

Ocimum Sanctum Ameliorates Valproic Acid – Induced Hepatotoxicity

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Research

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Abstract

OBJECTIVE: Evaluation of hepatoprotective potentials of *Ocimum sanctum* against Valproic-acid-induced-hepatotoxicity in Wistar-albino-rats.

METHOD: 70% of ethanolic extract of *Ocimum sanctum* was prepared under reduced pressure of rotary evaporator. Wistar albino rats were used as the experimental model and rats were divided into four groups (six animals each). The normal group received normal saline and group 2, 3 and 4 was injected valproic acid (500mg/kg) for four consecutive days respectively. Group 1 and 2 received normal saline throughout the period of study about 21 days while group 3 and 4 received different doses of extract of *OS* i.e. 200mg/kg and 300mg/kg. Through retro-orbital blood samples were collected on alternative days such as 0, 7, 21. By using one-way ANOVA, data was analyzed. Hepatotoxicity induced by valproic acid at the dosage of (500mg/kg) resulted in significant elevation in weight of animals and serum hepatic enzymes level of ALAT, ASAT, ALP and increase in the serum bilirubin.

RESULTS: *OS* at different doses (200mg/kg and 300mg/kg) considered statistically significant ($p \leq 0.05$) against all parameters. *OS* cause a significant reduction in weight of animals and serum enzymes biomarkers i.e. (ALAT, ASAT and ALP) including bilirubin content. *OS* may prove its hepatoprotective activity by increase a significant level of protein albumin.

CONCLUSION: antioxidant activity of *OS* and secondary metabolites such as flavonoids depicts hepatoprotective nature against valproic-acid-induced-hepatotoxicity.

Introduction

In year 1882, Valproic acid (2-propyl-pentanoic acid, 2 propylvaleric acid) was first synthesized by Burton [1]. In 1963, VPA (valproic acid) was used as a molecular carrier in anti-convulsive activities. Its pharmacological activities demonstrated that VPA was used in rodents against pentylenetetrazol-induced convulsions [2]. Today, worldwide valproic acid is used as an anti-epileptic drug to treat several forms of epilepsy and various types of seizures, that affecting both children's and adults [3]. The major undesirable side effects of VPA, included teratogenicity and hepatotoxicity [4]. Patients receiving valproic acid, causing hepatic failure resulting in fatalities [5]. Changes in serum cholesterol, triglycerides, fat glucose and gain of weight, have also been associated with side effects of VPA [6].

In rat hepatocytes, VPA is associated with biochemical disturbances such as inhibition of fatty acid oxidation, ketogenesis, gluconeogenesis, urea synthesis and reduced level of acetyl-co A (Becker and Harris, 1983) [7]. Administering high dose of VPA consistently, about 500-750mg/kg produces micro vesicular steatosis. The steatogenic effect is produced because of toxic metabolites of VPA. Mechanism of these effects is due to (a) depress the synthesis of proteins; (b) through mitochondrial injury depressing oxidation of fatty acids, or (c) enhancing mobilization to the liver from fat depots [8]. Medicinal plants as an antioxidant property of flavonoids and a tendency to protect the membrane, is

observed as a hepatoprotective medicinal plants [9] Some hepatoprotective medicinal plants includes, *Azadirachta indica*, *Cassia fistula*, *Silybum marianum* [10].

Ocimum sanctum commonly known as “Tulsi”. The word tulsi is a Sanskrit word which means “the inimitable one” [11]. In bygone literature, tulsi describe as “queen of herbs” [12]. In Ayurvedic and Unani system of medicines *ocimum sanctum* is also known as “Sweet Basil or Holy Basil” [13]. Worldwide, about 150 species or class of *Ocimum* is present but basil is the real one who developed fundamentally [14]. Plant of tulsi is about 30-60 cm tall with hairy stem. Leaves are green having petioles and up to 5 cm long. *Ocimum sanctum* is a flavoring remedy and enhanced many basic ailments [15]. Flavonoids, Alkaloids, Saponins, Tanins, Phenols, Anthocynins, Terpenoids and Steroils [16]. The hydro-ethanolic extract of *OS* leaves, orally administered at 200mg/kg dose or more than 200 mg/kg against ccl4, acetaminophen and lead-induced hepatotoxicity in albino rats, shows that *OS* induced protective effect in these animal models. *Ocimum sanctum* reduced the level of serum hepatic enzymes, such as ALAT, ASAT and ALAP in above animal models [17]. *OS* also induced hepatoprotective effect against anti-tubular drugs-induced hepatotoxicity in albino rats [18].

PLANT ANATOMY

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	Ocimum
Species	:	O.tenuiflorum
Botanical Name	:	Ocimum Tenuiflorum (Singh <i>et al.</i> , 2012a). [9].

IPNI Life Sciences Identifier (LSID)

urn:lsid:ipni.org:names:30252269-2

Kew Names and Taxonomic Backbone

The International Plant Names Index and World Checklist of Selected Plant Families.

Research Methadology

Ethical consideration

The study was approved by IRB of The Islamia University of Bahawalpur, Bahawalpur, Pakistan. Date of approval of this study was 03 September 2016 under the approval number of IUB/03-16/34059.

Material and Equipment's

For research purpose chemicals of analytical grade were used included valproic acid (platinum pharmaceutical LTD), ketamine (Indus pharma Lahore), xylazine (prix pharmaceutical Lahore), ether, aqueous ethanol all one of analytical grade.

Equipment's:

Table 3.1: Equipment's used

Apparatus	Modal and /or Manufacturer
Spectrophotometer UV-VIS	Irmeco , U2020
Centrifuge machine	Hettich –EBA 20
Rotary Evaporator	Heidolph Laborota 4000efficient Germany
Freezer	Haier, HF-240-T
Digital weighing balance	Shimadzu, AY62
Refrigerator	Dawlance, Pakistan
Grinder	National, Japan
Incubator	Memmert
Electric Mortar and pestle	
Vortex mixer	Mylab SLV-6
Water Bath	China
Micropipette	Huawei
Eppendorf	
Falcon tubes	
Beakers	Pyrex
Measuring cylinder	Pyrex

Pharmacological Section

In-vivo study

Wistar Albino rats weighting 220-270g were taken and kept in cages. Cages with polycarbonate layer secured by raw dust, under standard lab conditions it changed after every three days (temperature: $27\pm 2^{\circ}\text{C}$ in animal house of pharmacology research lab, faculty of pharmacy and alternative medicine, the Islamia university of Bahawalpur. Rats were provided with standard diet pellets and water ad libitum. Wistar Albino rats were acclimatized to a regimen of 12 hours of light and 12 hours of haziness. By ethical committee of the Islamia University of Bahawalpur, study was approved. According to study animal were divided.

Subject of study:

24 Wistar rats of either sex were assigned into four groups each having six animals and categorized as;

Group 1 was named as normal control and given normal saline.

Group 2 was named as valproic acid group and given (500 mg /kg)

Group 3 was named as VPA + extract 1 and given (200 mg/kg) of *O.S* extract.

Group 4 was named as VPA + extract 2 and given (300 mg/kg) of *O.S* extract

Plant collection:

Fresh, *Ocimum Sanctum* leaves were purchased from local nursery of Bahawalpur, Pakistan on May 2017. Information was collected about the medicinal uses of plants to treat hepatic injury.

Preparation of Crude Extract:

The leaves of *Ocimum Sanctum Linn* were separated, dried under shade for 15 days and powdered in a blender. It was grinded by electric grinder and then soaked in a mixture of 70% v/v ethanol and 30% v/v distilled water for three days with shaking and agitation occasionally. By muslin fabric, soaked material was separated for coarse filtration and afterward by Whattman filter paper (NO.1).

Then by using rotary evaporator (Heidolph Laborota 4000 efficient, Germany) at a temperature of 30-40°C the filtrate obtained was then evaporated under reduced pressure.

Until the filtrate become semi solid the process of evaporation was Continue and placed the filtrate into the incubator for further drying then stored in a refrigerator (-4°C) till further use.

Induction of Hepatotoxicity:

Valproic acid (500 mg/kg orally) once a day for four consecutive days was used to induce hepatotoxicity.

Experimental Protocol

Rats were assigned into four groups randomly and each group having six animals. Group-1 was normal control and animals were given normal saline as a vehicle for 21 days.

Group-2 group was intoxicated and toxicity was caused by valproic acid (500 mg/kg) once daily for four consecutive days given orally and received normal saline after further for 21 days. Group-3 was considered treatment group and treated with *Ocimum sanctum* (200 mg/kg) dose orally for 21 days and received (500 mg/kg) orally valproic acid for four consecutive days. Group-4 was considered treatment group and treated with *Ocimum sanctum* at dose (300 mg/kg) orally for 21 days and received valproic acid (500 mg/kg) orally for four consecutive days. The blood was collected via retro-orbital at day 0, 7 and 21 and serum was separated by centrifugation at 60 rpm about 20 minutes. Animals were sacrificed at the end of the experimental period.

Biochemical Parameters Studied

All parameters were carried out by human diagnostic kit Germany.

Liver Enzyme Test

Liver Protein Test

Bilirubin

Statistical Analysis

The obtained data were expressed as mean $\pm$ S.E.M. The data were subjected to one-way (ANOVA) Bonferroni's post hoc test for statistical significance between experimental groups. P-values <0.05 were examined as statistically significant.

Results

Results of control, VPA and VPA+ *O.S* (200mg/kg) treated group and VPA+ *O.S* (300mg/kg) treated group

Effect of *O.S* on Body weight

Variance in the body Weight of rats (all groups) was observed on day 0, 7 and 21 during the period of three weeks study. It was observed that the body weight among all groups at day "0" was not significantly different (all $p>0.05$). As the study continue, it was observed that on day "7" and "21" body weight of control group was significant higher as compared to the body weight of control group at day "0" (all $p<0.05$). Generally, incredibly the body weight of VPA treated group was showed significant increase on day "7" and day "21" as compared to the body weight at day 0 (all $p<0.05$). With the progression of days, VPA+*O.S*(200mg/kg) treated group and VPA+*O.S*(300mg/kg) treated groups of animals, exhibited significant reduction in body weight on day "7" and day "21" as compared to the body weight of VPA treated group on day 7 and day 21 respectively (all $p<0.05$).

Table 1: Effects of crude extract of O.S on body weight in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
Body weight (g)	Control	244 ± 7.2	285 ± 7.1(*)	320 ± 9.2(*)
	VPA (500mg/kg)	248 ± 7.8	322 ± 7.0(*)	336 ± 7.1(*)
	VPA+O.S (200mg/kg)	255 ± 8.4	294 ± 7.9(&)	283 ± 7.8(&)
	VPA+O.S (300mg/kg)	253 ± 8.9	281 ± 8.0(&)	265 ± 7.8(&)

Effect of O.S on Serum Alkaline phosphate (ALP)

Liver damage assessment was done by biochemical investigation of serum Alkaline phosphate (serum ALP) after three weeks of study. The ALP level was calculated on day "0", "7" and "21" respectively. The observations made no significant difference at day "0" among all groups (all $p > 0.05$). It was observed that on day "7" and day "21" the level of control group was not significantly different as compared to the control at day 0" (all $p > 0.05$). As the study proceeded, it was analyzed that the ALP level of VPA treated group on day "7" and "21" was significant increased as compared to the VPA group at day 0 (all $p < 0.05$). Interestingly, significant reduction in ALP level of VPA+O.S (200mg/kg) and VPA+ O.S (300mg/kg treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p < 0.05$). Prevention on the decrease in ALP levels at the dose 200 and 300 mg/kg was found highly significant.

Table 2: Effects of crude extract of O.S on Alkaline phosphate (ALP) in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
ALP (u/l)	Control	89 ± 6.5	107 ± 4.2	120 ± 6.8
	VPA (500mg/kg)	97 ± 9.1	225 ± 9.7(*)	226 ± 5.1(*)
	VPA+O.S (200mg/kg)	77 ± 3.7	194 ± 5.3(&)	172 ± 5.3(&)
	VPA+O.S (300mg/kg)	80 ± 3.4	179 ± 5.9(#)	160 ± 6.8(#)

Effect of O.S on Serum Alanine Aminotransferase (ALAT)

Liver damage evaluation was done by biochemical examination of serum Alanine Aminotransferase (serum ALAT) following three weeks of study. The level of ALAT was calculated on day “0”, “7” and “21” respectively. No significant difference at day “0” among all groups when observations were made (all $p>0.05$). It was noticed that on day “7” and day “21” the level of control group was not significantly different as compared to the control at day 0” (all $p>0.05$). As the study proceeded, it was evaluated that the ALAT level of VPA treated group on day “7” and “21” was significant increased as compared to the VPA group at day 0(all $p<0.05$). Incredibly, significant reduction in ALAT level of VPA+O.S (200mg/kg) and VPA+ O.S (300mg/kg treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p<0.05$). Anticipation on the decrease in ALAT levels at the dose 200 and 300 mg/kg was begun highly significant.

Table 3: Effects of crude extract of O.S on Alanine Aminotransferase (ALAT) in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
ALAT (u/l)	Control	29 ± 3.5	33 ± 4.5	30 ± 2.5
	VPA (500mg/kg)	28 ± 3.5	98 ± 4.9(*)	97 ± 8.9(*)
	VPA+O.S (200mg/kg)	23 ± 3.6	74 ± 6.3(&)	70 ± 4.2(&)
	VPA+O.S (300mg/kg)	30 ± 3.0	60 ± 6.9(#)	58 ± 6.0(#)

Effect of O.S on Serum Aspartate Aminotransferase (ASAT)

Liver anguish assessment was done by biochemical estimation of serum Aspartate Aminotransferase (serum ASAT) after three weeks of study. The ASAT level was calculated on day “0”, “7” and “21” respectively. The observations made no significant difference at day “0” among all groups (all $p>0.05$). It was analyzed that on day “7” and day “21” the level of control group was not significantly different as compared to the control at day 0” (all $p>0.05$). As the study proceeded, it was noticed that the ASAT level of VPA treated group on day “7” and “21” was significant increased as compared to the VPA group at day 0(all $p<0.05$). Interestingly, significant reduction in ASAT level of VPA+O.S (200mg/kg) and VPA+ O.S (300mg/kg treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p<0.05$). Deterrence on the decrease in ASAT levels at the dose 200 and 300 mg/kg was established highly significant.

Table 4: Effects of crude extract of O.S on Aspartate Aminotransferase (ASAT) in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
ASAT (u/l)	Control	79 ± 4.3	73 ± 6.4	76 ± 3.8
	VPA (500mg/kg)	63 ± 5.5	180 ± 11(*)	171 ± 6.4(*)
	VPA+O.S	71 ± 7.8	137 ± 11(&)	140 ± 5.6(&)
	(200mg/kg)			
	VPA+O.S	69 ± 8.4	25 ± 12(#)	116 ± 6.0(#)
	(300mg/kg)			

Effect of *O.S* on Serum Albumin

Liver injury assessment was done by biochemical evaluation of serum Albumin following three weeks of study. Level of Albumin was calculated on day “0”, “7” and “21” respectively. The observations made no significant difference at day “0” among all groups (all $p > 0.05$). It was observed that on day “7” and day “21” the level of control group was not significantly different as compared to the control at day 0” (all $p > 0.05$). As the study proceeded, it was analyzed that the Albumin level of VPA treated group on day “7” and “21” was significant decreased as compared to the VPA group at day 0 (all $p < 0.05$). Generally, significant increase in Albumin level of VPA+O.S(200mg/kg) and VPA+ O.S(300mg/kg treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p < 0.05$). Precaution on the increase in Albumin levels at the dose 200 and 300 mg/kg was created highly significant.

Table 5: Effects of crude extract of *O.S* on Albumin in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
Albumin (g/dl)	Control	4.2 ± 0.12	4.3 ± 0.15	4.2 ± 0.17
	VPA (500mg/kg)	4.2 ± 0.12	2.6 ± 0.15(*)	2.6 ± 0.099(*)
	VPA+O.S	4.3 ± 0.11	3.2 ± 0.10(&)	3.3 ± 0.14(&)
	(200mg/kg)			
	VPA+O.S	4.4 ± 0.11	3.4 ± 0.098(#)	3.5 ± 0.11(#)
	(300mg/kg)			

Effect of *O.S* on Serum Total Bilirubin (TBR)

Liver damage assessment was done by biochemical examination of serum total bilirubin (serum TBR) after three weeks of study. The level of TBR was calculated on day “0”, “7” and “21” respectively. The

observations made no significant difference at day “0” among all groups (all $p>0.05$). It was observed that on day “7” and day “21” the level of control group was not significantly different as compared to the control at day 0” (all $p>0.05$). As the study continue, it was analyzed that the TBR level of VPA treated group on day “7” and “21” was significant increased as compared to the VPA group at day 0(all $p<0.05$). Generally, incredibly significant reduction in TBR level of VPA+O.S(200mg/kg) and VPA+ O.S(300mg/kg) treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p<0.05$). Prevention on the decrease in TBR levels at the dose 200 and 300 mg/kg was begun highly significant.

Table 6: Effects of crude extract of O.S on TBR in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
TBR (mg/dl)	Control	0.39 ± 0.050	0.43± 0.029	0.46 ± 0.044
	VPA (500mg/kg)	0.42 ± 0.041	0.94± 0.031(*)	0.87± 0.044(*)
	VPA+O.S (200mg/kg)	0.43 ± 0.046	0.81 ± 0.028(&)	0.70 ± 0.038(&)
	VPA+O.S (300mg/kg)	0.37 ± 0.053	0.75 ± 0.029(#)	0.62 ± 0.023(#)

Effect of O.S on Serum Direct Bilirubin (DBR)

Liver damage assessment was done by biochemical examination of serum direct bilirubin (serum DBR) after three weeks of study. The level of DBR was calculated on day “0”, “7” and “21” respectively. The observations made no significant difference at day “0” among all groups (all $p>0.05$). It was observed that on day “7” and day “21” the level of control group was not significantly different as compared to the control at day 0” (all $p>0.05$). As the study continue, it was analyzed that the DBR level of VPA treated group on day “7” and “21” was significant increased as compared to the VPA group at day 0(all $p<0.05$). Generally, incredibly significant reduction in DBR level of VPA+O.S(200mg/kg) and VPA+ O.S(300mg/kg) treated groups on day 7 and 21 compared to the VPA treated group at day 7 and 21 respectively (all $p<0.05$). Prevention on the decrease in DBR levels at the dose 200 and 300 mg/kg was begun highly significant.

Table 7: Effects of crude extract of O.S on DBR in rats treated with VPA

		DAYS OF OBSERVATIONS		
Parameters	Groups	0 DAY	7 th DAY	21 DAY
DBR (mg/dl)	Control	0.11 ± 0.035	0.19 ± 0.023	0.16 ± 0.041
	VPA (500mg/kg)	0.15 ± 0.029	0.63± 0.048 (*)	0.58 ± 0.060 (*)
	VPA+O.S (200mg/kg)	0.18 ± 0.022	0.53 ± 0.051(&)	0.45 ± 0.057 (&)
	VPA+O.S (300mg/kg)	0.14 ± 0.031	0.47 ± 0.052 (#)	0.37 ± 0.049 (#)

Discussion

The objective of wild medicinal or therapeutic plants to treat human diseases is not recent. Since ancient time herbal medicine played an important role in curing diseases of humans [19]. Many environmental chemicals and drugs can cause some degree of injuries and leading cause of acute liver injury, is a drug-induced liver injury [20]. Generally, drugs can cause an imbalance between prooxidant and antioxidant levels in cells and tissues. Reactive oxygen species (ROS) are prooxidants which can cause tissue injury [21]. several medicinal plants with their antioxidant properties are effective to cure injuries of liver and beneficial as a hepatoprotective plant. Present study was designed to investigate the hepatoprotective effect of *ocimum sanctum linn* against valproic acid-induced hepatotoxicity in albino rats. Valproic acid (2-propyl-pentanoic acid, 2 propyl valeric acid), used as an anti-epileptic drug to treat several forms of epilepsy and various types of seizures [3]. The major undesirable side effects of VPA, included teratogenicity and hepatotoxicity [4]. In VPA-induced liver injury, pathogenesis involves the toxic metabolites of drug that effect directly the cell biochemistry or evoke an immune response. β -oxidation an important pathway for oxidative metabolism. In rat hepatocytes, VPA is associated with biochemical disturbances such as inhibition of fatty acid oxidation, ketogenesis, gluconeogenesis, urea synthesis and reduced level of acetyl-co A [22]. Valproyl-co A, helps to form esters with fatty acids [23]. A reactive metabolite of valproic acid that is 2,4-Diene-valproyl-CoA cause inhibition and inactivation of β -oxidation enzymes [24]. Several studies investigated that treatment with VPA, in case of liver injury, is associated with oxidative stress in many patients and in animal models too [25]. The etiology of oxidative stress is hypothesized because of overproduction of reactive oxygen species (ROS) and/or reduced antioxidant capacity [26]. Glutathione (GSH) is an important antioxidant cellular biomolecule. A reactive metabolite of VPA that is 4-ene-VPA cause depletion of GSH in rat hepatocytes and induced oxidative stress [27]. Valproic acid-induced hepatotoxicity showed by decrease or increase in the level of serum hepatic proteins and enzymes test [28]. So, my study was designed to examine the hepatotoxic effects of valproic acid in albino rats.

Ocimum sanctum play an important role in various pharmacological actions. Traditionally *ocimum sanctum* is fulfil actions in many other prevention and cure of illness like fever and common cold, coughs, sore throat, kidney stone, children's Ailments, mouth infection, insect bites, skin disorders, teeth disorders, headaches and colic pain [29]. *OS* shows antidote activity against several poisons [30]. *OS* potentiate significant effect to lower of sugar level in blood. It exerts hypoglycemic effect in streptozotocin-induced diabetic rats [31]. Moreover, *ocimum sanctum* induced their hepatoprotective activity in various animal models against CCl₄, acetaminophen, lead and anti-tubular drugs [32]. Antioxidant property of *ocimum sanctum* leaves have studied already. Chemical toxins and radiation induce biological damage because of forming reactive oxygen species, such as singlet oxygen and superoxide's, hydroxyl and hydroperoxyl radicals, hydrogen peroxide and organic peroxides. Extract of *OS* leaves and their flavonoids, such as orientin and vicienin have shown high antioxidant activity in vivo and anti-lipid peroxidative effect in vitro [33].

However, the antioxidant potential of herbs is due to the redox properties of their phenolic compounds, which allow them to act as a reducing agent, hydrogen donors and singlet oxygen species [34]. Phenolic compounds are secondary metabolites that possess high antioxidant activity [35]. It is strongly suggesting that free radicals scavenging a major mechanism, through which *ocimum sanctum* protect against tumor induction and cellular damage [36]. Also, *ocimum sanctum* reduced erythrocyte lipid peroxidation activity induced against hypercholesterolemia in albino rats and protect aortic and liver tissues against hypercholesterolemia-induced peroxidative damage [37].

Previous study has suggested that the plant extract contains constituents having hepatoprotective and strong antioxidant secondary metabolites that counteract the lethal impacts of valproic acid. So, my study was designed to determine hepatoprotective action of *ocimum sanctum* extract against valproic acid-induced hepatotoxicity. Hepatotoxicity was observed through some invitro study and by various liver function parameters in-vivo. In invitro study, gain of weight was observed in VPA treated group animals and weight was significantly reduced when animals treated with *ocimum sanctum* extract. One side effect of VPA is weight gain and during VPA treatment the amount of average weight gain is approximately 6 kg in human [38]. Invitro studies suggest that weight is gain when VPA initiates pancreatic insulin secretions that might increase energy storage and appetite. It concluded that possibly insulin resistance and hyperinsulinemia cause to weight gain [39]. Previous studies showed that in experimental animals, *ocimum sanctum* leaves induce hypoglycemic effects. A study was conducted with *OS* and neem stated that in diabetic patients this combination is good in lowering sugar level [40]. *OS* potentiate significant effect to lower of sugar level in blood. It exerts hypoglycemic effect in streptozotocin-induced diabetic rats.

The level of serum alkaline phosphatase (ALP), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) was increased after chronic administration of valproic acid in animals of toxicities group. The level of above serum hepatic enzymes was significantly reduced when extract of *ocimum sanctum* was given to treated group of animals. Previous studies showed that Valproic acid induced liver injury and increase levels of serum hepatic enzymes [41]. In the cell cytoplasm ASAT, ALAT

and ALP are located and after administration of *ocimum sanctum* extract improved toxicity and has intense effect in restoration of AST, ALT and ALP and decreased level of these enzymes towards their respective normal values. The hepatic efficiency improved, and regeneration of the hepatic tissue was indicate by the restoration of the bilirubin levels in previous studies [42].

The most generous circulating protein in the plasma is albumin and the vital protein synthesized by the liver [43]. In my study valproic acid induced toxicity by decreasing the level of albumin in group of animals treated by valproic acid. The extract OS was given in treated group animals and study shows that the level of albumin increased significantly. In previous studies it was discussed that valproic acid induced toxicity by decreasing the level of albumin [28]. Noteworthy, the level of albumin after administration of *OS* extract showed significant increase in their levels in previous studies.

My study was exhibits that after chronic administration of valproic acid the level of serum bilirubin (total and direct) was increased and shows that toxicity is induced in animals. In contrast the level was decreases and comes near to their normal values after giving *ocimum sanctum* extract in treated group of animals. In previous studies, after induction of toxicity by valproic acid the level of serum bilirubin (total and direct) was increased significantly compare to normal [28].

However, in vitro and in-vivo both studies show that valproic acid induced toxicities in hepatic enzymes and proteins biomarkers and disturbed their levels and in contrast of valproic acid, extract of *ocimum sanctum* induced its significant effects to normalize the level of serum hepatic enzymes. However, advance research is needed to determine the exact mechanism of action. Also, whether hepatotoxicity of valproic acid is cumulative remains contentious.

Conclusion

Present study may have concluded that valproic acid induces hepatic damage in rat liver and cause oxidative stress in hepatocytes of rat. Reactive oxygen species altered the hepatic antioxidant enzymes and proteins and depletion of free radicals may cause injury of liver in albino rats. In contrast, *ocimum sanctum linn* due to its strong antioxidant activity protect the liver from damage. *ocimum sanctum* can minimize the effect of hepatotoxic drugs and other chemicals. Therapeutic potential of *ocimum sanctum* as a hepatoprotective medicinal plant has been proven. We endorse further studies and more clinical trials conducted to underprop its hepatoprotective potential and other therapeutic uses.

Abbreviations

OS: Ocimum Sanctum

B.W: Body weight

NaOH: Sodium hydroxide

TCA: Trichloroacetic acid

VPA: Valproic acid

i.p: Intra peritoneal

ROS: Reactive oxygen species

BTW: Between

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable

Funding

Not applicable

Availability of data and materials

Not applicable

Authors' Contributions

Omer Iqbal, Qurratulain Rahim, Fiaz ud din Ahmad designed the study. Qurratulain Rahim performed the experiments and compiled the data. Faiza Naeem and Omer Iqbal analyzed the data and wrote the manuscript. Hania Mehboob Khan, Imran Akhtar, Quratulain Rahim, Omer Iqbal coordinated the project preparation of the final version of the manuscript.

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Conflict of interest: None

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Figures



Tulsi

Figure 1

Tulsi

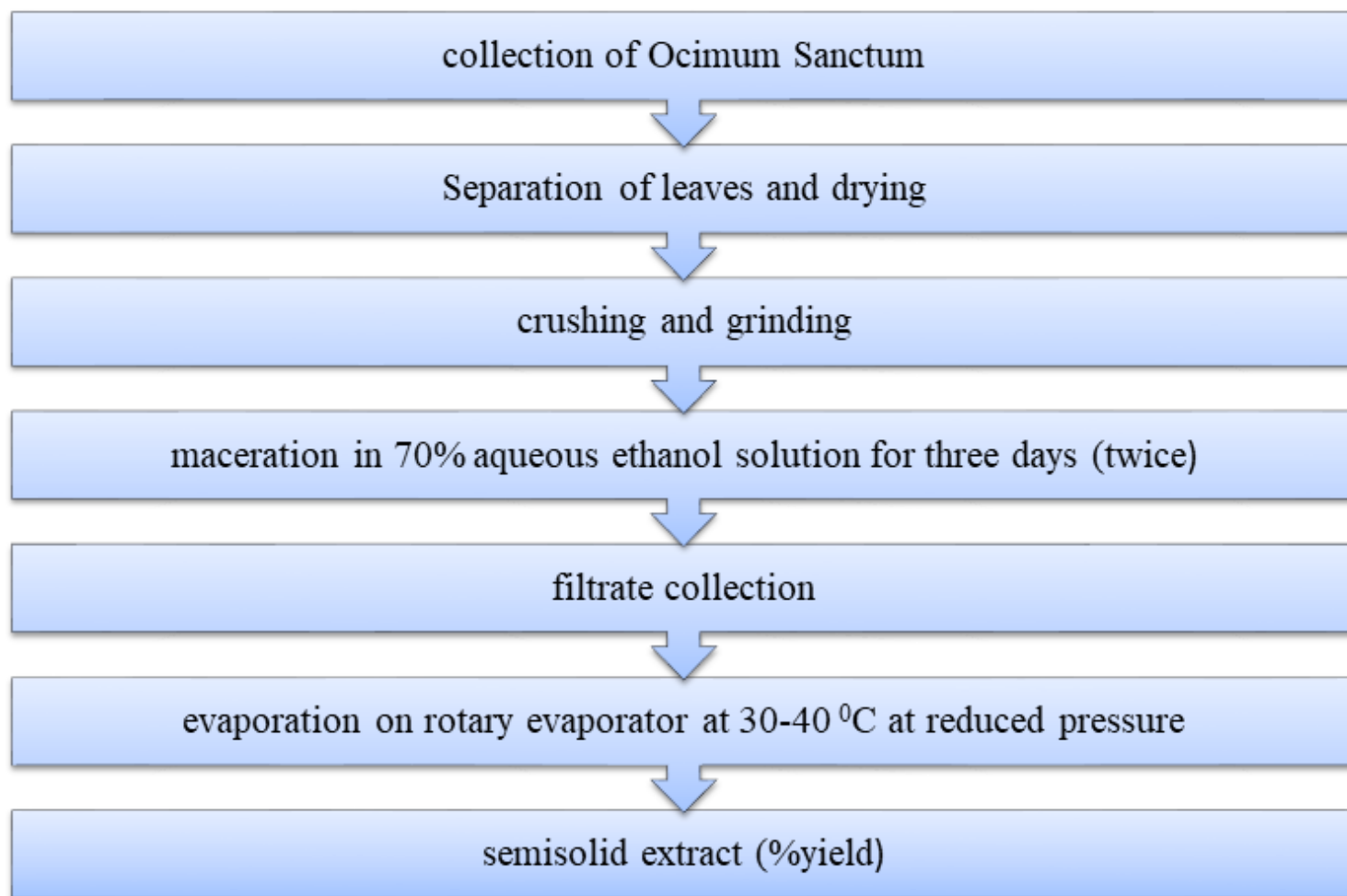


Figure 2

Steps involved in preparation of crude extract of *Ocimum Sanctum* linn

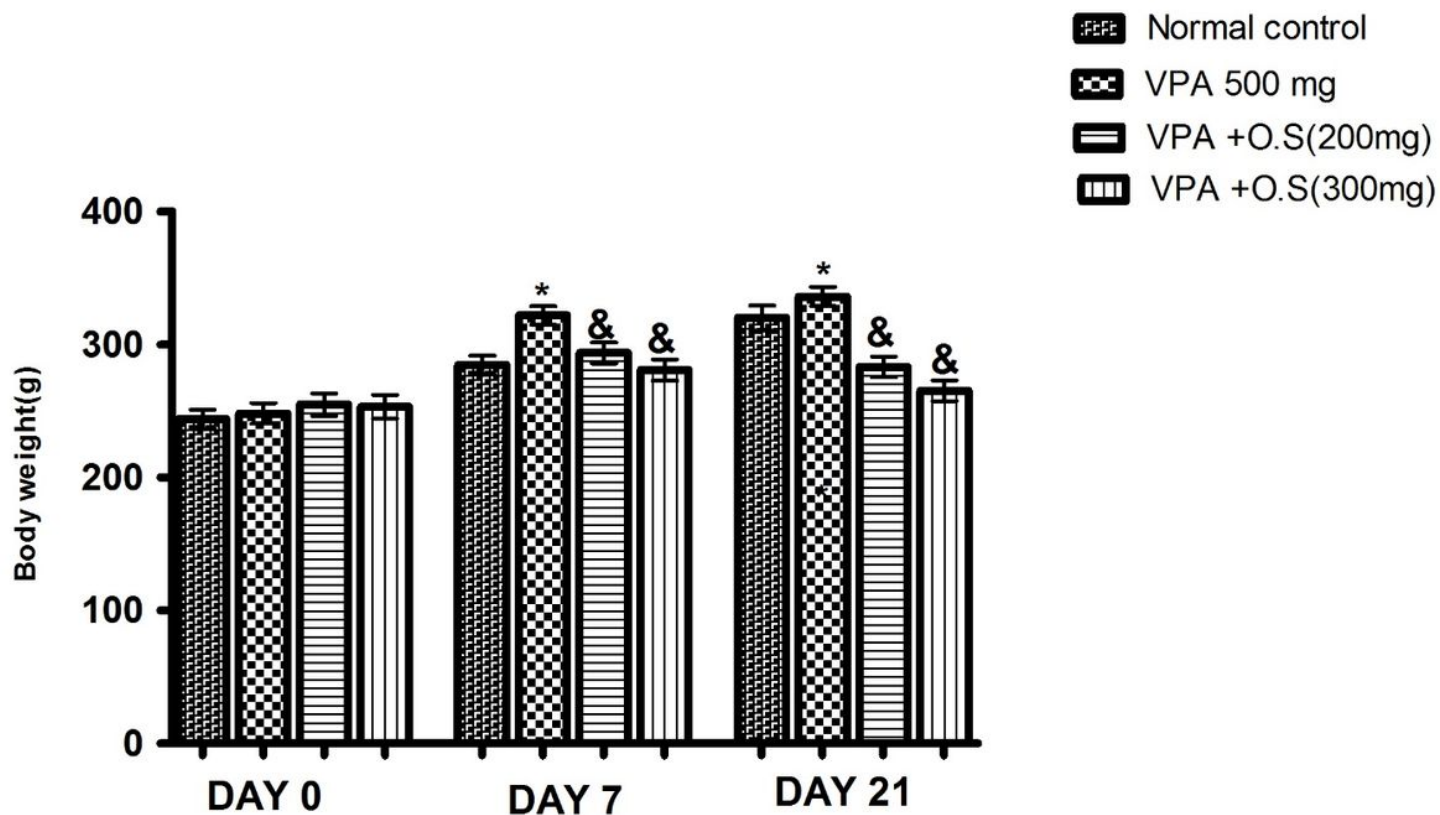


Figure 3

Body weight of normal control, VPA, VPA+O.S (200mg/kg), VPA+ O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($p < 0.05$). * indicates $p < 0.05$ vs normal. & indicates $p < 0.05$ vs VPA.

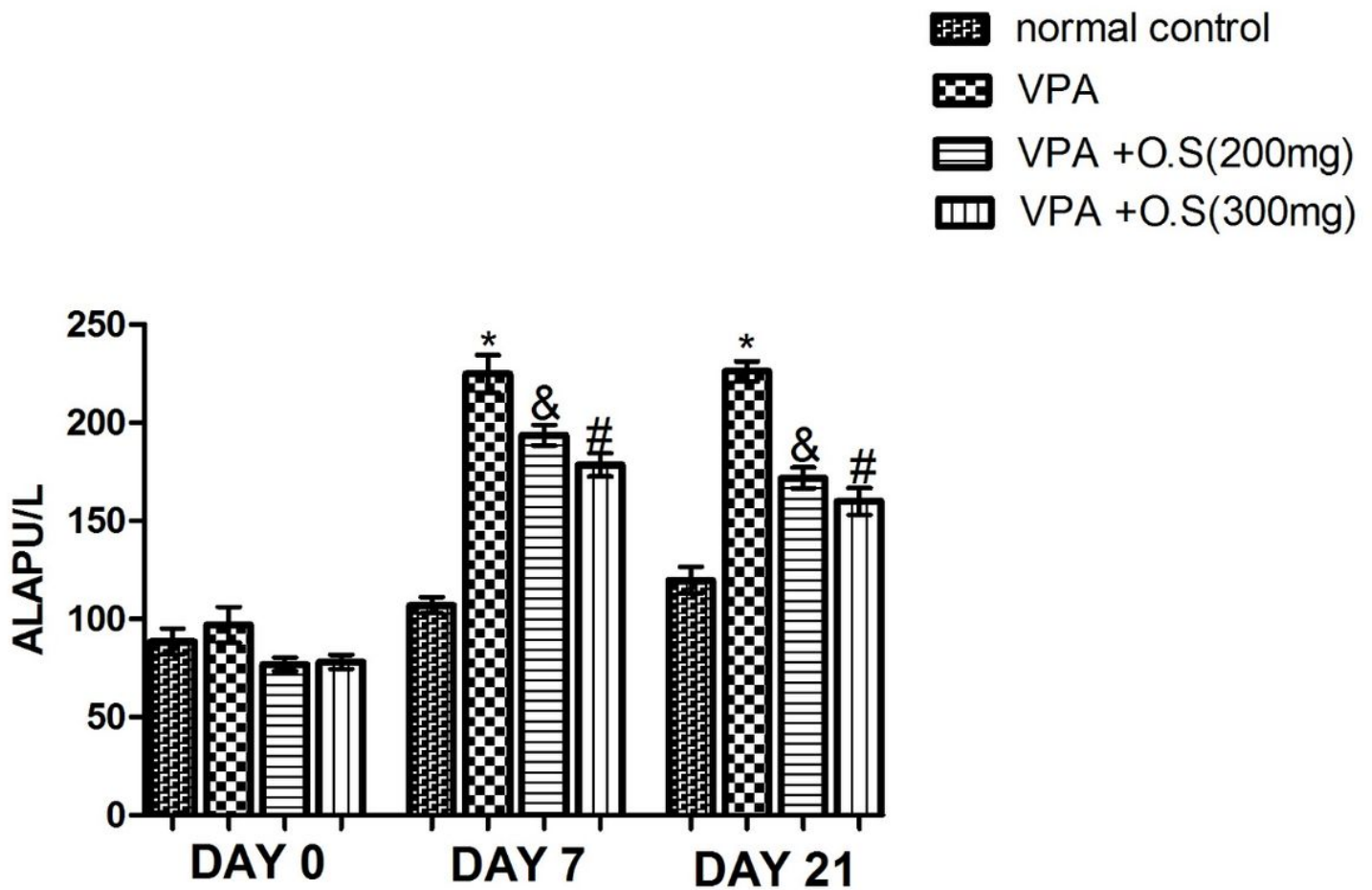


Figure 4

ALP of normal control, VPA, VPA+O.S (200mg/kg), VPA+ O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($p < 0.05$). * indicates $p < 0.05$ vs normal. & indicates $p < 0.05$ vs VPA. # indicates $p < 0.05$ vs VPA.

-  Normal control
-  VPA500 mg
-  VPA + O.S(200mg)
-  VPA + O.S(300mg)

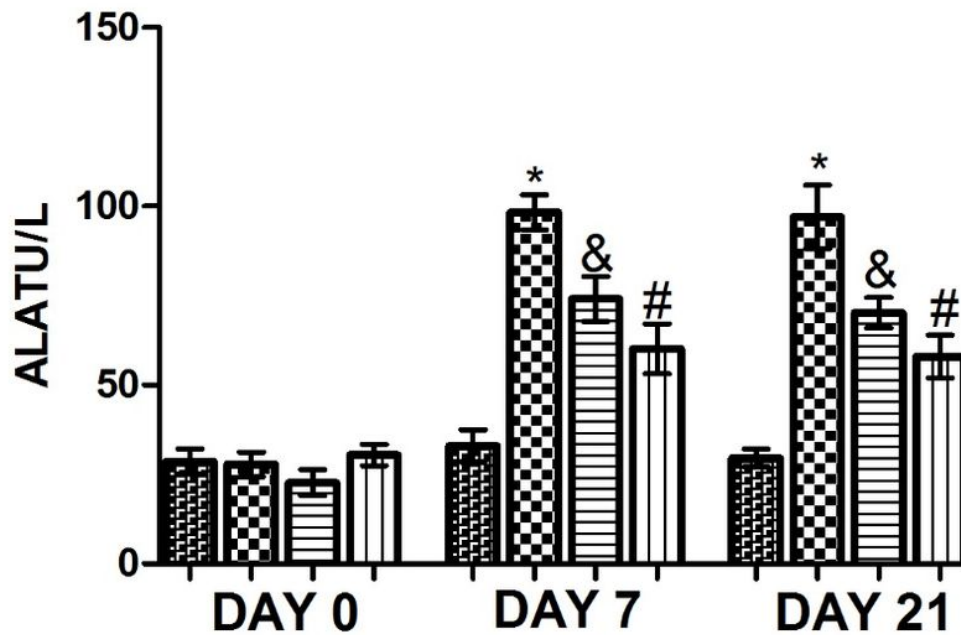


Figure 5

ALAT of normal control, VPA, VPA+O.S (200mg/kg), VPA+O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($P < 0.05$). *indicates $p < 0.05$ vs normal. &indicates $p < 0.05$ vs VPA. #indicates $p < 0.05$ vs VPA.

-  Normal control
-  VPA500 mg
-  VPA +O.S(200mg)
-  VPA +O.S(300mg)

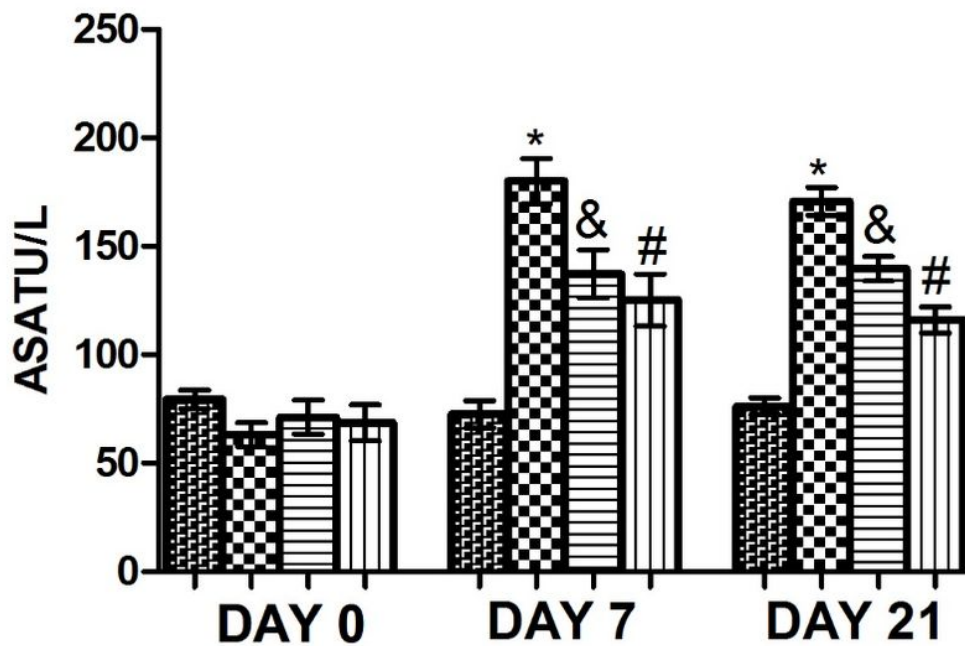


Figure 6

ASAT of normal control, VPA, VPA+O.S (200mg/kg), VPA+O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($P < 0.05$). *indicates $p < 0.05$ vs normal. &indicates $p < 0.05$ vs VPA. #indicates $p < 0.05$ vs VPA.

-  Normal control
-  VPA500 mg
-  VPA + O.S(200mg)
-  VPA + O.S(300mg)

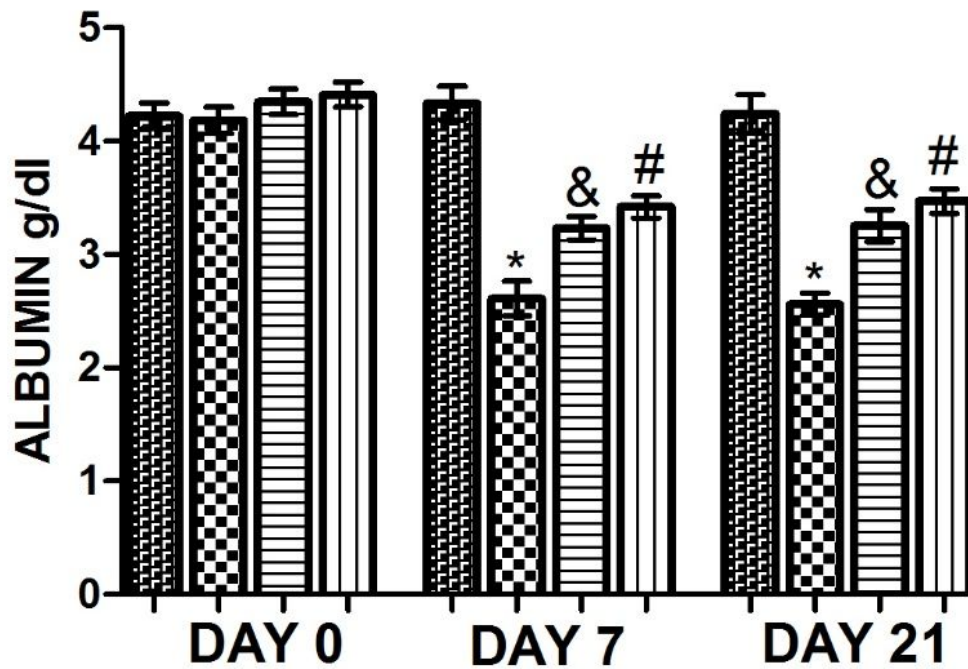


Figure 7

Albumin of normal control, VPA, VPA+O.S (200mg/kg), VPA + (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($P < 0.05$). *indicates $p < 0.05$ vs normal. &indicates $p < 0.05$ vs VPA. #indicates $p < 0.05$ vs VPA.

-  Normal control
-  VPA 500mg
-  VPA +O.S(200mg)
-  VPA +O.S(300mg)

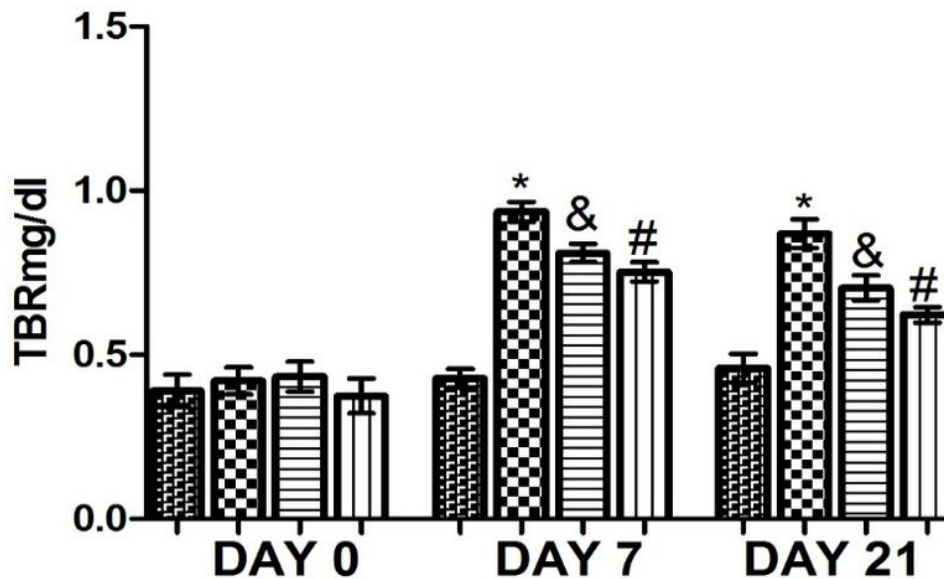


Figure 8

TBR of normal control, VPA, VPA+O.S (200mg/kg), VPA+O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for all gatherings in particular days. The results are considered significant ($P < 0.05$). *indicates $p < 0.05$ vs normal. &indicates $p < 0.05$ vs VPA. #indicates $p < 0.05$ vs VPA.

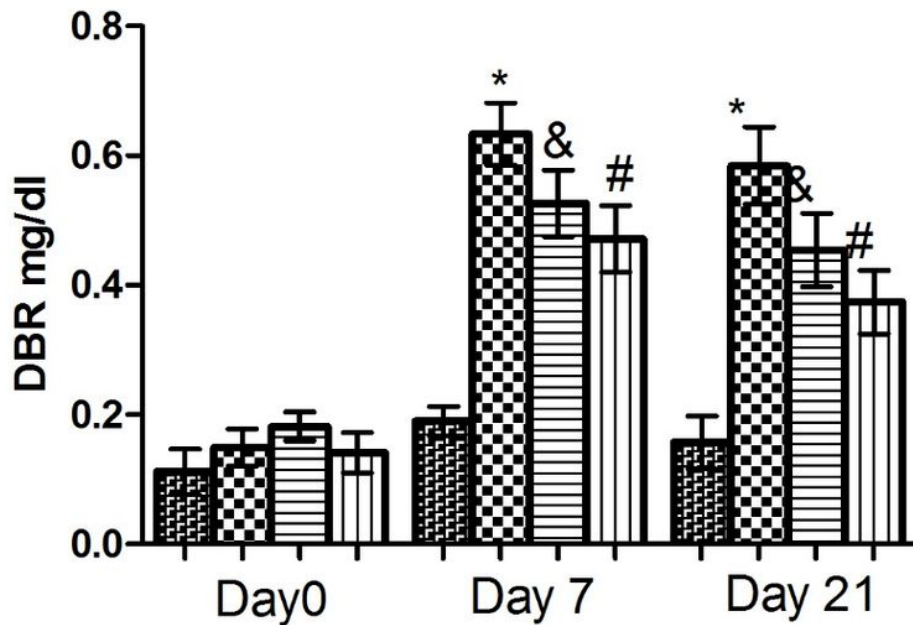
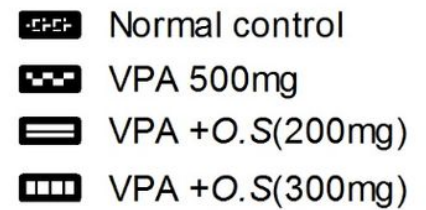


Figure 9

DBR of normal control, VPA, VPA+O.S (200mg/kg), VPA+O.S (300mg/kg). The values are mean $\pm$ SEM (n=6). Statistical analysis was done one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test for all gatherings in particular days. The results are considered significant ($P < 0.05$). *indicates $p < 0.05$ vs normal. &indicates $p < 0.05$ vs VPA. #indicates $p < 0.05$ vs VPA.