

**HEPATOPROTECTIVE ACTIVITY OF *MENTHA ARVENSIS*
LINN AGAINST ALCOHOL-CCl₄ INDUCED TOXICITY IN
ALBINO RATS**

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Summary

Liver is a versatile organ that regulates the internal environment of the body and hence becomes prone to toxicity. Despite the tremendous strides in modern medicine there is still a need for a drug that stimulates liver function. Aqueous extract of *M.arvensis* (AEMA) was prepared as per protocol. Hepatotoxicity was induced in the experimental models with alcohol. At the end of the experimental period the animals were sacrificed by cervical decapitation. Blood and tissues were collected for assessment of SGOT, SGPT, SALP, SGGT, S.Protein, S.Bilirubin, Tissue Glycogen, Lipid peroxide levels, SOD, GSH, GST and Gpx. The alcohol induction caused an elevation in the serum marker enzymes, bilirubin and Lipid peroxide levels. There was a decrease in the serum protein, tissue glycogen and the antioxidant status indicating hepatic damage. The treatment of the animals with AEMA depicted the restoration of these parameters in serum and liver. Preliminary phytochemical screening was done which showed the presence of flavanoids. The restoration of the hepatic functioning which was made evident from the levels of the serum and tissue markers suggests the protective activity of *M.arvensis* which may be attributed to the presence of flavanoids.

Key Words: Antioxidant, Hepatotoxicity, Lipid peroxidation, Marker enzymes.

Introduction

Hepatic system is the major organ system involved in the metabolism, detoxification and excretion of various endogenous and exogenously ingested substances like xenobiotics, pollutants etc. This physiological activity of liver results in the generation of highly reactive free radicals, which covalently bonds with membrane lipids causing lipid peroxidation[1] Drug induced hepatic injury is the most common reason cited for the withdrawal of an approved drug from the market[2] Alcohol is well known to potentiate the hepatotoxicity

of various xenobiotics and the information about interaction between alcohol and the hepatotoxins is well documented [3]. Alcoholic liver disease is a common consequence of prolonged and heavy alcohol intake. This disease encompasses a wide spectrum of lesions, the most characteristic being alcoholic steatosis (fatty liver), alcoholic hepatitis, alcoholic fibrosis and cirrhosis[4].

Mentha arvensis L. (Lamiaceae) is distributed throughout the western Himalayas and is cultivated throughout world for use as a vegetable. It is an erect aromatic herb that grows up to 60 cm in height with suckers; the stem is cylindrical and the leaves are simple and opposing type. *Mentha arvensis* L. is used as a carminative, anti-spasmodic, anti peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine [5]. According to several researchers the plant contains 90% mint oil. It contains monoterpenes such as (menthone, menthofuran, methyl acetate cineole and limonene); sesquiterpenes (viridiflorol); flavonoids (luteolin, menthoside, isorhoifolin, rutin hesperidin); phenolic acids (caffeic, chlorogenic and rosmarinic); triterpenes (squalene, a-amyrin, urosolic acid; sitosterol); phytol; tocopherols; carotenoids; choline; betaine; cyclenenes; rosmarinic acid; tannin; and minerals [6-8].

With no effective medication available in the modern medicine the focus is now on “Herbal Drugs” for treating many diseases particularly liver disease. The present study has been structured to evaluate the hepatoprotective potential of the aqueous extract of *Mentha arvensis* Linn.(AEMA) against alcohol induced hepatotoxicity in albino rats.

Materials and Methods

Collection of plant material:

The whole plant *Mentha arvensis* Linn was collected in and around Trichy identified and authenticated with the Flora of Presidency of Madras and confirmed with the voucher specimen deposited at the Rapinet herbarium of St.Joseph’s College, Trichy.

Preparation of aqueous extract:

The plant was shade dried and coarsely powdered. The powder was mixed thoroughly with 6 times the volume of water and stirred continuously until the volume reduced to 1/3rd. The extract was filtered with muslin cloth. The residue was re extracted. The filtrate was mixed and evaporated in a water bath till it reached a thick consistency. The extract was stored in refrigerator for further use.

Animal models:

Wistar strains of albino rats weighing approximately 150 g were used as the experimental animals. The animals were kept in well ventilated cages and were fed with commercial pelleted rat chow and water *ad libitum*.

Experimental design:

The rats were divided into 5 groups consisting of 6 rats each. Group I: untreated animals and served as normal control. Rats of Group II to VI were given 40% ethanol (2 ml/100 g. body wt.) for 21 days. On 20th day they were injected with CCl₄ (1:1 in olive oil, 0.1 ml/kg body wt.) and were sacrificed 48 hours later [9] and biochemical estimations were performed. Animals of group III, IV and V also were intoxicated in a similar way. Group III : treated with aqueous extract of *Mentha arvensis* Linn at a dosage of 100 mg/kgbw for 21 days. Group IV: treated with aqueous extract of *Mentha arvensis* Linn at a dosage of 200mg/kgbw for 21 days. Group V : treated with aqueous extract of *Mentha arvensis* Linn at a dosage of 300 mg/kgbw for 21 days. Group VI: treated with silymarin at a dose of 25mg/kgbw for 21 days.

At the end of the experimental period all the animals were sacrificed by cervical decapitation and the following estimations were carried out to assess the liver functions.

All the biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) [10], Alkaline phosphatase (ALP) [11], Total bilirubin (TB) [12], Total protein (TP) [13] and Tissue Glycogen

[14] were carried out using standard methods. The anti oxidant status of the models were also assayed by studying the Lipid peroxide levels(LPO) [15], Superoxide Dismutase (SOD) [16],Reduced Glutathione (GSH) [17], Glutathione peroxidase (GPx)[18] and Glutathione S Transferase (GST) [19].

Histopathological study

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in 10% neural buffer formalin. After 12 hours liver was embedded in paraffin using conventional methods [20] and cut into 5µm thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical Analysis

The data obtained was expressed as mean \pm SEM. The data were subjected to one way ANOVA. The p value < 0.05 was considered statistically significant.

Results

Mentha a commonly used culinary is non toxic. From Table 1 it is evident that Alcohol –CCl₄ intoxication resulted in an elevation in the marker enzyme levels in the serum. The restoration the marker enzyme levels in the serum on treatment with *M.arvensis* clearly depicts the protective nature of the selected drug source.

Table 1. Effect of the aqueous extract of *Mentha arvensis* on the serum marker enzyme levels

| Parameter | Groups | | | | | |
|--------------|----------------|-----------------|-----------------|-----------------|-----------------|---------------------|
| | Normal control | Disease Control | AEMA 100mg/kgbw | AEMA 200mg/kgbw | AEMA 300mg/kgbw | Silymarin 25mg/kgbw |
| S GOT | 10.1±0.28 | 47.22±1.67 | 34.52 ± 1.22* | 27.23 ± 0.64* | 11.3 ± 1.88 | 9.5 ± 0.7** |
| S GPT | 15.21±0.34 | 91.21±0.68 | 64.22 ± 0.47 | 46.6 ± 0.28* | 22.2 ± 0.34 | 19.44± 1.89** |
| S ALP | 16.92±0.36 | 71.24±3.10 | 64.86 ± 0.38 | 46.72 ± 2.47 | 25.91±2.97** | 18.96±0.22 |
| S GGT | 31.46±0.31 | 64.88±1.23 | 58.22 ± 2.54 | 45.23 ± 0.25 | 38.27±1.24** | 35.42± 1.22** |

Values are mean ± S.E.M (n=6)

* p<0.05 statistically significant when compared with normal control

**p< 0.05 statistically significant when compared with CCl₄ treated group

From the results obtained it is clear that the intoxication causes a decrease in the levels of biochemical parameters such as Tissue glycogen, Serum protein and a marked elevation in the serum bilirubin levels. The aqueous extract of *M.arvensis* restored the levels back to near normal and was in par with that of the silymarin treated groups. (Table 2)

Table 2. Effect of *Mentha arvensis* on Biochemical Parameters

| Parameter | Groups | | | | | |
|--------------------|----------------|------------------|-----------------|------------------|-------------------|---------------------|
| | Normal control | Disease Control | AEMA 100mg/kgbw | AEMA 200mg/kgbw | AEMA 300mg/kgbw | Silymarin 25mg/kgbw |
| T.Glycogen | 38.31± 0.85 | 11.71 ± 0.42* | 24.38 ± 0.78 | 32.05 ± 0.75 | 36.48 ± 0.57** | 36.10± 0.96** |
| S.Bilirubin | 0.98± 0.045 | 5.46± 0.15* | 3.6± 0.02 | 1.98± 0.005** | 1.12 ± 0.005** | 1.06 ± 0.88** |
| S.Protein | 7.2± 0.03 | 2.5± 0.022* | 4.6± 0.01 | 5.67± 0.01** | 6.8± 0.06** | 6.34 ± 1.23 |

Values are mean ± S.E.M (n=6)

* p<0.05 statistically significant when compared with normal control

**p< 0.05 statistically significant when compared with CCl₄ treated group

Table 3 depicts the antioxidant status of the alcohol –CCl₄ intoxicated and the AEMA treated animals. An elevation in the LPO levels with a concurrent decrease in the SOD, GSH, GPx and GST activity in the disease control indicates the extent of toxicity. A reduction in the LPO and an improvement in the antioxidant status of the animals was noticed in AEMA and silymarin treated groups.

Table 3. Antioxidant status of the disease control and test drug treated groups

| Parameter | Groups | | | | | |
|------------|----------------|-----------------|-----------------|-----------------|-----------------|---------------------|
| | Normal control | Disease Control | AEMA 100mg/kgbw | AEMA 200mg/kgbw | AEMA 300mg/kgbw | Silymarin 25mg/kgbw |
| LPO | 2.09 ± 1.09 | 6.77 ± 0.14* | 5.18 ± 0.23 | 4.29 ± 1.13 | 2.25 ± 0.19 | 2.19 ± 1.11** |
| SOD | 2.77 ± 0.11 | 0.83 ± 0.11* | 1.91 ± 0.12 | 1.58 ± 2.09 | 3.13 ± 0.29** | 3.33 ± 1.82** |
| GSH | 43.63 ± 0.74 | 23.22 ± 0.50* | 23.10 ± 0.65 | 33.93 ± 0.95** | 42.50 ± 1.13 | 46.32 ± 1.01** |
| GPx | 46.81 ± 0.97 | 26.10 ± 0.76* | 25.57 ± 1.30 | 36.70 ± 1.31 | 44.02 ± 1.28 | 44.86 ± 1.05** |
| GST | 165.85 ± 2.93 | 79.29 ± 2.77* | 88.83 ± 2.34 | 122.33 ± 5.41 | 153.56 ± 3.8** | 155.25 ± 2.96** |

Values are mean ± S.E.M (n=6)

* p<0.05 statistically significant when compared with normal control

**p< 0.05 statistically significant when compared with CCl₄ treated group

The histopathological examination of liver of control and treated animals confirmed the hepatoprotective activity of AEMA. The liver sections of control group showed normal cellular architecture with prominent hepatic cells, and a central vein. Vacuolization and fatty changes were severe in the alcohol- CCl₄ treated group compared control groups [Fig 2]. The liver of the rat treated with AEMA at a dose of 200 mg/kg and 300 mg/kg,[Fig.4], [Fig.5] and silymarin [Fig.6] showed significant recovery from alcohol- CCl₄ induced liver damage evidenced by normal hepatocytes with nuclei. Vacuolization and fatty degeneration were prevented by the treatment with extract and silymarin.

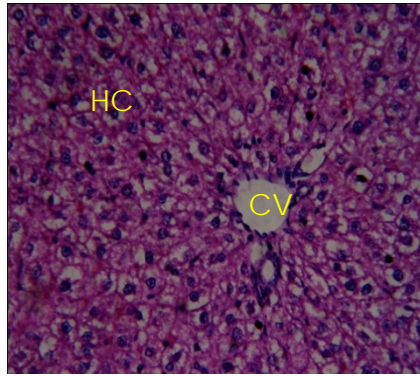


Fig 1 Normal Control group showing distinct hepatic cell (HC) and central vein (CV)

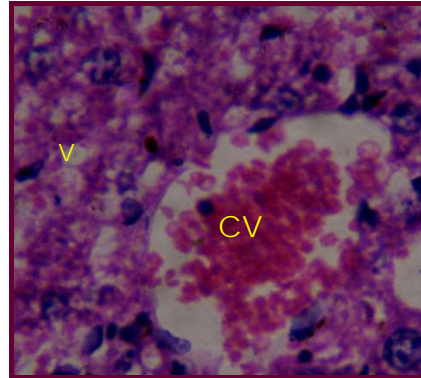


Fig 2 Degeneration of normal hepatic cells with, vacuole (V) formation with centrilobular necrosis

H

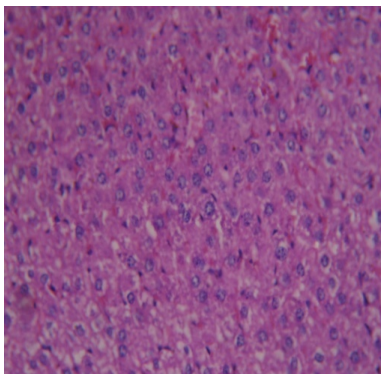


Fig 3 Rats treated with AEMA 100mg/kgbw showing less degeneration of hepatocytes, and fatty changes compared to hepatotoxin treated group

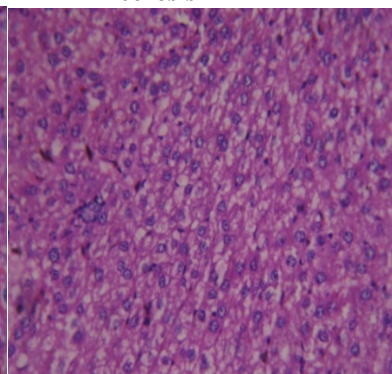


Fig 4 Rats treated with AEMA 200mg/kgbw showing regeneration of hepatocytes with lesser fatty changes

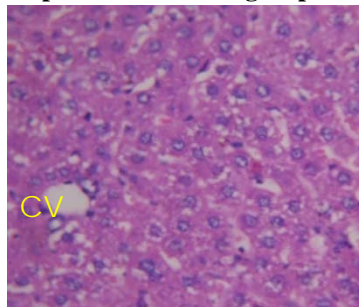


Fig5 Rat treated with AEMA 300mg /kgbw showing regeneration of hepatocytes, and lesser fatty changes

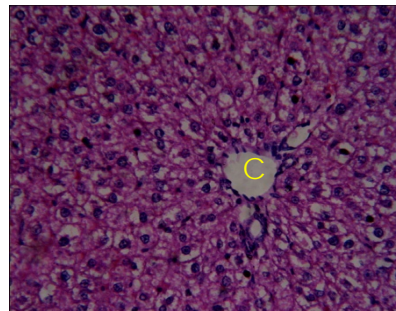


Fig6 Rat treated with silymarin showing regeneration of hepatocytes, with CV

Discussion

Alcohol is well known to potentiate the hepatotoxicity. Toxicity in liver due to ethanol and CCl₄ is attributed to the toxic metabolites formed, responsible for the initiation of CCl₄ dependent lipid per oxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the tri chloro methyl radical [9]. These activated radicals bind covalently to the macromolecules and induce per oxidative degradation of membrane lipids. The degradation of bio membranes due to lipid per oxidation is one of the principal causes of hepatotoxicity [21, 22]. This eventually leads to hepatocellular necrosis and it is reflected in our experiment by marked changes in various enzymatic parameters of alcohol and CCl₄ treated rats.

Assessment of liver function was be made by estimating the activities of serum SGOT, SGPT and ALP which are enzymes originally present in higher concentration in cytoplasm[23,24]. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. GGT is a sensitive marker of alcohol ingestion and certain hepatotoxic drugs. An elevation in the ALP levels along with an elevation in the GGT levels is a clear indication of a persisting liver damage. Measurement of these enzyme levels has proved to be powerful tools in the assessment of hepatotoxicity [25]. The elevated level of all these marker enzymes observed in alcohol – CCl₄ treated rats in the present study corresponded to the extensive liver damage induced by the toxin. The tendency of these enzymes to return towards a near - normalcy in Group III, IV and V was clear manifestation of the anti – hepatotoxic effect of *Mentha avensis* Linn. A significant reduction in the marker enzyme levels was also seen in the silymarin treated groups.

Liver damage caused by acute exposure to alcohol- CCl₄ shows clinical symptoms such as jaundice, swollen and tender liver and elevated levels of liver enzymes in the blood[26,27]. Bilirubin, a major breakdown product of haemoglobin rises when there is liver injury or damage; Elevation of total bilirubin which results from decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct or

conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts[28]. In the present study it was found that there was a significant elevation in the serum bilirubin level which was restored to normalcy on treatment with the AEMA.

Most protein found in the plasma are synthesized by the hepatocytes and secreted into circulation. Total protein concentration of the alcohol- CCl₄ treated rats (Table 2) was significantly reduced as compared to control. This suggests a reduction in the protein synthetic function of the liver, which could be as a result of possible damage to the hepatocytes induced by the ingested toxins. The administration of the plant extract improved the serum protein levels in the animals which proved the role of the plant extract in enhancing the synthetic function of the liver.

The ingestion of toxins results in an increased demand for energy and hence a greater rate of glycogenolysis leading to depletion in the glycogen stores in the liver. In addition the number of active hepatocytes involved in the synthesis of glycogen is also reduced due to intoxication with CCl₄[29]. Hence there is a significant reduction in the synthesis of glycogen in the disease control group. The plant extract activated the synthetic machinery of the hepatic tissue thus improving the glycogen stores of the animals.

Ethanol and CCl₄ are capable of generating oxygen radicals, inhibiting glutathione synthesis, producing glutathione loss from the tissue, increasing malonyldialdehyde levels and impairing anti oxidant defense systems in experimental animals. Lipid peroxidation results from the increased oxygen radical production which may be due to the activity of the induced cytP4502E1 [30]. The accumulation of the lipid peroxides and the depletion of SOD, GSH, GPx and GST indicate the necessity of these enzymes for the quick removal of the accumulated toxins from the system. The removal of the toxic metabolites is believed to be the vital initial step in providing cell survival during ethanol-CCl₄ intoxication [31]. Super oxide dismutase is most important anti-oxidant enzyme. The rat induced with alcohol-CCl₄, shows minimal activity of super oxide dismutase resulting in free radical accumulation and tissue damage [32].

GSH and its dependent systems – GPx and GST efficiently scavenge the free radicals. GST binds to lipophilic compounds and acts as an enzyme for GSH conjugation reactions. The decrease in the activity of GST may be due to the decreased availability of GSH and suggests a total inhibition of drug metabolism during CCl₄-intoxication. Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption. The depletion of GSH leads to the inactivation of GPx also. Accumulation of GSSG is seen in the intoxicated animals which may be due to inactivation of glutathione. This subsequently leads to depletion of GSH [33]. The activity of the antioxidant defense system was found to be very low in the group 2 animals and was restored in the plant drug and silymarin treated groups.

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. Centrilobular necrosis, a common feature of CCl₄ induced toxicity was noticed along with vacuolization. Treatment of rats with AEMA exhibited regenerative changes like normal appearance of hepatic cells with nucleus, less vacuolization, disappearance of necrotic lesions and decreased fat deposit. These changes viewed in the histological sections of the animal tissues supplements the protective effect of the extract.

Conclusion

The results strongly suggest an initiation in the process of liver regeneration, which is evident from the restoration of various biochemical parameters and the histological studies.

References

1. Scott Luper ND. A review of plants used in the treatment of liver disease: Part I. *Alternative Med Rev* 1998; 3: 410-421.
2. Sailor GU, Dudhrejya AV, Seth AK, Maheshwari R, Nirmal Shah, Chintan Aundhia. Hepatoprotective effect of *Leucas cephalotes* Spreng on CCl₄ induced liver damage in rats, *Pharmacologyonline* 2010; 1: 30-38.

3. Zimmerman HJ, Effects of alcohol on other hepatotoxins, A/C Clini Exp Res 1986; 10:3-15
4. Kai OL. Alcohols liver disease; pathobiological aspects. J Hepatol 1995; 23:7-15.
5. Ramesh L Londonkar, Pramod V Poddar. Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals World J Gastrointest Oncol 2009; 1(1): 82-88.
6. Pino S. Phytochemical studies of medicinal plants. Int JPlant Sci 1996; 68: 130-142.
7. Buneton J. Phytochemical studies of medicinal plants. Int JPlant Sci 1995; 20: 155-158
8. Liest, Hrhammer. Phytochemical studies of medicinal plants. Int J Plant Sci 1998; 68: 130-142.
9. Arulkumaran KSG, Rajasekaran A, Ramasamy R, Jegadeesan M, Kavimani S, Somasundaram A. *Cassia roxburghii* seeds protect Liver against Toxic effects of Ethanol and Carbontetrachloride in rats. International Journal of PharmTech Research 2009; 1(2): 273-246.
10. Reitman S, Frankel AS. A colorimetric method for the determination of Serum glutamate oxaloacetate and glutamate transaminase. J Clin Pathology 1957; 7: 322.
11. Mac Comb RB, Bowers GN. Alkaline phosphatase activity in serum. Clin Chem 1972; 18: 97.
12. Malloy HT, Evelyn KA, The determination of Bilirubin, J.Biol.Chem 1937;119: 481
13. Lowry OH, Roserbrough NJ, Farr AL, Randall RJ, Protein measurement with Folin Phenol Reagent. J.Biol.Chem 1951;193: 265-275
14. Morales MA, Jabbagy AJ and Terenizi HR. Mutations affecting accumulation og glycogen. Neurospora Newsletters 1973; 20: 24-25.
15. Ohkawa H, Ohishi N and Yagi K. Assay of Lipid peroxides in animal tissues for thiobarbituric acid reaction. Annal Biochem 1979; 95: 351-358.
16. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. J.Biol Chem 1972; 247: 3170-3175
17. Beutler E, Duron C and Kelly BM. Improved method for the determination of blood glutathione. J lab Clin Med 1963; 65: 782-797.

- 18 Rotruck JT, Pope AL, Ganther H, Swanson AB, Hafeman DG and Hoeksira WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973; 179: 588-590.
- 19 Habig WH, Pabst MJ, Jakoby WB. Glutathione- S-transferase –The first enzymatic step in mercapturic acid formation, *J. Biol Chem* 1974, 249: 7130-7139.
- 20 Sujai Suneetha. Handbook of CMAI medical Laboratory Technology by Robert H Carman, Christian Medical Association of India, 1993.
- 21 Kapur V, Pillai KK, Hussain SZ, Balani DK. Hepatoprotective activity of Jigrine on liver damage caused by alcohol-carbontetrachloride and paracetamol in rats. *Ind J Pharmacol* 1994;26:35-40
- 22 Sheila Sherlock, James Dooley. Assessment of liver function, in *Diseases of the liver and biliary system*, vol.9 (oxford Blackwell Scientific Publications, London),1993,17.
- 23 Wells FE, Tests in liver and biliary tract disease is Varley's *Practiccal Clinical Biochemistry*, edited by A H Gowenlock, (Heinemann Medicinal Books, London)1988,744
- 24 Castillo T, Koop DR, Kamimura S, Triadafilopoulos G, Tsuleanoto H. Role of cytochrome P-450 2E1 in ethanol-CCl₄ and iron dependent microsomal lipid peroxidation. *Hepatology* 1992; 16: 992-996
- 25 Ambiga S, Sivabalan, Madhavan S. Hepatoprotective activity of *Phyllanthus emblica* Linn. extract against paracetamol induced hepatic damage in rats. *J.Sci.trans Environ technov* 2007; 1(1): 19-22.
- 26 Allis JW, Ward TR, Seely JC, Simmons JE Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats. *Fundam Appl Toxicol* 1990; 15:558-570.
- 27 Tirkey, Pukhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbontetrachloride in rat liver and kidney. *BMC Pharmacol* 2005 5:2-6
- 28 Sanjiv C. *The liver book: A comprehensive guide to diagnosis, treatment and recovery*. Atria Jimcafe Company 2002.
- 29 Ajay Kumar Gupta, Neelam Misra. Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in

- paracetamol intoxicated albino rats. Am J Pharmacol & toxicol 2006; 1: 17-20.
- 30 Muller A, Sies H. Role of alcohol dehydrogenase activity and the acetaldehyde in ethanol-induced ethane and pentane production by isolated perfused rat liver. Biochem J 1982; 206: 153-156
 - 31 Nordmann R, Ribiere C, Rouach H. Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radic Biol Med 1992; 12: 219-240
 - 32 Amin A, Hazma A A, Hepatoprotective effects of *Hibiscus*, *Rosmarinus* and *Salvia* on Azathioprine induced toxicity in rats. Life Science 2005; 77(3): 266 – 78.
 - 33 Anandan R, Deepa Rekha R, Devaki T, Protective effect of *Picrorrhiza kurroa* on mitochondrial glutathione antioxidant system in D galactosamine induced hepatitis in rats, Current Science. 1999; 76: 1543-1545.