Hepatoprotective and antioxidant activity of methanolic extract *of vetiveria zizanioides* roots against paracetamol-induced liver damage in rats

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Abstract: To investigate the hepatoprotective and antioxidant activity of methanolic extract of *Vetiveria Zizanioides* roots (MEVZ) against paracetamol (3 g/kg, p.o. for 3 days) induced liver damage in rats. Animals were pretreated with MEVZ (300 and 500 mg/kg, p.o) 30 min prior to paracetamol ingestion for three days. The degree of protection was measured using levels of serum enzymes like Alanine and Aspartate aminotransferase (ALT & AST) alkaline phosphatase (ALP), total and direct bilirubin (TBL & DBL), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT) and total protein (TP). Also oxidative stress parameters such as levels of malondialdehyde (MDA), reduced glutathione (GSH) and activity of superoxide dismutase (SOD) and catalase (CAT) along with histopathological examination of liver sections was carried out to support the induction of liver damage and hepatoprotective activity. The substantially elevated serum enzyme levels of ALT, AST, ALP, TBL, DBL, GGT LDH and TP, also Oxidative stress parameters MDA, GSH levels and SOD, CAT activities were found to be restored towards normalization by MEVZ comparable with silymarin. Histopathological changes were in same direction supports finding of biochemical evidences of Hepatoprotection. MEVZ possess a highly promising antioxidant and hepatoprotective activity against paracetamol induced liver damage.
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1. Introduction

Liver is the vital organ of metabolism and excretion. Because of unique features, liver is also an important target of the toxicity of xenobiotics and oxidative stress [1]. Excessive consumptions of alcohol and viral infections, environmental pollution, hepatic viruses, parasitic infections, and chemotherapeutics are the most common factors known to cause liver damage in developed countries [2]. Drug induced liver damage is a potential complication of nearly every medication that is prescribed. Paracetamol is a well-known antipyretic and analgesic. Its hepatotoxicity is more common, caused by the reaction metabolite N-acetyl-p-benzoquinoneimine (NAPQI) [3].

Liver diseases are the biggest threat to the world which is characterized with impaired metabolic and secretary functions of liver clinically as jaundice, cirrhosis, hepatitis, liver cancer and ultimately liver failure [4]. About 20,000 deaths found every year due to liver disorders [5]. A common chronic disease known as liver fibrosis may lead to end-stage liver cirrhosis and liver cancer [6]. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50, 000 new cases each year [7].

There are many natural products such as plant and traditional herbal formulation available for the protective effect on liver against damage induced by hepatotoxin. More than 600 commercial herbal products with claimed hepatoprotective role are being sold in all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to show hepatoprotective role. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy [8].

Vetiveria zizanioides Linn. root (Commonly known as: Ushira, Family: Poaceae), is a perennial herb which found throughout the plains and lower hills of India, particularly on the river banks and in rich marshy soil. The plant used as digestive, carminative,

stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, antigout [9]. It possesses various pharmacological activities such as anthelmintic [10], antimicrobial [11], diuretic [12] and *in-vitro* antioxidant activity [13]. Recently, in our recent study *Vetiveria zizanioides* was demonstrated to possess hepatoprotective action against ethanol and carbon tetrachloride intoxication in rats [15]. Plant derived natural products such as phenolic compounds (flavonoids), terpenoids, steroids, glycosides, saponins, volatile oils etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [16-20]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against various diseases.

Silymarin, a standardized extract obtained from seeds of *Silybum marianum*, is widely used in treatment of liver diseases of varying origins [21]. Seeds of *S. marianum* have been shown to treat liver and gall bladder disorders, including hepatitis, cirrhosis and jaundice and to protect the liver against poisoning from chemicals, environmental toxins, snake bites, insect stings, mushroom poisoning and alcohols [22]. Due to its proven hepatoprotective and antioxidant properties, silymarin is being used in the current study as a standard agent for comparison with of methanolic extract of *V. zizanioides* [23].

However, no comprehensive evidence has yet been documented for the hepatoprotective activity of *V. zizanioides* against paracetamol induced liver damage. Keeping this in view, the present study was designed to evaluate the hepatoprotective and antioxidant activity of *V. zizanioides* and its putative mechanisms using paracetamol induced liver damage in rats.

2. Materials and methods

2.1. Plant material and extraction

The plant material *V. zizanioides* was collected from forest area of Jodhpur, Rajasthan, India in the month of July 2007. The plant was identified and authenticated by Dr A. S. Reddy, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India where a voucher specimen (No. MP-2**:** 28/7/07) was kept for future reference. The powder of *V. zizanioides* roots was defatted with petroleum ether (60-80°C), and cold extracted with methanol. The methanol crude extract (10.5 % yields) was obtained by evaporation using Rotavapour ® (BÜCHI, Switzerland) under reduced pressure. The dry methanol extract was stored in cool and dry place which further used for the evaluation of hepatoprotective activity. All the test and standard suspensions were prepared in the distilled water.

2.2. Experimental Animals

Studies were carried out using either sex Wistar albino rats (200-250 g). They were obtained from the animal house, Anand pharmacy college (APC), Anand, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 22 + 2°C), relative humidity (55 ± 5 %) with dark and light cycle (12/12 h).They were allowed free access to standard pellet diet (Amrut feed, Sangli, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. Animal studies were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conducted according to the regulations of Institutional Animal Ethics Committee (Protocol no. 7004).

2.3. Drugs and Chemicals

Silymarin was obtained as a gift sample from Micro labs, Bangalore, India. AST, ALT, ALP, Bilirubin kits were procured from Span Diagnostics, Surat, India. LDH, GGT kits were procured from Coral Clinical Systems, Goa, India. All other chemicals and reagents used were of analytical grade.

2.4. Acute Toxicity Studies

Rats were divided into three different groups (n = 6) and assigned either as vehicle (distilled water, p.o, 5 ml/kg), low and high dose of Methanolic extract of *V. zizanioides* (MEVZ) at 3 g/kg and 5 g/kg, p.o. respectively. The rats were not fed overnight prior to the treatments. After treatments, the rats were observed for toxicity symptoms and behavioural changes for a period of 48 hr. The observations continued up to day 14. Then, the rats were sacrificed after overnight fasting on day 15. Livers were excised for gross necropsy and histopathological examination. There was no lethality in any of the groups. One tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity, i.e., 300 & 500 mg/kg [24].

2.5. Experimental design

2.5.1. Paracetamol-induced liver damage in rats **[25]**

Wistar albino rats were randomly divided into five groups six of each: Group I (Normal control) was received distilled water, p.o, and Group II (Paracetamol control) was treated with paracetamol (3 g/kg/d, p.o.), for 3 days. Group III (Test-1) and Group IV (Test-2) were pretreated with MEVZ at a dose of 300, 500 mg/kg and Group V (Standard) silymarin at a dose 200 mg/kg/d, p.o., respectively 30 min prior to paracetamol ingestion for 3 days.

2.5.2. Effects of MEVZ on Serum enzymes

At the end of last treatment, blood was collected from retro-orbital plexus from all groups of rats after 24 h overnight fasting, on 4th day between 10.00 AM

and 11.00 AM. All blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 4 ºC for 15 min and used for the estimation of various serum enzymes like Alanine and Aspartate aminotransferase (ALT & AST) [26], alkaline phosphatase (ALP) [27], total and direct bilirubin (TBL & DBL) [28], lactate dehydrogenase (LDH) [29], gamma glutamyl transferase (GGT) [30] and total protein (TP) [31].

After collection of blood samples, the rats were sacrificed by light ether anesthesia and their livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A small 10% (w/v) portion of the Liver was homogenized in chilled Phosphate buffered saline (50 mM, pH 7.4) using a Potter Elvehjehm Teflon homogenizer. The homogenate obtained was centrifuged in a cooling centrifuge at 1,000×*g* for 10 min at 4°C to remove nuclei and unbroken cells. The pellet was discarded and portion of supernatant was again centrifuged at 12,000×*g* for 20 min at 4°C obtain a post-mitochondrial supernatant which was used for enzyme analysis [32]. The contents of malondialdehyde (MDA) [33], reduced glutathione (GSH) [34], Superoxide dismutase (SOD) [35] and Catalase (CAT) activity [36] were estimated spectrophotometrically using above post-mitochondrial supernatant.

2.5.3. Histopathological studies

A Small piece of liver were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. A transverse section of 5 µm was cut from each sample and stained with haematoxylin and eosin. Histopathological assessment (light microscopy) was performed on randomized sections of liver [37].

2.6. Statistical Analysis

The experimental results were expressed as Mean ± SEM for six animals in each group. All parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (DMCT) using Graph Pad prism 5.0 software [38]. Data were considered statistically significant at $P < 0.05$.

3. Results

3.1. Acute Toxicity Study

There was no morbidity and mortality observed throughout the study. *Vetiveria Zizanioides* was found not toxic to the experimental rats up to the high dose of 5 g/kg.

3.2. Effects of MEVZ on Serum enzyme levels in Different groups

Significantly increased levels of AST, ALT, ALP, TBL, DBL, LDH and GGT whereas decreased level of TP were observed in paracetamol control (3 g/kg, p.o. for 3 days) compared to normal control. Treatments with MEVZ (Test-1 & Test-2) received 300 and 500 mg/kg, p.o. as well as standard (silymarin) received 200 mg/kg, p.o. for 3 days caused significant reduction in above serum biomarkers and significantly enhanced the level of TP (Table 1).

Table 1: Level of serum enzyme in different groups

Group	AST (IU/L)	ALT (IU/L)	ALP (KAU/dl)	TBL (mg/dl)	DBL (mg/dl)	LDH (U/L)	GGT (U/L)	TP (mg/ml)
I-Normal control	37.2±2.5	24.6±2.1	9.2±0.3	0.7 ±0.05	0.4±0.1	325.6±9.3	18.8±0.9	10.6±0.05
II-Paracetamol control	170.4±6.4 ^{a}***	120.0±9.6 ^{a}***	59.2±0.8 ^{a}**	11.5 ±3.2 ^{a}*	6.6±0.8 ^{a}**	1766.2±12.9 ^{a}***	89.8±2.0 ^{a}**	4.4±0.04^{a}**
III-Test-1	123.17±2.62 ^{b}*	80.58±2.10 ^{b}*	44.13± 0.15 ^{b}*	3.39±0.09 ^{b}*	1.88 ±0.08 ^{b}*	1163.26±5.19 ^{b}*	65.74±1.68 ^{b}*	9.62±0.06 ^{b}*
IV-Test-2	92.33 ±3.48 ^{b}*	59.75±2.68 ^{b}*	43.08± 0.24 ^{b}*	1.64±0.09 ^{b}*	1.35 ±0.37 ^{b}*	933.34±15.60 ^{b}*	57.96±1.64 ^{b}*	8.95±0.06 ^{b}*
V-Standard	48.6±1.5 ^{b}**	39.0±1.4 ^{b}**	11.0±0.04 ^{b}**	1.3±0.1 ^{b}**	1.2± 0.1 ^{b}**	471.2±6.5 ^{b}**	37.4±2.2 ^{b}**	10.2±0.03^{b}**

Values are expressed as mean ± SEM for six rats in each group. [a] Different from normal control, [b] Different from paracetamol control (*p< 0.05, **p< 0.01, ***p< 0.001).

3.3. Effects of MEVZ on Oxidative stress parameters in Different groups.

Oxidative stress parameters of post mitochondrial supernatant were measured. A significant ($P < 0.01$) increase in MDA while reductions in GSH levels, SOD and CAT activities were found in paracetamol control as compared to normal control. Treatments with MEVZ (Test-1 & Test-2) and standard (silymarin) exhibited significant decrease in MDA levels while significant increase in GSH level, SOD and CAT activity as compared to paracetamol control (Table 2).

Table 2: Level of oxidative stress parameters in different groups

Groups	GSH (nM/mg protein)	MDA (nM/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
I-Normal control	11.12±0.1	2.4±0.002	1.62 ±0.01	24.87 ± 1.8
II -Paracetamol control	8.40±0.1 [a]**	16.8±0.01 [a]**	0.50 ±0.01 [a]**	5.74 ±1.3 [a]**
III-Test-1	9.80±0.07 [b]*	4.6±0.06 [b]*	1.57 ±0.16 [b]*	11.59 ± 0.3 [b]*
IV-Test-2	10.68±0.15 [b]*	5.0±0.01 [b]*	1.63± 0.07 [b]*	15.80 ± 1.5 [b]*
V-Standard	10.48±0.4 [b]**	4.3±0.03 [b]*	1.67± 0.14 [b]**	24.52 ± 3.5 [b]**

Values are expressed as mean ± SEM for six rats in each group. [a] Different from normal control, [b] Different from paracetamol control (*p< 0.05, **p< 0.01).

3.4. Liver histopathology

Histopathological examination of normal control animals revealed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig 1A). In paracetamol control, there was a severe disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation (Fig 1B). Treatment with MEVZ (Test-1 & Test-2) and silymarin showed evidence of preservation of normal structure & architecture of hepatocytes (Fig 1C, 1D and 1E).

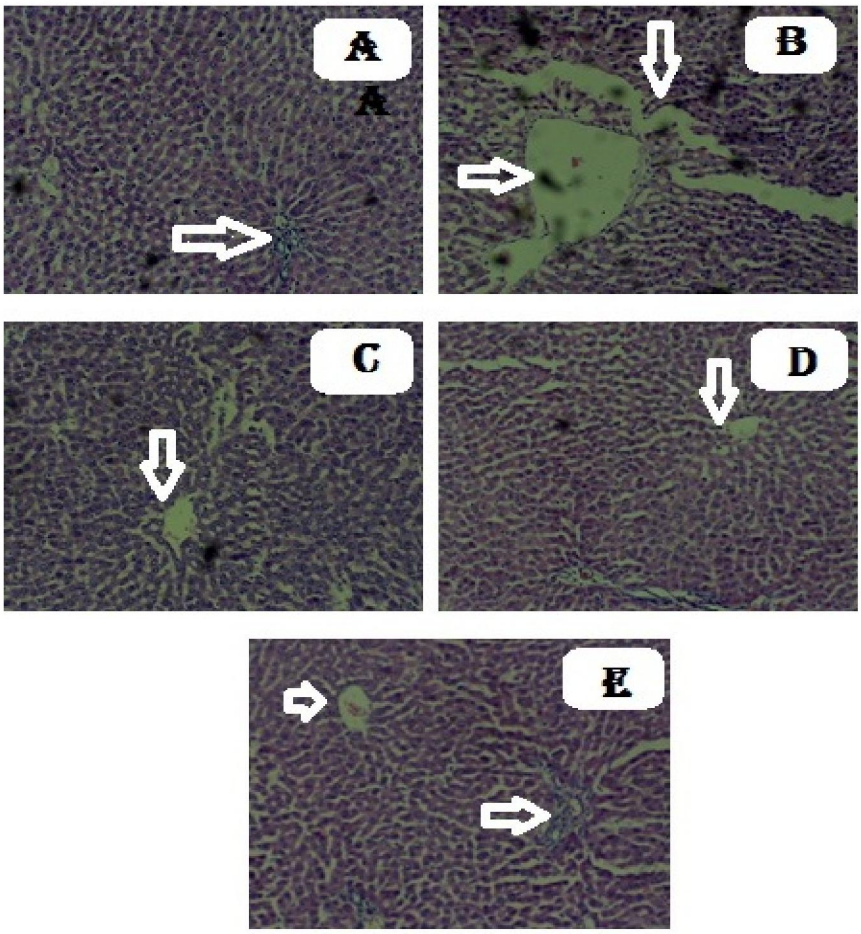

Figure 1: Histological examination of liver tissue section in different groups

4. Discussion

Protection against paracetamol-induced liver damage has been taken as a test for potential hepatoprotective agent by several investigators [39-40]. Paracetamol is a common antipyretic agent which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses [41-42]. Hepatotoxicity of paracetamol has been attributed to the formation of highly reactive toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) [43-44].

The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver [45], because these are cytoplasmic in location and are released into circulation after cellular damage [46]. Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP, which are enzymes originally present in higher concentration in cytoplasm [47], when there is hepatopathy, these enzymes leak into blood stream in confirmity with the extent of liver damage [48]. ALT is frequently included in biochemical profiles for the purpose of assessing hepatic injury [49]. The elevation of ALP indicates the disturbed excretory function of liver [50]. GGT and ALP are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. The elevation of serum GGT concentrations is regarded as one of the most sensitive indices of hepatic damage [51]. LDH is catalyses the conversion of lactate to pyruvate using NAD^+ as coenzyme of NAD [52]. The increase in LDH activity in serum may be due to leakage of the enzyme from the tissues into the blood on account of cellular injury. An elevation in the levels of the serum marker enzymes in generally regarded as one of the most sensitive index of the hepatic damage [53]. In the present study administration of paracetamol treated rats showed an increase in the levels of AST, ALT, ALP, GGT and LDH when compared with control rats. Oral administration of MEVZ (Test-1 & Test-2) and silymarin to paracetamol treated rats showed an inhibition in the elevated levels of serum AST, ALT, ALP, LDH and GGT levels than paracetamol alone treated rats.

Bilirubin is well known metabolic breakdown product of blood heme with great biological and diagnostic values. Bilirubin is one of the most clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. In the present study paracetamol hepatotoxic rats showed a significant increase in the level of serum TBL and DBL when compared with control rats. Oral administration of MEVZ (Test-1 & Test-2) and silymarin to paracetamol treated rats showed an inhibition in the elevated levels of serum TBL and DBL than paracetamol alone treated rats. This decrease in serum bilirubin after treatment with these extracts in liver damage induced by paracetamol, indicated the effectiveness of these extracts in normal functional status of the liver.

Proteins are important organic constituents of the animal cells playing a vital role in the process of interactions between intra and extra cellular media. Being a part of cell membrane and as an enzyme, protein participates the intricately balanced sub cellular fractions. The depletion in the protein levels might be because of their metabolism to liberate energy during paracetomol toxicity. Protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants, which enter into the animal body [54]. In the present study administration of paracetamol treated rats showed decrease in the level of TP when compared with control rats. Oral administration of MEVZ (Test-1 & Test-2) and silymarin to paracetamol treated rats showed an increase the level of serum TP than paracetamol alone treated rats.

NAPQI is initially detoxified by conjugation with GSH to form mercapturic acid [55]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of proteins and alters the homeostasis of calcium after depleting GSH. The non enzymic antioxidant, GSH is one of the most abundant tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical (FR) species such as hydrogen peroxide (H_2O_2), super oxide radicals (O^-_2) and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) and glutathione –s-transferase (GST) [56]. In the present study, the decreased level of GSH has been associated with elevations in the levels of end products (MDA) of LPO in paracetamol control. The increase in MDA level in liver suggests enhanced LPO leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive FR. Treatment with MEVZ (Test-1 & Test-2) and silymarin significantly reverse the above changes.

SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system [57]. Catalase is an enzymatic antioxidant widely distributed in all animal tissue and the highest activity is found in the red cells and in liver [58]. SOD offers protection form highly reactive O^-_2 and converts them to form H_2O_2 and O_2. Because H_2O_2 is still harmful to cells, CAT and GPx further catalyse the decomposition of H_2O_2 to H_2O [59]. Thus, the coordinate actions of various cellular antioxidants in mammalian cells are critical for effectively detoxifying free radicals. Decrease in activity of SOD and CAT is a sensitive enzymatic index in liver damage, might reduce protection against free radicals. In the present study, it was observed that treatment with MEVZ (Test-1 & Test-2) and silymarin caused a significant increased in the hepatic SOD and CAT activity which were decreased in paracetamol control. This show MEVZ prevents the accumulation of excessive FR that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme. The hepatoprotective effect MEVZ (Test-1 & Test-2) was further substantiated by histopathological assessment in comparison with paracetamol control.

Preliminary phytochemical investigations of MEVZ revealed the presence of phenolic compounds (flavanoid), saponins, glycosides, volatile oils and terpenoids which may be responsible for the protective effect on paracetamol-induced liver damage in rats, probably by its antioxidative effect on liver by eliminating the deleterious effects of toxic metabolites of paracetamol.

In conclusion, the acute toxicity study showed that *V. zizanioides was* not toxic to the experimental rats up to an oral dose of 5 g/kg body weight. Furthermore, the results of this study demonstrate that MEVZ has a hepatoprotective and antioxidant activity upon paracetamol-induced liver damage in rats comparable to the effects of silymarin, a standard drug used to treat liver diseases. Further, investigation is underway to determine the exact phytoconstituents that is responsible for its hepatoprotective activity. These results can be useful as a starting point of view for further applications of this plant or its constituents in pharmaceutical preparations after performing clinical researches.

Authors Contributions

MP and TG have performed experimental designed, literature search and animal treatment. MP and PS have carried out biochemical and statistical analysis as well as interpretation of the data. MP and VT participated in histopathological investigation. MP, PS and TG involve in writing of the manuscript. MP, AA and SR have review and edited manuscript.

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Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Huo HZ, Wang B, Liang YK, Bao YY, Gu Y. Hepatoprotective and Antioxidant Effects of *Licorice* Extract against CCl_4-Induced Oxidative Damage in Rats. *Int J Mol Sci* 2011; **12** (10): 6529-6543.

[2] Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of *Orthosiphon stamineus* extract on thioacetamide-induced liver cirrhosis in rats. *Evid Based Compl Alt Med* 2011; 1-6.

[3] Shah VV, Deval K. Hepatoprotective activity of leaves of *Parkinsonia aculeata* linn against paracetamol induced hepatotoxicity in rats. *Int J Pharmacol* 2011; **1** (2): 59-66.

[4] Goyal R, Ravishankar B, Shukla VJ, Singh M. Hepatoprotective Activity of *Rohitaka ghrita* against Paracetamol Induced Liver Injury in Rat. *Pharmacologia* 2012; **3** (7): 227-232.

[5] Sharma B, Sharma UK. Hepatoprotective activity of some indigenous plants. *Int J Pharm Tech Res* 2010; **2** (1): 568-572.

[6] Ao ZH, Xu ZH, Lu ZM, Xu HY, Zhang XM, Dou WF. Niuchangchih (*Antrodia camphorata*) and its potential in treating liver diseases. *J Ethnopharmacol* 2009; **121** (2): 194-212.

[7] Meganathan M, Madhana MG, Sasikala P, Mohan J, Gowdhaman N, Balamurugan K. Evaluation of antioxidant effect of Omega 3-fatty acid against paracetamol induced liver injury in albino rats. *Global J Pharmacol* 2011; **5** (1): 50-53.

[8] Nabavi SM, Hajizadeh Moghaddam A, Fazli M, Bigdellou R, Mohammadzadeh S, Nabavi SF, Ebrahimzadeh MA. Hepatoprotective activity of *Allium paradoxum. Europ Rev Med Pharmacol Sci* 2012; **16** (3): 43-46.

[9] Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants used in Ayurveda, Edn 1, Vol. 5, CCRAS, New Delhi, 2002, 445-477.

[10] Gilbert B. Anthelmintic activity of essential oils and their chemical components. *An Acad Bras Cienc* 1992; **44**: 423-428.

[11] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol* 1999; **86**: 985-990.

[12] Rao RC, Serradeil-Le GC, Granger I, Gleye J, Augereau JM, Bessibes C. Khusimol, a non peptide ligand for vasopressin VI a receptors, *J Nat Prod* 1994; **57**: 1329-1335.

[13] Kim HJ, Chen F, Wang X, Chung HY, Jin Z. Evaluation of antioxidant activity of vetiver (*Vetiveria zizanioides L.*) oil and identification of its antioxidant constituents, *J Agric Food Chem* 2005; **53**: 7691-7695.

[14] Parmar MY, Shah PA, Thakkar VT, Gandhi TR. Hepatoprotective activity of *V. zizanioides* Linn. against ethanol-induced liver damage in rats, *Pharmacog Mag* 2008; **4** (16): S 216-221.

[15] Parmar MY, Shah PA, Thakkar VT, Salim R, Gandhi TR. Hepatoprotective potential of methanolic extract of *Vetiveria zizanioides* roots against carbon tetrachloride induced Acute liver damage in rats. *Digest J Nanomat Biostr* 2013; **8** (2): 835-844.

[16] Defeudis FV, Papadopoulos V, Drieu K. *Ginkgo biloba* extracts and cancer: a research area in its infancy. *Fundam Clin Pharmacol* 2003; **17**: 405-417.

[17] Takeoka GR, Dao LT. Antioxidant constituent of almond [*Prunus dulcis* (Mill.) Webb DA] Hulls. *J Agric Food Chem* 2003; **51**: 496-501.

[18] Quyang MA, He ZD, Wu CL. Antioxidative activity of glycosides from *Ligustrum sinense*, *Nat Prod Res* 2003; **17**: 381.

[19] Banskota AH, Tezuka Y, Adnyana IK, Xiong Q, Hase K, Tran KQ. Hepatoprotective effect of *Commbretum quadrangulare* and its constituents. Biol Pharm Bull 2000; **23**: 456-460.

[20] Yohikawa M, Morikawa T, Kashima Y, Ninomiya K, Matsuda H. Structure of New dammarane type triterpene saponins from the flower buds of *Panax notoginseng* and hepatoprotective

effects of principal ginseng saponins. *J Nat Prod* 2003; **66**: 922. 2003.
[21] Janbaz KH, Gilani AH. Studies on preventive and curative effects of berberine on chemical induced hepatotoxicity in rodents. *Fitoter* 2000; **71**: 25.
[22] Gilani AH, Janbazz KH, Preventive and curative effects of *Artemisia absinthium* on paracetamol and CCl_4-induced hepatotoxicity. *Gen Pharmacol* 1995; **26**: 309-315.
[23] El-Samaligy MS, Afifi NN, Mahmoud EA. Evaluation of hybrid liposomes-encapsulated silymarin regarding physical stability and in vivo performance. Int J Pharm 2006; **319**:121-129.
[24] Organization for Economic Co-operation and Development, "Guidelines on acute oral toxicity, No-425". Revised Document. October 2001.
[25] Kumar SV, Sujatha C, Syamala J, Nagasudha B, Mishra SH. Protective effect of root extract of *Operculina turpethum* linn. against paracetamol-induced hepatotoxicity in rats. *Ind J Pharm Sci* 2006; **68** (1): 32-35, (Modified).
[26] Bergmeyer HU, Scelibe P, Wahlefeld AW. Optimization of methods for aspirate aminotransferase and alanine aminotransferase. *Clin Chem* 1978*;* **24**: 58-61.
[27] King J. The hydrolases acid and alkaline phosphatase. *Pract Clin Enz*. Van, D (ed.), London: Nostrand company Ltd, pp. 191-208, 1965.
[28] Malloy HJ, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem* 1937*;* 119: 481.
[29] Amador E, Dorfman LE, Wacker WE. Serum lactic dehydrogenase activity: an analytical assessment of current assays. *Clin Chem* 1963; 9: 391.
[30] Szasz G, Persyn JP. New substrates for measuring G-glutamyl transpeptidases activity. *Clin Chem and Clin Biohem* 1974; 12: 228.
[31] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193** (1): 265-275.
[32] Singh KS, Kumar VR, Rajat S. N-acetylcysteine inhibits hyperglycemia-induced oxidative stress and apoptosis markers in diabetic neuropathy. *J Neurochem* 2010, **112** (1): 77-91.
[33] Ohkawa H, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal Biol Chem* 1979, **95**: 351-358.
[34] Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophy* 1959; **82** (1): 70-77.
[35] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophy* 1984; **21**: 131-132.
[36] Aebi H. Catalase In: Bergmeyer, editor, Mehtods in enzymatic analysi. New York, 1974; **2**: 674-684.
[37] Galighor AE, Kozloff EN. In Essentials of practical micro techniques. 2nd ed. Lea and Febiger, Philadelphia, 1971: 77-210.
[38] Bolton S, Bon C. Pharmaceutical statistics: Practical and clinical applications, 4th ed. Marcel Dekker, Inc. New York, 2004: 229-230.
[39] Dwivedi Y, Rastogi R, Garg NK, Dhawan BN. Prevention of paracetamol-induced hepatic damage in rats by Picroliv, the standardized active fraction from *Picrorhiza kurroa*. *Phytotherapy Research* 1991; **5** (3): 115-119.
[40] Shukla B, Visen PK, Patnaik GK, Dhawan BN. Choleretic effect of *andrographolide* in rats and guinea pigs. *Planta Medica* 1992; **58** (2): 146-149.
[41] Mitchell JR, Jollow DJ, Potter WZ, Gillettee JR, Brodie. Acetaminophen-induced hepatic necrosis, Role of drug metabolism. *J Pharmacol ExpTher* 1973; 187: 185-194.
[42] Prescott LF, Wright N, Roscoe P, Brown SS. Plasma paracetamol half life and hepatic necrosis in patients with paracetamol over dosage. *Lancet* 1971; **297** (7698): 519-522.
[43] Savides MC, Oehme FW. Acetaminophen and its toxicity'. *J Appl Toxicol* 1982; **3** (2): 95-111.
[44] Vermeulen NPE, Bessems JGM, Vande R. Molecular aspects of paracetamol-induced hepatotoxicity and it mechanism based prevention. *Drug Metabol Rev* 1992; **24**: 367-407.
[45] Chenoweth MB, Hake CL. The smaller halogenated aliphatic hydrocarbons. *Ann Rev Pharmacol* 1962; **2**:363-398.
[46] Sallie R, Tredger JM, William R. Drug and liver. *Biopharmaceutical Drug Disposition* 1991; **12**: 251-259.
[47] Wells FE. Tests in liver and biliary tract disease. In: Gowenlock, H.A. eds. Varley's Practical Clinical Biochemistry. CRC Press, Florida. 1988.
[48] Venukumar MR, Latha MS. Effect of *Coscinium fenestratum* on hepatotoxicity in rats. *Indian J Exp Biol* 2004; **42**:792–797
[49] Willianson EM, Okpako DT, Evans FJ. England (Chichester): John Wiley and Sons Ltd. Selection, Preparation and Pharmacological Evaluation of Plant Material 1996; 131–54.
[50] Kothavade RJ, Joylekar SN, Barodavalla SA. Protective effect of indigenous drug livomyn on Ketoconazole induced hepatotoxicity. *Indian J Pharm Sci* 1996; **58**: 142-146.
[51] Szczeklik E, Orlowski M, Szewes A. Serum gamma glutamyl peptidase activity in liver disease. *Gastroenterol* 1961; **41**: 353-359.
[52] Burtis LA, Ashwood ER. *Textbook for Clinical Chemistry*. W.B. Saunders Company, Philadelphia, Pennsylvania 1986.
[53] Kapil A, Suri OP, Koul IB. Antihepatotoxic effects of chorogenic acid from *Anthrocephalus cadamba*. Phytother Res 1995; **9**:189-193.
[54] Ramasamy R. Effects of sevin on blood free amino acids level of the fish sarotherodon mossambicus. *Environ Ecol* 1987; **5**: 633-637.
[55] Moore M, Thor H, Moore G, Nelson S, Moldeus P, Orrenius S. The toxicity of acetaminophen and N-acetyl P-benzoquinone-imine in isolated hepatocytes is associated with thio depletion and increased cystosolic Ca^{2+}. *J Biol Chem* 1985; **260**: 13035-13040.
[56] Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S. Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumors in Swiss albino mice. *Phytother Res* 2001; **15** (3): 240-244.
[57] Curtis SJ, Moritz M, Snodgrass PJ. Serum enzymes derived from liver cell fraction and the response to carbon tetrachloride intoxication in rats. *Gastroenterol* 1972; **62** (1): 84-92.
[58] Chance B, Green DS, Stein, Roughton RJW. The mechanism of catalase action steady state analysis. *Arch Biochem Biophy* 1952; **37**: 301-339.
[59] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annl Rev Pharmacol and Toxicol* 2005; **45**: 51–88.

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