effect ($p \leq 0.05$) with decreased amylase activity and increased serum insulin levels. Restoration of renal profile, lipid profile with increase in HDL-C ($p \leq 0.05$) along with reversal of pro-apoptotic Bax and anti –apoptotic Bcl-2 were well observed with 300 mg/kg mixture showing synergistic activity of the extracts compared to individual low dose of 150 mg/kg. Collectively our results indicate that, combination of chamomile and oregano extracts will form a new class of drugs to treat diabetic complications.

Key words: Diabetes Mellitus, Matricaria chamomilla, Origanum vulgare, HbA$_1$C, Insulin, Lipid Profile, Synergism.
1. Introduction

Diabetes mellitus is characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein which in turn leads to chronic complications including diabetic nephropathy (Davis and Granner 1996). As diabetes mellitus cannot be cured, managing the disease becomes the only choice of treatment. The plants with anti-diabetic activities provide important sources for the development of new drugs in the treatment. Many herbs and spices from traditional medicine have been used in most developing countries as a valuable alternative for treating diabetes mellitus (Anderson 2004). The major advantages of herbal medicine seem to be their efficacy, low incidence of side effects, and the low cost (Manal 2012).

Chamomile (Matricaria chamomilla L.), is one of the medicinal herbs known in ancient Egypt, Greek and Rome (Najla et al.2012). It contains a large group of therapeutically interesting and active compound classes like sesquiterpenes, flavonoids, coumarins, and polyacetylenes (Ompal et al.2011). Eleven bioactive phenolic compounds, (Ompal et al.2011) such as herniarin and umbelliferone (coumarin), chlorogenic acid and caffeic acid (phenylpropanoids), apigenin, apigenin-7-O-glucoside, luteolin and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavonols), and naringenin (flavanone) are found in chamomile extract. Studies recorded that chamomile ameliorates the hyperglycemia and diabetic complications via suppressing blood glucose levels and increase the serum insulin and C-peptide levels in diabetic rats (Kato et al.2008). The pharmacological activity of chamomile reveals its protective effect on pancreatic beta cells in diminishing hyperglycemia-related oxidative stress (Zemestani et al 2016).

Oregano (Origanum vulgare), is a native plant widely distributed throughout the Mediterranean, Euro-Siberian and Irano-Siberian regions. The main known active compounds
are camphene, carvacol, gamma-terpiene, thymol, Terpinin-4-ol, myricine and Linalyl-acetate. Oregano leaves are traditionally used as potent hypoglycemic agents in diabetes control and treatment; (Jouad et al.2003) however it had no effect on the basal plasma insulin concentrations in both normal and diabetic rats and the hypoglycemic effect is facilitated by inhibiting the production of glucose in the liver.

Though rich background information on the anti-diabetic and medicinal properties of these herbs are available, the combined aqueous extracts of these plans in managing DM and its complications in alloxan induced diabetic animal models has not been studied. It is because of the high degree of overlap between different signaling pathways and the redundancy within a cell, agents with multiple mechanisms of action and particularly the use of combination treatments have become increasingly important. In this study we therefore hypothesize on two herbal extracts and their combination with different mechanisms in alloxan induced diabetes mellitus and hyperlipidaemic model.
2. Materials and Methods

2.1 Chemicals:

Kits for assessing blood (serum) glucose, glycated hemoglobin (HbA\textsubscript{1C}), total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), urea, creatinine, uric acid, total protein and amylase were obtained from Human Co. Annexin V kit was purchased from e-Bioscience USA. Bcl-2, Bax and β-actin antibodies were purchased from Cell Signaling, USA. All other chemicals were purchased from Sigma.

2.1.1 Preparation of plant extracts:

Chamomile and oregano were obtained from local markets. Dry chamomile (flowers, leaves, stems or all aerial parts) and oregano leaves extract has been prepared as describe previously (Srivastava and Gupta 2009; Williamson et al 1996) to have a concentration of 200 mg/ml.

2.2 Experimental Animals:

All animal research was conducted in accordance and approval by the Institutional Animal Care and Use Committee. Eighty male albino (Wistar) rats weighing 150-200 gram were purchased from the animal house of National Research Center, Cairo-Egypt and housed for one week under controlled condition and provided with standard diet and water ad libitum to allow them acclimatize before conducting the study. Body weight and physical signs were monitored during the experiments.
2.2.1 Experimental Design:

Rats were divided into eight groups of ten rats per each group as per the below schematic figure.

2.2.2 Alloxan induced diabetic rats:

Group 1 and 2 were uninduced animals. Group 3 to 8 animals were induced with diabetes mellitus by intra-peritoneal injection of 120mg/Kg body weight of alloxan dissolved in normal saline. The development of diabetes was checked by measuring serum glucose level after four days of alloxan injection. DM was confirmed by elevated fasting serum glucose over 300 mg/dl (Misra and Fridovich 1972).
2.2.3 Treatment procedure for DM induced rats:

Group 1 was sham control animals which were not disturbed. Group 2, 3 rats were administered with vehicle without drug by stomach tube for 6 weeks. Group 4, 6 rats were given aqueous suspension of chamomile at dose level of 150 mg/kg and 300 mg/kg body weight respectively by stomach tube for 6 weeks. Group 5, 7 rats received aqueous suspension of oregano at dose level of 150 mg/kg and 300 mg/kg body weight respectively by stomach tube for 6 weeks.

Group 8, rats were treated with an aqueous suspension of chamomile and oregano extracts as combination of 50% each and together totalling 300 mg/Kg body weight. The group received this at dose by same route and time period as that of individual treatments. No evidence of side effects was observed after drug administration in all treated groups.

After 3 weeks of treatment, five rats from each group were selected; blood samples collected by vein puncture using standard protocol (Parasuraman et al.2010) in plain centrifuge tube and serum was separated for measurement of all biochemical parameters. The treatment was continued with remaining animals for 3 more weeks. At the end of 6th week, blood samples from remaining 5 rats in each group were collected as before and serum was separated for analysis. After blood collection at each period, the animals were sacrificed. The kidneys were rapidly dissected and collected in RPMI1640 medium placed on ice. Kidney cells were isolated as described in section 2.4. Prior to the blood collection procedure at the end of 3rd and 6th weeks, the body weight of animals in each group was noted.
2.3 Biochemical analysis:

Blood samples were assessed for several bio-indicators of diabetes among which are: insulin, glucose, amylase, lipid, renal etc. and renal cellular apoptosis. Blood (Serum) glucose, glycated hemoglobin (HbA$_1$C), total cholesterol (TC), triglycerides (TG), high density lipoprotein- cholesterol (HDL-C), urea, creatinine, uric acid, total protein and amylase were estimated by kit protocols of Human Co. according to manufacturer’s instructions. The calculation of serum very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) were carried out according to the standard methods from the equations: $\text{VLDL-c (mg/dl)} = \frac{\text{TG}}{5}$ and $\text{LDL-c (mg/dl)} = \text{TC} - \text{HDL} - \text{VLDL}$ (Lee and Nieman 1996). Total protein was measured according to Bradford method (Bradford 1976). The serum insulin level was assayed with an ELISA kit (Linco Research Inc., USA) according to the manufacturer’s instructions.

2.4 Preparation of kidney cells for analysis:

The kidneys were washed and cut up with scissors into fine pieces, and minced with a sterile blade to yield 2 × 2-mm pieces in sterile RPMI 1640 medium on ice. After thorough washes with serum-free HBSS, to obtain single cell suspensions, cells were mixed with ultra-pure collagenase III in medium 199 (200–250 units of collagenase per ml) and allowed to incubate at 37°C for 3–4 h. Further, cells were filtered through a 45-µl nylon mesh and washed with RPMI/20% FBS media; then washed twice with HBSS. Cells were counted using hemocytometer and transferred to a 5-ml tube, washed twice with HBSS with 2% heat-inactivated calf serum (HICS; 5 min at 1,000 rpm) and re suspended in 100 µl (per 10$^6$ cells) of HBSS with 2% HICS.
2.6 Annexin V Assay:

The assay was performed using Annexin V detection kit from e-Biosciences, USA as per the manufacturer's instructions as follows. 0.5 X 10^6 of isolated kidney cells from control and treated groups were washed twice with wash buffer (WB) and incubated with 0.25 µg/ml Annexin V reagent in 1 X binding buffer for 15 minutes. After couple of washes with WB, cells were re-suspended back in binding buffer containing 0.5 µg/ml propedium iodide and 10,000 events acquired on a guava easyCyte™ flow cytometer. Data were analyzed using InCyte software-Millipore. Apoptotic cells were segregated with a quadri plot graph and total percentage of apoptotic cells represented using Graphpad Prism software.

2.7 Western immunoblotting for apoptotic downstream signaling:

The isolated kidney cells were lysed in RIPA buffer containing 50 mMTris, 150 mM NaCl, 0.5% deoxycholate, 0.1% SDS, 2 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM phenyl methyl sulfonyl fluoride, 10 µg/ml aprotinin and 5 µg/ml pepstatin A. After the insoluble materials were removed by centrifugation at 14,000 × g for 10 min at 4°C, protein were quantified using Coomassie plus Protein Assay Reagent kit (Pierce; Rockford, IL, USA). Total cellular proteins (20–40 µg) in the cell lysate were separated by 8–15% SDS polyacrylamide gel electrophoresis. The proteins on the gel were then transferred onto a nitrocellulose membrane, which was probed with the respective primary antibodies [(BCL-2; Santa Cruz Biotechnology); BAX; Santa Cruz Biotechnology; anti-β-actin (Sigma-Aldrich, St Louis, MO, USA)] followed by addition of the corresponding horseradish peroxidase (HRP) conjugated secondary antibodies. The membrane was then stripped by stripping buffer containing 100 mM β-mercaptoethanol, 2% SDS and 62.5 mMTris- HCl at 50°C for 30 min for quantification and normalization with β-actin
Protein bands were visualized by using ECL reagents (Amersham Bioscience; Piscataway, NJ, USA) and exposed to Kodak X-Omat Blue XB-1 films (Rochester, NY, USA). Bands were quantified using Image J (Ver. 1.46, NIH) and normalized to actin.

2.8 Statistical analysis:

Statistical analyses were performed using Graphpad Prism 6.0 (La Jolla, USA). The results are expressed as mean ± SE. The data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s procedure for multiple comparisons. Any value of $p \leq 0.05$ was considered significant. All experiments were conducted in duplicates to ensure consistency (Tamhane and Dunlop 2000).

3. Results:

3.1 Anti hyperglycemic effects of chamomile, oregano and their combination:

Results illustrated in Figure -1 a, shows the changes in body weight, blood glucose levels, HbA$_1$C, insulin and amylase activity in all experimental groups. The body weight of the diabetic control rats were significantly decreased as compared to the vehicle control group. Diabetic rats treated with 300 mg/kg body weight of either oregano or chamomile extracts relatively maintained the body gain compared with the diabetic group. While 150 mg/kg of either chamomile or oregano treated groups did not show significant weight gain, the equal combination of extracts treated group had significant results post treatment. In addition, the combination treated group was observed to gain more weight than 300 mg/kg treated individual extracts. These results were consistent at both 3 weeks and 6 weeks of treatment.
The intra-peritoneal injection of alloxan effectively induced diabetes as reflected by significant elevation of blood glucose, HbA1C, amylase activity and diminution of insulin level when compared to vehicle control group (Fig. 1). Treatment of diabetic rats with 150 mg/kg or 300 mg/kg of chamomile extract showed significant increase in insulin, while oregano treatment was ineffective at both doses (Fig. 1 b). The treatment of chamomile or oregano at 300 mg/kg and 150 mg/kg reduced blood glucose (Fig. 1 c), HbA1C (Fig. 1 d) and amylase activity (Fig. 1 e) when compared to diabetic control group. Our results revealed that the mixture of both extracts significantly decreased the blood glucose when compared with the effect of 150 mg/kg each extract alone on diabetic animals (Fig 1 c). The effect of glucose reduction by the mixture was observed to be synergistic when compared with individual extracts (Table–1). On the same hand, the mixture significantly decreased HbA1C and amylase activity (Fig. 1 d and e) and increased insulin level (Fig. 1 b) when compared with the effect of 150 mg/kg chamomile or oregano treated diabetic animals.

3.2 Effects of extracts and their combination in renal function profile:

Figure 2 shows significant increase ($p \leq 0.05$) in the levels of blood urea, creatinine and uric acid, with a decrease in the total protein as markers of renal dysfunction in the diabetic group compared to control group. Treatment of diabetic rats with 300 mg/kg of each extract alone decreased blood urea, creatinine and uric acid levels and increased total protein levels (Figure 2 a-d). The effect of oregano treatment was observed to be little higher than that of chamomile on these parameters (Figure 2 a-d). While 150 mg/kg chamomile or oregano treated groups did not respond to the reversal of urea, uric acid, creatinine or total protein as compared to diabetic group, the mixture treatment almost renormalized these levels (Figure 2). The
observations reveal that mixture of both extracts was more effective in the improvement of renal parameters compared to the effect of both doses of each extract alone on diabetic animals.

3.3 **Anti hyperlipidaemic activity of chamomile, oregano and their mixture:**

The results of lipid profile are shown in Figure 3. Serum TC, TG LDL-c and VLDL-c levels were significantly (P ≤ 0.05) increased in diabetic group compared to the control, while HDL-c levels were decreased (Fig 3 a-e). Treatment of diabetic rats with 300 mg/kg oregano or chamomile extracts lowered the levels of total cholesterol (Fig. 3 a), triglycerides (Fig. 3 b), LDL-c (Fig. 3 d), VLDL-c (Fig. 3 e) and elevated HDL-c (Fig. 3 c) compared to the diabetic group. As observed in the kidney profile, the results of lipid profile also showed 150 mg/kg of either extract treatment were trace effective, while the combination of extract was more effective as anti-hyperlipidaemic compared with 300 mg/kg treatment of chamomile or oregano extracts (Fig 3 a-e).

3.4 **Renal protective effects of extracts and their combination in diabetic model:**

To check if the extracts or their combination had a renal protective effect manifested by decreasing apoptosis in kidney cells, we performed Annexin V assay using flow cytometry. As observed in Fig. 4 a, kidney cells from diabetic control rats showed significant increase in apoptotic cell population when compared with vehicle control group. 300 mg/kg chamomile or oregano treatment decreased early and late phase apoptotic cells compared to diabetic control (Fig 4 a). Apoptosis reducing effect was more prominent in mixture treated group when compared with the diabetic control and single extract treated groups (Fig. 4 a, b).
To substantiate Annexin V assay results, we next evaluated the pro and anti apoptotic protein signaling of kidney cells by western blotting. The anti apoptotic Bcl-2 protein levels were increased in 300mg/kg single extracts or mixture treated group compared with diabetic rats (Figure 4 c). The Bax protein levels were decreased in all treatment groups compared with diabetic control rats (Figure 4 c). As a consequence, the Bcl-2/BAX ratio significantly increased in treatment groups with special reference to the mixture treated group (Fig. 4 d).

Sham animals did not have significant difference in any of the parameters tested when compared to vehicle control group (data not shown).

4. Discussion

Plants and plant products both as extracts and derived compounds are gaining importance as effective and versatile agents against various diseases (Qattan et al.2008). The demand for herbal remedies is growing as various types of hypoglycemic agents produce number of side effects (Mutalik et al.2003). Herbal formulations have been effectively prescribed for the treatment of many diseases including diabetes mellitus. Before isolation of the active compounds from the herbs, the crude extract must be tested for its effectiveness in animal models (Karashima and Schally 1988). The hypoglycemic activity of some plants extracts have been evaluated and confirmed in animals and in human beings (Sharma et al.2010). Also, complex and interactive combination therapies have become more popular and are proved to be more efficacious. The present study was therefore planned to elucidate the anti-hyperglycemic and anti-hyperlipidaemic and renal protective properties of chamomile, oregano aqueous extracts and their combination in alloxan induced-diabetic rats besides estimation of other related biochemical profiles.
Weight loss has been known to be one of the chief DM symptoms (Sellamuthu et al.2009). Figure 1 a, shows body weight of the untreated diabetic rats significantly decreased as compared to control and diabetic treated group. However, the diabetic rats treated with 300 mg/kg oregano/chamomile/ mixture relatively maintained the body gain with special reference to the mixture treated group. Similar observation was obtained by other co workers, who reported that the administration of plant extracts showed significant gain in body weight which may be due to its protective effect in controlling muscle wasting, where loss of body weight associated with diabetes could be a consequence of increased muscle wasting due to the loss of tissue proteins (Kholoud and Manal 2012). It is postulated that the deficiency of insulin in the diabetic rats lead to decrease in amino acids uptake by tissues with a consequent reduction in the level of protein synthesis(Kato et al.2008). Therefore the increase in insulin levels observed (Fig. 1 b) can also be allied to the weight gain observed in these groups (Fig. 1 a).

Diabetic rats treated with 300 mg/kg chamomile or oregano extracts showed significant reduction of blood glucose, glycated hemoglobin (HbA1C) and amylase activity as compared to diabetic group (Figure 1 b - d). Studies show that chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus (Rafraf et al.2015). Chamomile extracts are proven to lower the blood glucose (Manal 2012), HbA1C levels and amylase activity (Darvishpadok et al.2012). Oregano extracts has been proven for its anti- hyperglycemic activity (Vujicic et al.2015). Ethanolic extract of the plant is also shown to inhibit α-amylase activity \textit{in vitro} (Patrick et al.2007). Therefore the observed effects of chamomile or oregano stand well with reported literature. However the efficacy was achieved with 300 mg/kg of chamomile or
oregano treatment, where 150 mg/kg treatments were less effective. Interestingly, 300 mg/kg dose, which contained equal mixture of chamomile and oregano extracts showed significant decrease of blood glucose, HbA1C and amylase activity when compared with the effect of 150 mg/kg either extracts treated or the diabetic control group (Fig 1). The hypoglycemic activity exhibited by the mixture was observed to be synergistic (Table-1). We have previously established methods to calculate synergistic index either by calculating the ratio of expected percentage to observed percentage or by arriving at ratio of average percentage to observed percentage (Prasanna et al.2011). In the current study, both methods showed synergistic effect of the mixture treatment in reducing the blood glucose levels of diabetic rats as observed in Table-1a and 1b.

Treatment of chamomile extract at 150mg/kg or 300 mg/kg body weight showed significant insulin increase, with higher dose being more effective than lower one (Fig. 1 b). On the other hand, oregano treatments at either dose did not have an effect on insulin secretion (Fig. 1 b). It has been observed that, oregano extract had no effect on the basal plasma insulin concentrations in both normal and diabetic rats (Lemhadri et al.2004), while chamomile is proven to significantly increase the serum insulin and C-peptide levels in diabetic rats (Kholoud and Manal 2012). The observed effects of chamomile for its hypoglycemic action in diabetic rats may be due to potentiating the effect of insulin in serum or by increasing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form. Contrarily, reports show the hypoglycemic activity of the oregano extract is facilitated by the inhibition of hepatic glucose production and/or stimulation of glucose utilization by peripheral tissues, especially muscle and adipose tissue (Lemhadri et al.2004). This action of oregano extract, when combined with insulin secretion activity of chamomile, would have contributed to the observed
anti-hypoglycemic effects in mixture treatment group where individual low doses were less effective.

Reports indicate that, the treatment of diabetic rats with chamomile extract decreased levels of both serum creatinine and blood urea to the normal level (Asghari et al.2015). The decrease in blood urea and serum creatinine in chamomile treated diabetic rats may be due to the antioxidant activity of chamomile (Najla et al.2012). Moreover, studies also reveal that, the oral administration of the aqueous extract of oregano leaves improved the elevation in blood urea, uric acid and creatinine levels in diabetic rats (Nema and Omimah 2013). Oregano extract is also showed to have anti-urolicthtic activity both in vitro and in vivo models in addition to its antioxidant, cell protective, antispasmodic and diuretic activities (Khan et al.2011). Our results were in line with above literature where diabetic rat treated with 300 mg/kg each extract alone decreased the levels of urea, uric acid, creatinine and increased total protein compared to diabetic group. While 150 mg/kg individual treatments were less effective, the mixture of both extracts on diabetic rat was more effective in the improvement of renal parameters compared to the effect of 300 mg/kg each extract on diabetic animals.

Diabetic rats treated with oregano and/or chamomile extracts indicated decrease of total cholesterol, triglycerides and LDL-c with increase HDL-c compared to the diabetic rats. Oregano is proven to be effective in reducing the serum lipid levels significantly in NIDDM rats (Pimple et al.2012). There are studies to show aqueous chamomile extracts decreases TC, TG and LDL-c with raise in HDL-c in diabetic rats (Asghari et al.2015; Najla et al.2012). The decrease in serum levels of TC and TG may be attributed due to the uninhibited actions of lipolytic hormones on the fat depots (Goodman and Gilman 1985) or to the increase in the metabolism of free fatty
acids from the peripheral fat depots (Pari and Latha 2002). The results of lipid profile showed that the treatment of diabetic rats with the mixture of both extracts were significantly effective as anti-hyperlipidaemic compared to the effect of each extract alone on the diabetic animals. Overall, above results could be translated into restoration of diabetes-induced lipo-toxicity by either extracts or their combinations in diabetic rats.

Apoptosis is an important mechanism of cell death associated with diabetes induced renal damage (Marcia et al.2015). Annexin V assay showed the decrease in apoptotic cell population observed in extracts or mixture treatment (Fig. 4 a, b) confirmed the anti apoptotic effects of chamomile, oregano and their combination in diabetes kidney cells. The event of apoptosis is tightly regulated by anti apoptotic Bcl-2 protein and pro apoptotic Bax protein (Moretti et al.2010). The ratio of Bcl-2/Bax is therefore critical in determining the susceptibility of cells to induce apoptosis(Rasiova 2002). From fig 4 d, it is evident that up regulation of anti-apoptotic Bcl-2 caused decrease in the pro-apoptotic Bcl-2/Bax ratio in all treatments, suggesting chamomile, oregano and their mixture had a protective effect against diabetes induced renal apoptosis. Collectively our results clearly show that both extracts and their combination play a protective role in diabetes induced renal damage.

Diabetic lipotoxicity manifested by down regulation of fatty acid oxidation with altered expression of its downstream lipogenic transcription factors is well linked with diabetic nephropathy(Murea et al.2010). There are studies to show that lipid metabolism may serve as a target for specific therapies of human diabetic nephropathy (Herman et al.2014; Kim et al.2013). As both chamomile and oregano extracts have established anti oxidant activities, it is noteworthy to have a protective effect on diabetes induced renal toxicity as observed in figure 4.
These results also correlated well with anti hyperlipidaemic activity of the extracts and their mixture observed in this study. There are many examples where synergy was observed, when individual agents showed little or no effect (Khafif et al.1994; Suganuma et al.1999).

Collectively these results give us an insight on the combinational use of the plant extracts, further the dose ratio and variations in batch to batch extraction process may alter drug efficacy. However all these pitfalls can be overcome with cGMP facility standards, the dose for the big animal’s especially non-human primates and clinical trials with humans may pose great challenge with the concentration of the crude extracts.

5. Conclusion:

In conclusion, the present study clearly demonstrated that aqueous extracts of chamomile and oregano could be potentially useful in treating hyperglycemia and related diabetic complications. In addition a mixture of Chamomile and Oregano extracts may produce a therapeutic product with a potent anti-diabetic activity.

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6. References:


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Figure legends:

**Figure 1:** Effect of chamomile, oregano and their mixture on (a) body weight, (b) insulin, (c) blood glucose, (d) HbA1C and (e) serum α-Amylase in alloxan-induced diabetic rats for 3 and 6 weeks treatment. Results expressed as mean ± SD (n=5). * p ≤ 0.05 Vs uninduced control rats; ** p ≤ 0.05 Vs diabetic control rats.

**Figure 2:** Renal function profile of alloxan induced diabetic rats on treatment with chamomile, oregano, individually or as mixture. (a) Blood urea nitrogen, (b) Creatinine, (c) Uric Acid (d) Total protein levels. Results expressed as mean ± SD (n=5). * p ≤ 0.05 Vs uninduced control animals; ** p ≤ 0.05 Vs diabetic control animals

**Figure 3:** Anti hyperlipidaemic effects of chamomile, oregano extracts either individual or mixture. (a) Total cholesterol [TC], (b) triglycerides [TG], (c) high density lipoproteins [HDL], (d) low density lipoproteins [LDL], (e) very low density lipoproteins [VLDL] in alloxan-induced diabetic rats. Results expressed as mean ± SD (n=5). * p ≤ 0.05 Vs uninduced control rats; ** p ≤ 0.05 Vs diabetic control rats.

**Figure 4:** Apoptotic profile of isolated rat kidney cells with or without treatment. (a) representative histograms showing flow cytometric enumeration of the apoptotic cells by Annexin V staining, (b) decrease of total apoptosis in kidney cells with chamomile, oregano individual or combined treatment, (c) representative western blots of anti-apoptotic Bcl-2 and pro-apoptotic BAX expression in rat kidney cells. (d) Quantitative analyses of the Bcl-2/BAX ratio. Results expressed as mean ± SD (n=5). * p ≤ 0.05 Vs uninduced control rats; ** p ≤ 0.05 Vs diabetic control rats.
Figure 1

Body weight (grams)

Insulin (IU/l)

Glucose (mg/dl)

HbA1C %

\( \alpha \)-Amylase (U/l)

C - Uninduced control
DC - Diabetic control
1 - 150 mg/kg chamomile
2 - 150 mg/kg oregano
3 - 300 mg/kg chamomile
4 - 300 mg/kg oregano
5 - 300 mg/kg mixture

3 weeks post treatment
6 weeks post treatment

599x629mm (96 x 96 DPI)
Figure 2

599x511mm (96 x 96 DPI)
Figure - 3

634x660mm (96 x 96 DPI)
Figure - 4

603x479mm (96 x 96 DPI)
Table-1: Hypoglycaemic effect of chamomile, oregano and their combination in 3 and 6 weeks treatments. Data are represented as mean ± SD (n=5 per group).

Table-1 a

<table>
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<td>150 mg/kg chamomile</td>
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<td>6 weeks</td>
<td>Diabetic Control (DC)</td>
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a Expected value of chamomile and oregano combination = ([observed chamomile treatment value]/ [control value]) × ([observed oregano treatment value]/ [control value]) × (control value).

b Ratio = (expected value/observed value).
A ratio of > 1 indicates a synergistic effect, a ratio of 1 indicates additive effect and a ratio of < 1 indicates less than additive effect.

### Table-1 b

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin reduction (Normalised with DC)</th>
<th>Observed (%)</th>
<th>Average(%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Control (DC)</td>
<td>100.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 mg/kg chamomile</td>
<td>88.892</td>
<td>85.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 mg/kg oregano</td>
<td>82.075</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture</td>
<td>44.461</td>
<td>1.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>100.00</td>
<td>70.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 mg/kg chamomile</td>
<td>80.856</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>150 mg/kg oregano</td>
<td>77.239</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture</td>
<td>35.453</td>
<td>1.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Average value of chamomile and oregano combination

<sup>b</sup> Ratio = (average value / observed value).

A ratio of > 1 indicates a synergistic effect, a ratio of 1 indicates additive effect and a ratio of < 1 indicates less than additive effect.