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Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2016-0447.R1
Manuscript Type:	Article
Date Submitted by the Author:	08-Sep-2016
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Keyword:	liver injury, sodium valproate, sulforaphane, TNF α , haemoxygenase-1.

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Sulforaphane Protects against Sodium Valproate - Induced Acute Liver Injury

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Abstract:

Drug-induced hepatotoxicity is one of the most commonly encountered obstacles in the field of medical practice. Sodium valproate (VPA) is amongst many drugs with reported hepatotoxic effects. Sulforaphane (SFN) is a thiol compound of wide abundance in cruciferous plants and numerous reported therapeutic efficacies. The current investigation sheds light on the potential hepatoprotective effect of SFN against VPA-induced liver injury in rats. Twice daily I.P. VPA (700 mg/kg) for 7 days induced significant biochemical alterations and hepatic histopathological damage. SFN (0.5 mg/kg, orally) for 7 days significantly boosted liver functions biomarkers; it reduced serum ALT, AST and ALP and restored serum albumin concentration in a significant manner. Meanwhile, SFN significantly mitigated VPA-induced histopathological alterations. To highlight the mechanisms implicated in the observed hepatoprotective action, hepatic MDA and TNF α contents significantly declined with concomitant increase in hepatic haemoxygenase-1(HO-1) content and GSH concentration with SFN treatment. In conclusion; SFN can significantly ameliorate VPA-induced hepatotoxicity and liver injury mainly by direct association between antioxidant and anti-inflammatory properties.

Key words; liver injury, sodium valproate, sulforaphane, TNF α , haemoxygenase-1.

1. Introduction:

The liver is the largest organ within the human body. It has metabolic synthetic and excretory functions supporting almost every other organ within the body. Multiple critical metabolic functions eliminating toxins and other xenobiotics are mediated primarily by the liver (Bogdanos et al. 2013). Because of its strategic location and multidimensional functions, the liver is prone to many forms of injury ranging from acute liver injury, hepatitis, cholestasis, non-alcoholic fatty liver diseases, fibrosis and cirrhosis to ultimately hepatocellular carcinoma (Byrne and Targher, 2015). The human body cannot dispense any alteration of these vital functions occurring secondary to significant liver damage.

Several xenobiotics are believed to play a key role in induction of hepatic injury, of particular concern, drugs and pharmaceutical compounds. Administration of antiepileptics, antituberculous agents, halothane, corticosteroids, non-steroidal anti-inflammatory drugs, antifungals and recreational drugs have been correlated with incidence of several forms of hepatic injury (Pyleris et al. 2010)

Valproic acid (VPA) and its salts are mood stabilizing and anticonvulsant agents (Akhondian et al. 2015). Once it has been introduced into clinical use as an antiepileptic, VPA has rapidly become one of the widely prescribed antiepileptic medications (Silva et al. 2008). Various side effects have been reported alongside chronic VPA administration; gastrointestinal complaints, vision disturbance, hormonal impairments, hair loss and most importantly, mild to severe hepatotoxicity (Búdi et al. 2015). In spite of the undeniable pharmacological merits and effectiveness of VPA, its associated hepatotoxicity is still posing a major obstacle in clinical practice (Watkins and Seeff 2006).

Aminotransferases levels have been reported to increase rapidly in 11% of patients with hepatic macrosteatosis following VPA administration. At least 4 months of regular VPA administration are required for progression of hepatic injury. Predisposing factors include: infancy, multi antiepileptics administration, mental retardation, developmental delay and congenital anomalies (Akhondian et al. 2015).

VPA-induced hepatotoxicity usually manifests with hepatitis-like symptoms; malaise, anorexia, nausea and vomiting or with fever, coagulopathy and icterus. In chronic forms of hepatic failure, features like ascites and hypoglycemia are the predominant signs. These cases usually have poor prognosis and death happens due to hepatic failure and its associated further complications (Akhondian et al. 2015).

VPA administration has also been linked to other metabolic disturbances, of which; metabolic syndrome, dyslipidemia, insulin resistance and nonalcoholic fatty liver disease (NAFLD) (Farinelli et al. 2015). Estimations of NAFLD prevalence in developed world countries has been reported to be between 20% to 30% (Tarantino 2007).

Such adverse reactions and specifically hepatotoxicity are presumed to result in interruption of the medical course of treatment and failure of control of epileptic episodes mainly due to reduced patient compliance and reduced adherence. Considering the significant impact of chronic VPA administration on the patients' quality of life and health especially those in younger age population, the search for new and effective approaches that can confer significant protection against VPA-induced hepatotoxicity has become inevitable.

Antioxidants have been repeatedly reported to offer significant protection against various forms of liver injury (Said et al. 2012; Shoeib et al. 2015; Stravitz and Kramer 2009). Endogenous antioxidant defenses are reported to be activated to protect against oxidative injury (Bir et al. 2012).

Sulforaphane (SFN) is an organosulfur isothiocyanate molecule abundant in several cruciferous plants such as Brussels sprouts, cabbages and broccoli (de Figueiredo et al., 2015). SFN has been reported to be a potent anti-proliferative, anti-inflammatory, anti-oxidant and anti-cancer molecule (Thakur et al. 2014).

This study was designed to evaluate the hepatoprotective potential of SFN against VPA-induced hepatic injury. Antioxidant and anti-inflammatory activities of SFN were evaluated as well as the impact of its administration on VPA-induced hepatic injury.

2. Materials and methods:

2.1. Experimental Animals:

Twenty four male Sprague–Dawley rats, 8 weeks old, 210 ± 20 g, were obtained from "Experimental Research Centre, Nephrology and Urology Center", Mansoura University, Mansoura, Egypt. The research protocol complies with the ethical guidelines of experimental research; "Research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt in accordance with "Guide for the Care and Use of Laboratory Animals", 1996.

2.2. Drugs and chemicals:

Sodium valproate (VPA) and sulforaphane (SFN) were purchased from Sigma Aldrich Chemicals, (St. Louis, Missouri, USA). VPA was dissolved in 0.9% w/v saline for intraperitoneal (I.P.) injection and SFN was prepared for oral administration as a suspension in 0.5 % carboxymethylcellulose (CMC).

2.3. Experimental design:

Rats were randomly grouped (6 rat/group). Liver injury was induced as described by Lee et al. (2008), by injection of VPA (700 mg/kg, I.P.) twice daily for 7 days. Rats in the negative control group received (0.5 ml of 0.5% CMC orally, once daily) and twice daily I.P normal saline for 7 days; SFN control rats received SFN (0.5 mg/kg, orally) (Pan et al. 2014) once daily for 7 days without VPA administration, VPA-control rats received (0.5 ml of 0.5% CMC orally, once daily) and VPA (700 mg/kg, I.P.) twice daily for 7 days; SFN-treated rats received SFN (0.5 mg/kg, orally) (Pan et al. 2014) once daily for 7 days together with VPA (700 mg/kg, I.P.) twice daily for 7 days.

Rats were sacrificed 24 hours following the last VPA dose using I.P. thiopental sodium (40 mg/kg). Blood samples were withdrawn from the retro-orbital venous plexus; sera were separated for assessment of liver functions biomarkers. Liver samples were rapidly excised and weighed for calculation of liver/body weight index. The right lobes of the liver were harvested, weighed and preserved in 0.5% KCl for preparation of liver homogenate, evaluation of oxidative stress biomarkers and hepatic inflammatory cytokines and antioxidant elements contents while, the left

lobes were separated, fixed in 10% buffered formaldehyde and further preceded for histopathological examination.

2.4. Assessment of hepatic functions biomarker; serum alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, albumin concentration, bilirubin, (ALP) activity, and γ -glutamyl transferase (γ -GT) activity:

Serum (ALT), (AST), (ALP) activities and albumin concentration were colorimetrically assessed using Biomerieux assay kit (Marcy-l'Etoile, France), total bilirubin contents were assessed using Biocon assay kit (Vöhl/Marienhagen, Biocon Diagnostik, Germany), and γ -glutamyl transferase activity was assessed using kinetic method (Fortress Diagnostics, Ltd., UK) assay kits according to enclosed manufacturers' instructions.

2.5. Preparation of liver homogenate and assessment of hepatic (MDA) content, (SOD) activity, reduced (GSH) concentration, tumor necrosis factor α (TNF α) and hemoxygenase-1 (HO-1) contents:

Liver homogenate was prepared as described by Buege and Aust (1978). The homogenate was centrifuged (500g for 15 min) and the supernatant was separated and used immediately for the assessment of hepatic MDA content, SOD activity and GSH concentration using Biodiagnostic assay kits (Giza, Egypt) in accordance to supplied manufacturer instructions.

Hepatic TNF α and HO-1 contents were assessed using ELISA technique using rat platinum ELISA assay kits (Bender Med Systems GmbH, Austria) and (Cloud-Clone Corp., Houston, USA) respectively in accordance to enclosed manufacturers' instructions.

2.6. Histopathology:

Left lobes of the livers were excised and preceded as previously described. Specimens were embedded in paraffin blocks, sectioned and stained using hematoxylin and eosin (H&E) stain. The prepared slides were examined randomly under light microscope to assess VPA-induced histopathological changes and responses to SFN administration. The histopathologist was blinded to the

experimental groups and at least two different specimens were examined per liver sample. Modified HAI grading was used for quantification of: focal lytic necrosis, apoptosis, focal inflammation and portal inflammation (Ishak et al. 1995; Knodell et al. 1981).

2.7. Statistical analysis:

Values are presented as mean $\pm$ SDM. One-way analysis of variance (ANOVA) followed by Tukey–Kramer post *hoc* test and linear regression for the best fitting line for all of the standard points were used for statistical evaluation. Statistical significance was accepted at $p < 0.05$. Instat-3 software computer program (version 2.04; Graph Pad Software, Inc., San Diego, California, USA) was used to conduct statistical evaluation.

3. Results

3.1. Effect of sodium valproate (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on liver/body weight index:

As seen in figure (1), VPA administration (700 mg/kg, I.P.) twice daily for 1 week significantly increased liver/body weight index in comparison to normal control by about 1.2 folds. On the other hand, daily oral co-administration of SFN (0.5 mg/kg) for 7 days significantly reversed VPA-induced increase in liver/body weight index which significantly declined by 11% in comparison to VPA control. Such reduction in liver/body weight index with SNF administration can signify the retraction of hepatic inflammation and edema due to VPA injection.

3.2. Effect of sodium valproate (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on serum ALT, AST activities and albumin concentration:

Daily (700 mg/kg, I.P.) significantly increased serum ALT, AST activities with a significant decline in serum albumin concentration in comparison to normal control. Serum ALT and AST significantly increased by approximately 5 and 2.4 folds respectively and serum albumin significantly declined by about 1.36 fold in comparison to normal control. However, SFN co-administration (0.5 mg/kg, orally)

for 7 days significantly reduced serum ALT and AST activities by about 73%, 63% respectively (figure, 2) and serum albumin significantly increased by about 11 % in comparison to VPA control (table 1).

3.3. Effect of sodium valproate (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on serum ALP and γ GT activities and bilirubin concentration:

VPA (700 mg/kg, I.P.) twice daily, induced a significant increase in serum ALP activity without significant increase in either serum bilirubin or γ -GT activities compared to normal control. Serum ALP significantly increased by 1.3 folds in comparison to normal control. Co-administration of oral SFN (0.5 mg/kg) for 7 days significantly reduced serum ALP activity by about 15%, while, serum albumin significantly increased by about 11 % compared to VPA control, (table, 1).

3.4. Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on hepatic MDA content, SOD activity and GSH concentration:

As demonstrated in table (2), VPA (700 mg/kg, I.P.) twice daily, induced a significant increase in both of MDA content and SOD activity by about 1.8 and 1.33 folds respectively, with concomitant reduction in hepatic GSH concentration by about 1.35 folds compared to normal control. Co-administration of SFN (0.5 mg/kg, orally) for 7 days significantly reduced hepatic MDA content by approximately 23%, and SOD activity by 14% and significantly increased hepatic GSH concentration by 34% in comparison to VPA control.

3.5. Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on hepatic TNF α content:

VPA (700 mg/kg, I.P.) twice daily, significantly increased hepatic TNF α content compared to normal control by approximately 3.6 folds, but, co-administration of SFN (0.5 mg/kg, orally) significantly reduced hepatic TNF α content by about 48% compared to VPA control, (figure, 3).

3.6. Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on Hepatic HO-1 content:

VPA (700 mg/kg, I.P.) twice daily induced a significant decrease in hepatic HO-1 content compared to normal control by approximately 2 folds, on the other hand, SFN (0.5 mg/kg, orally) for 7 days significantly increased hepatic HO-1 content compared to VPA control, (figure, 4).

3.7. Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) on histopathological changes in H& E stained specimen:

Histopathological examination of liver specimen from normal control rats and SNF control revealed normal hepatic architecture, regular cords of hepatocytes (arrows) radiating from central vein (star), score 0 in modified HAI grading, (figure 5; A & B). On the other hand, liver specimen from VPA controls revealed destructed normal hepatic architecture, marked vacuolization and hydropic degeneration of hepatocytes (arrows). Areas of piecemeal necrosis and portal inflammatory infiltrate are seen (crossed arrows). (figure 5; C) with areas of focal lytic necrosis, inflammatory infiltrate (arrows) and apoptotic bodies (crossed arrows) (figure 5; D). Specimen scored 3 in modified HAI grading. Histopathological examination of liver specimen of the VPA control also revealed neocholangioles: proliferation of ductile like structures (arrows), areas of focal necrosis and inflammatory infiltrate (crossed arrows), congestion (C) and fibrosis (star) (figure 5; E). Daily oral SFN (0.5 mg/kg, orally) for 7 days significantly ameliorated VPA-induced hepatocellular damage, minimal neocholangioles (star), mild portal inflammatory infiltrate (arrow) and minimal focal lytic necrosis (crossed arrow) were detected in the examined specimen with score 1 in modified HAI grading with resolution of VPA-induced changes and restoration of normal hepatic architecture (figure 5; f, G and H) respectively.

4. Discussion:

The current study provides evidence on the hepatoprotective efficacy of sulforaphane (SNF) against sodium valproate (VPA)-induced hepatotoxicity and highlights the underlying mechanisms implicated.

Drug-induced hepatotoxicity is one of the most frequently encountered problems in the field of medical practice. Nevertheless, some patients are more prone than others to drug-induced hepatotoxicity, especially those ones with altered pharmacokinetic parameters; elderly patients, patients with pre-existing kidney or hepatic impairments, immuno-compromised patients and children. Moreover, several drugs have been withdrawn from markets following general public use due to their hepatotoxic effects.

VPA is an important medication for epileptic patients. Unfortunately, its hepatotoxicity might greatly curb its valuable pharmacological use. Thus it is very important to seek medications and hepatic supportive agents that can help in reducing VPA-induced hepatotoxicity and hence maximize its associated therapeutic outcomes.

SFN is a thiol compound found in many cruciferous vegetables. It has previously demonstrated detoxifying and anti-oxidative properties and prominent clinical values in the prevention and treatment of certain diseases related to oxidative stress (Chi et al. 2015). The observed hepatoprotective effect of SNF reported in the current study is in agreement with Chi et al. (2015), reporting amelioration of impaired hepatic function and improvements in oxidants/antioxidants biomarkers following ischemia reperfusion injury to the liver.

VPA is metabolized primarily in the liver by three routes; cytochrome P450, fatty acid and β -oxidation then it is conjugated to yield active and inactive metabolites (Sadeghi Niaraki et al. 2013). VPA has mainly two major hepatotoxic components; Hypoglycin and Pantoic acid. Pantoic acid is strongly believed to inhibit β -oxidation (Akhondian et al. 2015) and inhibition of β -oxidation is believed to be strongly associated with hepatotoxicity (Silva et al. 2002).

SFN has been reported to modify phase I detoxification reactions in the liver mainly by inhibiting the activity of several cytochrome P450 enzymes (Fahey et al., 2002). Meanwhile, SFN has also been reported to be a potent inducer of phase II detoxifying enzymes *in vitro* and *in vivo* (Geisel et al. 2016; Gerhäuser et al. 1997; Hu et al. 2002).

Such interference with hepatic metabolic machinery with SFN treatment can be presumed to contribute to the observed hepatoprotective effect of SFN. SFN can be presumed to interfere with the metabolism of VPA into its hepatotoxic metabolites with concomitant enhancement of excretion of those toxic metabolites, protecting the liver.

Acute liver injury is believed to proceed not only through direct injury to the hepatocytes but also through activation of innate immune-mediated responses with activation of signal transduction pathways and cytokines release, initially locally within the liver itself initiating hepatocyte death mainly through necrosis (Privitera et al. 2014). In association, oxidative stress has been reported to contribute significantly to pathophysiology of liver injury (Greco et al. 2011).

A complete and intricate system of a wide array of endogenous antioxidants is responsible for antagonizing oxidative stress. When stimulated by reactive oxygen species (ROS), nuclear factor-erythroid 2-related factor2 (Nrf2)/ARE signaling pathway as one of the several antioxidant pathways is stimulated to initiate the expression of distinct genes curbing the production of ROS or facilitating their reduction and eradication endogenously, strengthening anti-oxidative defense system (Chi et al. 2015).

Most ROS are generated in mitochondria which generate ATP supplying cells with energy. Mitochondria have been reported to be a primary target of oxidative stress (Greco et al. 2011). Evidence suggests that within mitochondria, ROS and their derivatives initiate oxidative injury of membranous proteins. They suppress electron transport of the respiratory chain and reduce ATP generation (Chi et al. 2015). Increased mitochondrial oxidative stress, in turn, induces opening of the mitochondrial permeability transition pore (PTP) (Takeyama et al. 1993)

incapacitating mitochondrial ATP formation, leading to metabolic failure, oncosis and apoptosis (Greco et al. 2011; Lee et al. 2001).

In agreement, in the current study, SFN administration successfully reduced hepatic MDA content and restored hepatic GSH concentration and SOD activity. It is worth mentioning that hepatic SOD activity significantly increased in VPA-control, which can be accounted for as a compensatory mechanism combating increased oxidative stress observed with onset of VPA-induced hepatotoxicity.

SFN has been reported to inhibit oxidative stress-induced PTP opening in rat liver mitochondria mainly by direct oxidation of sulfhydryl groups. Additionally, SFN has been reported to increase several important mitochondrial proteins and molecules serving as direct antioxidants, or generating the reducing power as NADPH, necessary for driving antioxidant activities (Greco et al. 2011).

Activation of Nrf2 signaling mainly by low molecular weight molecules has drawn significant attention in the past few years (Zhou et al. 2014). Following its absorption, SFN undergoes conjugation to GSH, enhancing intracellular SFN accumulation, generating intracellular stress in turn, activating various signaling pathways including kelch-like ECH-associated protein 1 (Keap1)- Nrf2 (Hayes et al. 2010; Wang et al. 2015).

Expression of many cytoprotective enzymes protecting against a variety of damages produced by either electrophilic or oxidative chemicals has been reported to be associated with Nrf2 activation. Up regulation/activation of Nrf2 promoted protection against variety of drugs-induced organ toxicities, inflammation and carcinogenesis (Osburn and Kensler 2008). Up-regulation of Nrf2 and subsequently other critical protective enzymes can be presumed to offer significant protection against hepatotoxicity.

In context with the association between oxidative stress and inflammation in pathogenesis of acute liver injury, thioredoxin is believed to regulate the action of several gene transcription factors including NF- κ B (Hintze et al. 2003) which tightly controls expression of various cytokines playing important role in the pathogenic pathway of acute liver injury, including TNF α .

In agreement with the observed reduction in hepatic TNF α content with SFN treatment in the current study, SFN has been reported to have anti-inflammatory effects attributed mainly to its ability to decrease the expression of innate cytokines like IL-6, TNF α , and IL-1 by macrophages *in vitro* in response to LPS (Lin et al. 2008) and to impair induction of IL-6 and TNF α -expression *in vivo* in response to LPS (Innamorato et al. 2008).

Further confirming implication of antioxidant and anti-inflammatory effects of SFN in its hepatoprotective activity, Heiss et al. (2001) proposed that the inactivation of NF-kB by SFN is likely to be mediated through the interaction with cellular redox regulators like glutathione or thioredoxin. SFN-mediated effects on cellular systems regulating oxidative stress and activation of several cytoprotective genes, is believed to be mediated through the Nrf2/ARE signaling pathway (Geisel et al. 2016).

As a Nrf2 agonist, SFN induces Nrf2 nuclear localization and antioxidant response elements (ARE) transactivation, enhancing phase II detoxifying enzyme activities and hence free radicals elimination (Guerrero-Beltrán et al. 2012). Nrf2 regulates the transcription of cytoprotective genes promoting cell protection against oxidative damage. SFN directly activates Nrf2, therefore, SFN is considered to be an indirect antioxidant (Geisel et al., 2016). It has further been shown that SFN-induced activation of phase II detoxification is also mediated through the induction of release of Nrf2 from the Keap 1-Nrf2 cytoplasmic complex (Wang et al. 2015).

In the current study, VPA-intoxication was accompanied by significant decline in hepatic haemoxygenase-1 (HO-1) content. HO-1 is one of many Nrf2-ARE driven antioxidant enzymes involved in protection against oxidative stress (Wang et al. 2015). HO-1 has been reported to show protective activity in different experimental models (Chora et al. 2007; Hegazi et al. 2005). Moreover, nuclear HO-1 interferes with activity of NF-kB (Levine and Kroemer 2008).

SFN administration restored hepatic HO-1 contents in the current study. Similar to HO-1 inducer dimethyl fumarate, SFN is a potent Nrf2 activator inducing HO-1 *in vitro* and *in vivo* (Harvey et al. 2011; Innamorato et al. 2008). SFN has been reported to induce HO-1 and other Nrf2-dependent genes *in vivo* (Li et al. 2013). It

can be assumed that SFN-induced induction of HO-1 directly links its anti-oxidative properties to its anti-inflammatory potentials.

In conclusion, sulforaphane can offer significant protection against sodium valproate induced toxicity mainly through its anti-inflammatory and immune-modulating antioxidant properties.

5. Conflict of interest:

Authors of the current manuscript confirm absence of any conflict of interest amongst each other or any other parties.

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Table 1: Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on serum ALP and γ GT activities, bilirubin and albumin concentration:

Experimental groups	Serum ALP (units/ml)	γ -GT (units/ml)	Bilirubin (mg/dl)	Serum Albumin (g/dl)
negative control (0.5% CMC, orally)	210 $\pm$ 7.806	2.494 $\pm$ 0.271	0.504 $\pm$ 0.035	4.16 $\pm$ 0.152
Sulforaphane control (0.5 mg/kg, orally)	212 $\pm$ 3.974	2.436 $\pm$ 0.194	0.510 $\pm$ 0.025	4.10 $\pm$ 0.131
VPA control (700 mg/kg, I.P.) twice daily	270.6 $\pm$ 36.678*	2.66 $\pm$ 1.491	0.558 $\pm$ 0.010	3.04 $\pm$ 0.207 *
Sulforaphane (0.5 mg/kg, orally) + VPA	230 $\pm$ 17.593 [†]	2.14 $\pm$ 1.146	0.47 $\pm$ 0.039	3.72 $\pm$ 0.205 [†]

Values are the mean $\pm$ SD of 6 rats/group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control

† Statistically significant VS VPA control

Table 2: Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on Hepatic MDA content, SOD activity and GSH concentration:

Experimental groups	MDA (nmol/ml)	SOD (U/ml)	GSH (mmol/L)
negative control (0.5% CMC, orally)	8.46 ± 0.192	235.4 ± 7.301	0.66 ± 0.079
Sulforaphane control (0.5 mg/kg, orally)	8.32 ± 0.974	233.46 ± 3.194	0.65 ± 0.031
VPA control (700 mg/kg, I.P.) twice daily	15.14 ± 2.091*	311.94 ± 21.949*	0.494 ± 0.122*
Sulforaphane (0.5 mg/kg, orally) + VPA	11.66 ± 3.610 [†]	267.56 ± 11.380 [†]	0.66 ± 0.103

Values are the mean ± SD of 6 rats/ group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control

[†] Statistically significant VS VPA control

Figure (1): Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on liver/body weight index:

Values are the mean $\pm$ SD of 6 rats/group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control;

† Statistically significant VS VPA control

Figure (2): Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on serum ALT, AST activities:

Values are the mean $\pm$ SD of 6 rats/group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control;

† Statistically significant VS VPA control

Figure (3): Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on hepatic TNF α content:

Values are the mean $\pm$ SD of 6 rats/group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control;

† Statistically significant VS VPA control

Figure (4): Effect of sodium valproate alone (700 mg/kg, I.P.) twice daily and in combination with sulforaphane (0.5 mg/kg, orally) for 7 days on hepatic HO-1 content:

Values are the mean $\pm$ SD of 6 rats/group.

Statistical analysis of mean values was evaluated using ANOVA followed by Tukey–Kramer's test

* Statistically significant VS negative control;

† Statistically significant VS VPA control

Figure (5): Photomicrograph of histological section of liver (A): Normal control, (B); SNF control, (C), (D) and (E); VPA-control, (F), (G) and (H); SFN-treated group (Magnification 400x)

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Figure (1)

Figure (2)

Figure (3)

Figure (4)

Figure (5)

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