ISSN: 2640-4133

Research Article

Advances in Bioengineering & Biomedical Science Research

Hepatoprotective Activity of *Amomum Subulatum* (Roxb.) Seeds against Paracetamol-Induced Liver Damage in Rats

Mihir Y Parmar[1,2*], Purvi A Shah[2], Vaishali T Thakkar[2] and Tejal R Gandhi[2]

[1]*Professor, Department of Pharmacology, Bharat Institute of Technology, Mangalpally, Jawaharlal Nehru Technological University, India*

[2]*Anand Pharmacy College, Shree Ram Krishna Seva Mandal, Opp. Town Hall, Sardar Patel University, India*

[*]Corresponding author
Mihir Y Parmar, Professor, Department of Pharmacology, Bharat Institute of Technology, Mangalpally, Jawaharlal Nehru Technological University, India, Tel: 9638330050; E-mail: mihirparmar4uonly@yahoo.com

Submitted: 06 Mar 2019; **Accepted**: 14 Mar 2019; **Published**: 21 Mar 2019

Abstract

Hepatoprotective activity of methanolic extract of Amomum subulatum seeds (MEAS) was evaluated against paracetamol (3 g/kg, p.o. for 3 days) induced liver damage in rats. The degree of protection was measured using serum biomarkers like Alanine and Aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin, lactate dehydrogenase, gamma glutamyl transferase and total protein; oxidative stress parameters such as malondialdehyde, glutathione, super oxide dismutase and catalase along with histopathology. Significant higher levels of above serum biomarkers, whereas lower level of total protein in paracetamol control as compared to normal control was observed. Treatments with MEAS and silymarin caused significant reversal in above biomarkers which was altered by paracetamol. Similarly, significant increase in MDA while decrease in GSH level, SOD and CAT activities in paracetamol control as compared to normal control. Treatment with MEAS and silymarin brings these stress parameters changes significantly towards normal level as compared to paracetamol control. Histopathological changes were in the same direction supports finding of above biomarkers and stress parameters. These results suggest that MEAS may have potential therapeutic value to reduce the severity of paracetamol induced liver damage, probably by its antioxidative effect on liver by eliminating the deleterious effects of toxic metabolites of paracetamol.

Keywords: *Amomum subulatum*, Hepatoprotection, Oxidative stress, Paracetamol

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].

Amomum subulatum Roxb. (Commonly known as Greater cardamom, Family: Zingiberaceae) is a perennial herb which grows widely in moist tropical countries [9]. 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 [10-15]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against various diseases. In our previous study we already reported hepatoprotective activity of *Amomum subulatum* against ethanol and Carbon tetrachloride induced liver damage in rats [16-17]. However, no comprehensive evidence has yet been documented for the hepatoprotective activity of *Amomum subulatum* against paracetamol induced liver damage. Keeping this in view, the present study was designed to evaluate the protective effect of *Amomum subulatum* and its putative mechanisms using paracetamol induced liver damage in rats.

Materials and Methods

Plant Material and Extraction

Amomum subulatum fruits were purchased from a local market of Anand, India. The fruits were identified and authenticated by Dr. AS 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 seeds were dried at room temperature and mechanically powdered to obtain a coarse powder; defatted with petroleum ether (60-80°C) and Soxhlet extracted with methanol. The solvent was evaporated in vacuo to give a dark brown semisolid residue (yield 9.5% w/w). The dry methanol extract was stored in cool and dry place which further used for the evaluation of hepatoprotective and antioxidant activity. All the test and standard suspensions were prepared in the distilled water.

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).

Drugs and Chemicals

Paracetamol was purchased from S.D. Fine Chem. Ltd, Mumbai. 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.

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-dose MEAS (1 g/kg, p.o), and high-dose MEAS (3 g/kg, p.o). 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 and kidneys 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 and antioxidant activity, i.e., 100 and 300 mg/kg [18-19].

Experimental Design

Paracetamol-Induced Liver Damage in Rats [20]

Wistar albino rats were randomly divided into five groups six of each: Group I (Normal control) received distilled water, p.o, and Group II (Paracetamol control) received paracetamol (3 g/kg, p.o.), for 3 days. Group III (Test-1) received MEAS (100 mg/kg, p.o.); Group IV (Test-2) received MEAS (300 mg/kg, p.o.) And Group V (Standard) received silymarin (200 mg/kg, p.o.) followed by a dose of paracetamol (3 g/kg, p.o.) for 3 days.

Effects of MEAS on Biochemical Parameters

At the end of treatment, blood was collected from retro-orbital plexus of overnight fasted rats and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 4ºC for 15 min and used for the estimation of various serum biomarkers 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) [21-26].

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 [27]. The contents of malondialdehyde (MDA), reduced glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) activity were estimated spectrophotometrically using above post-mitochondrial supernatant [28-31].

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 [32].

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 [33]. Data were considered statistically significant at $P < 0.05$.

Results

Acute Toxicity Study

There was no morbidity and mortality observed throughout the study. *Amomum subulatum* was found not toxic to the experimental rats up to the high dose of 3 g/kg.

Effects of MEAS on Body Weight, Liver Weight and Food Utilization Rate of Different Groups

The initial and final body weight and liver weight of the rats after three day of different treatments are shown in Table 1. Overall, there were no significant differences in body weight between the rats in the different groups. However, the rats treated with paracetamol showed significant ($P < 0.01$) higher liver weight compared to normal control group. Treatments with MEAS (Test-1 & Test-2) received 100 and 300 mg/kg, p.o. as well as standard (silymarin) received 200 mg/kg, p.o significantly ($P < 0.01$) lower the liver weight compared to paracetamol control. There was no significant difference in average body weight gain and food utilization rate between different groups (Table 1).

Table 1: Average body weight gain, food utilization rate and liver weight in different groups

Group	Initial body weight (g)	Final body weight (g)	Average body weight gain (g)	Food utilization rate (%)	Liver weight (g)
I-Normal control	236.6 ± 29.9	246.5 ± 30.1	9.8 ± 5.1	14.7 ± 3.7	10.4±0.6
II-Paracetamol control	241.7 ± 35.3	250.9 ± 28.9	9.3 ± 5.3	13.9± 2.2	11.1±0.6 a **
III-Test-1	238.8 ± 27.4	248.6 ± 30.6	10.0 ± 4.8	13.5 ± 2.9	9.8±1.1 b***
IV-Test-2	239.5 ± 26.2	248.2 ± 32.8	9.8 ± 5.2	15.0 ± 3.1	10.1±0.9 b**
V-Standard	240.5 ± 31.1	250.1 ± 30.7	10.1 ± 4.5	14.1 ± 3.1	9.9±0.7 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).

Effects of MEAS on Serum Biomarkers 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 MEAS (Test-1 & Test-2) received 100 and 300 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 2).

Table 2: Level of serum biomarkers 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	0.4±0.1	0.7 ±0.05	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**	6.6±0.8 a**	11.5 ±3.2 a*	1766.2±12.9 a***	89.8±2.0 a**	4.4±0.04 a **
III-Test-1	69.1±3.1 b**	54.00 ±1.6 b**	13.5± b*	1.7±0.2 b**	2.0±0.2 b**	692.7±22.9 b**	53.2±1.08 b**	8.9± 0.04 b*
IV-Test-2	58.1±2.0 b**	46.2±1.4 b**	13.2±0.3 b*	1.3±0.1 b**	1.2±0.1 b**	634.3±16.2 b**	49.7±1.7 b**	8.1± 0.4 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).

Effects of MEAS on Oxidative Stress Parameters in Different Groups

Similarly, 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 MEAS (Test-1 & Test-2) and standard (silymarin) exhibited significant decrease in MDA levels by 80.56%, 76.39% and 86.81% while significant increase in GSH level by 115.44%, 166.54% and 76.47% respectively as compared to paracetamol control. Also a significant increase in SOD activity by 99.55%, 103.14% and 104.93%; CAT activity by 43.02%, 92.37% and 98.17% when treated with MEAS (Test-1 & Test-2) and standard (silymarin) respectively (Table 3).

Table 3: 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	11.54±0.3 b**	5.2±0.02 b*	1.61±0.01 b**	13.97±0.6 b*
IV-Test-2	12.93±0.6 b**	5.8±0.04 b*	1.65±0.01 b**	23.41±2.1 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, ***p< 0.001).

Liver Histopathology

Histopathological examination of normal control animals revealed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 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 (Figure 1B). Treatment with MEAS (Test-1 & Test-2) and silymarin showed evidence of preservation of normal structure and architecture of hepatocytes (Figure 1C, 1D and 1E).

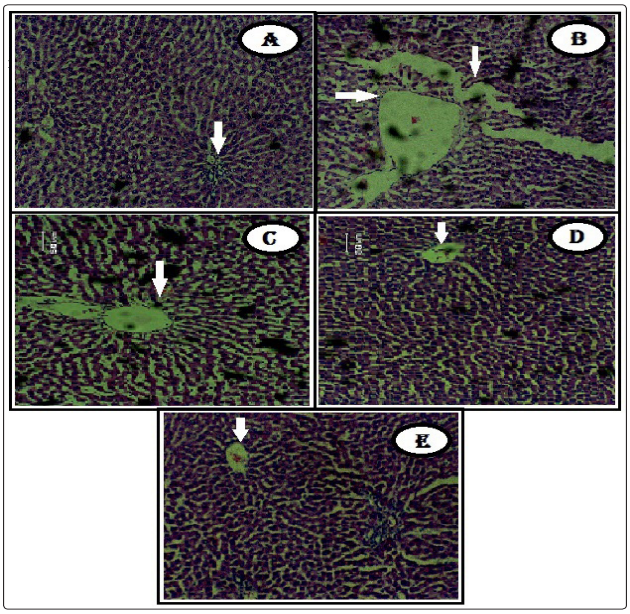

Figure 1: Histological examination of liver tissue section in different groups

Discussion

Protection against paracetamol-induced liver damage has been taken as a test for potential hepatoprotective agent by several investigators [34,35]. 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 [36,37]. In the present study, the liver damage produced by paracetamol is evident by altered levels of serum biomarkers. Treatment with MEAS (Test-1 & Test-2) and silymarin reverse the altered enzyme levels towards the normal limit.

Hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites, when a part of paracetamol is activated by hepatic cytochrome P 450, to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) [38,39]. NAPQI is initially detoxified by conjugation with GSH to form mercapturic acid [40]. 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) [41]. 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 MEAS (Test-1 & Test-2) and silymarin significantly reverse the above changes.

Increase in activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [42]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the O-2 to form H_2O_2, hence diminishing the toxic effect caused by this radical. CAT is an enzymatic antioxidant widely distributed in all animal tissue and the highest activity is found in the red cells and in liver. CAT decomposes H_2O_2 and protects the tissue from highly reactive hydroxyl radicals (OH-) [43]. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of O-2 and H_2O_2. In the present study, it was observed that treatment with MEAS (Test-1 & Test-2) and silymarin caused a significant increased in the hepatic SOD and CAT activity compared to paracetamol control. This shows MEAS prevent 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 MEAS (Test-1 & Test-2) was further substantiated by histopathological assessment in comparison with paracetamol control.

Preliminary phytochemical investigations of MEAS 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.

Conclusion

In conclusion, the acute toxicity study showed that *A. subulatum* was not toxic to the experimental rats up to an oral dose of 3 g/kg body weight. Furthermore, the results of this study demonstrate that MEAS has a hepatoprotective 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.

Acknowledgement

The authors express sincere thanks to Dr. AS Reddy, Professor, BRD School of biosciences, S.P. University, V.V. Nagar for the authentication of the plant. The authors also thank Dr. Prera Desai, Ayurvedic practitioner for her interest and encouragement.

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No RGP-VPP-179.

References

1. HZ Huo, B Wang, YK Liang, YY Bao, Y Gu (2011) Hepatoprotective and Antioxidant Effects of Licorice Extract against CCl4-Induced Oxidative Damage in Rats. International Journal Molecular Science 12: 6529-6543.
2. MA Alshawsh, MA Abdulla, S Ismail, ZA Amin (2011) Hepatoprotective effects of Orthosiphon stamineus extract on

thioacetamide-induced liver cirrhosis in rats. Evidence-Based Complementary and Alternative Medicine 2011: 103039.
3. VV Shah, K Deval (2011) Hepatoprotective activity of leaves of Parkinsonia aculeata linn against paracetamol induced hepatotoxicity in rats. International Journal of Pharmacology 1: 59-66.
4. R Goyal, B Ravishankar, VJ Shukla, M Singh (2012) Hepatoprotective Activity of Rohitaka ghrita against Paracetamol Induced Liver Injury in Rat. Pharmacologia 3: 227-232.
5. Sharma B, Sharma UK (2010) Hepatoprotective activity of some indigenous plants. International Journal of Pharmaceutical Technology Research 2: 568-572.
6. ZH Ao, ZH Xu, ZM Lu, HY Xu, XM Zhang, et al. (2009) Niuchangchih (Antrodia camphorata) and its potential in treating liver diseases. Journal of Ethnopharmacology 121: 194-212.
7. M Meganathan, MG Madhana, P Sasikala, J Mohan, N Gowdhaman, et al. Evaluation of antioxidant effect of Omega 3-fatty acid against paracetamol induced liver injury in albino rats. Global Journal of Pharmacology 5: 50-53.
8. SM Nabavi, A Hajizadeh Moghaddam, M Fazli, R Bigdellou, S Mohammadzadeh, et al. Hepatoprotective activity of Allium paradoxum. European Review for Medical and Pharmacological Sciences 16: 43-46.
9. PC Sharma, MB Yelne, TJ Dennis (2002) Database on Medicinal Plants used in Ayurveda. Central council for research in ayurveda and siddha 1: 454-461.
10. FV De Feudis, V Papadopoulos, K Drieu (2003) Ginkgo biloba extracts and cancer: a research area in its infancy. Fundamental Clinical Pharmacolology 17: 405-417.
11. GR Takeoka, LT Dao (2003) Antioxidant constituent of almond [Prunus dulcis (Mill.) D.A. Webb.] Hulls. Journal of Agriculture and Food Chemical 51: 496-501.
12. AH Banskota, Y Tezuka, IK Adnyana, Q Xiong, K Hase, et al. (2000) Hepatoprotective effect of commbretum quadrangulare and its constituent. Biology Pharmaceutical Bulletin 23: 456-60.
13. R Amarowicz, Z Egarska, RB Pegg, M Karamaæ, A Kosiñska (2007) Antioxidant and radical scavenging activities of a barley crude extract and its fractions. Journal of Food Science 25: 73-80.
14. H Madani, M Talebolhosseini, S Asgary, GH Naderi (2008) Hepatoprotective activity of Silybum marianum and Cichorium intybus against thioacetamide in rat. Pakistan Journal of Nutrition 7: 172-176.
15. S Prabhu (2006) Protective effect of Tefroli on cadmium chloride induced hepatotoxic rats. Pharmacognosy Magazine 2: 112-118.
16. MY Parmar, PA Shah, VT Thakkar, TR Gandhi (2009) Hepatoprotective activity of A. Subulatum Roxb seed against ethanol-induced liver damage in rats. International Journal of Green Pharmacy 3: 250-254.
17. MY Parmar, PA Shah, J Gao, TR Gandhi (2011) Mitochondrial protection: Mechanism of Amomum subulatum Roxb. Seeds extract against liver damage in mice. Indian Journal of Pharmacology 43: 671-675.
18. Organization for Economic Co-operation and Development (2001) Guidelines on acute oral toxicity, No-425.
19. SS Handa, A Sharma (1990) Heptoprotective activity of andrographolide from Andrographis paniculata against CCl4. Indian Journal of Medical Research 92: 276.
20. SV Kumar, C Sujatha, J Syamala, B Nagasudha, SH Mishra (2006) Protective effect of root extract of Operculina turpethum linn. against paracetamol-induced hepatotoxicity in rats. Indian Journal of Pharmaceutical Sciences 68: 32-35.
21. HU Bergmeyer, P Scelibe, AW Wahlefeld (1978) Optimization of methods for aspirate aminotransferase and alanine aminotransferase. Clinical Chemistry 24: 58-61.
22. King J (1965) The hydrolases acid and alkaline phosphatase. Practical Clinical Enzymology. Van D (ed.) pp. 191-208.
23. HJ Malloy, KA Evelyn (1937) The determination of bilirubin with the photoelectric colorimeter. Journal of Biological Chemistry 119: 481.
24. E Amador, LE Dorfman, WE Wacker (1963) Serum lactic dehydrogenase activity: an analytical assessment of current assays. Clinical Chemistry 9: 391.
25. G Szasz, JP Persyn (1974) New substrates for measuring G-glutamyl transpeptidases activity. Clinical Chemistry and Clinical Biochemistry 12: 228.
26. OH Lowry, NJ Rosebrough, AL Farr, RJ Randall (1951) Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265-275.
27. KS Singh, VR Kumar, S Rajat (2010) N-acetylcysteine inhibits hyperglycemia-induced oxidative stress and apoptosis markers in diabetic neuropathy. Journal of Neurochemistry 112: 77-91.
28. H Ohkawa, K Yagi (1979) Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analysis of Biological Chemistry 95: 351-358.
29. GL Ellman (1959) Tissue sulphydryl groups. Archives of Biochemistry and Biophysics 82: 70-77.
30. P Kakkar, B Das, PN Viswanathan (1984) A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry Biophysics 21: 131-132.
31. H Aebi (1974) Catalase In: Bergmeyer. Mehtods in enzymatic analysis 2: 674-684.
32. AE Galighor, EN Kozloff (1971) In Essentials of practical micro techniques. 2nd ed. Lea and Febiger, Philadelphia, pp. 77-210.
33. S Bolton, C Bon (2004) Pharmaceutical statistics: Practical and clinical applications. 4th ed. Marcel Dekker, Inc. New York, pp. 229-230.
34. Y Dwivedi, R Rastogi, NK Garg, BN Dhawan (1991) Prevention of paracetamol-induced hepatic damage in rats by Picroliv, the standardized active fraction from Picrorhiza kurroa. Phytotherapy Research 5: 115-119.
35. B Shukla, PK Visen, GK Patnaik, BN Dhawan (1992) Choleretic effect of andrographolide in rats and guinea pigs. Planta Medica 58: 146-149.
36. JR Mitchell, DJ Jollow, WZ Potter, JR Gillettee, BN Brodie (1973) Acetaminophen-induced hepatic necrosis, Role of drug metabolism. Journal of Pharmacology Experimental Therapeutics 187: 185-194.
37. LF Prescott, N Wright, P Roscoe, SS Brown (1971) Plasma paracetamol half life and hepatic necrosis in patients with paracetamol over dosage. Lancet 1: 519-522.
38. MC Savides, FW Oehme (1982) Acetaminophen and its toxicity. Journal of Applied Toxicology 3: 95-111.
39. NPE Vermeulen, JGM Bessems, R Vande (1992) Molecular aspects of paracetamol-induced hepatotoxicity and it mechanism based prevention. Drug Metabolic Review 24: 367-407.
40. M Moore, H Thor, G Moore, S Nelson, P Moldeus, et al. The toxicity of acetaminophen and N-acetyl P-benzoquinone-imine in isolated hepatocytes is associated with thio depletion and increased cystosolic Ca^{2+}. Journal of Biology and Chemical

260: 13035-13040.
41. SK Gupta, V Kochupillai, N Singh, YK Gupta, S Joshi (2001) Chemopreventive activity of Withania somnifera in experimentally induced fibrosarcoma tumors in Swiss albino mice. Phytotherapy Research 15: 240-244.
42. SJ Curtis, M Moritz, PJ Snodgrass (1972) Serum enzymes derived from liver cell fraction and the response to carbon tetrachloride intoxication in rats. Gastroenterology 62: 84-92.
43. B Chance, DS Green Stein, RJW Roughton (1952) The mechanism of catalase action steady state analysis. Archives of Biochemistry and Biophysics 37: 301-339.