Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

HEPATOPROTECTIVE ACTIVITY OF *ACLYPHA INDICA* LINN AGAINST THIOACETAMIDE INDUCED TOXICITY

S V SURESH KUMAR*, C VIJAYA KUMAR, A VISHNU VARDHAN

Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool 518218, Andhra Pradesh. India.
Email: sureshsolleti@yahoo.co.in

Received: 05 Sep 2013, Revised and Accepted: 05 Oct 2013

ABSTRACT

Objective: Evaluation of hepatoprotective activity of *Acalypha indica* linn against thioacetamide induced toxicity.

Methods: Methanol extract and methanolic fraction of methanol extract were evaluated for hepatoprotective activity against thioacetamide-induced liver damage in albino rats. Levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatise (ALKP), total bilirubin (TBL) total cholesterol (CHL), total protein (TPTN) and albumin (ALB) in serum were evaluated and were supported by histopathological examination of liver sections.

Results: Administration of thioacetamide (100 mg/kg i.p.) induced a marked increase in the serum levels of GOT, GPT, ALKP, TBL, and CHL; and decrease in the levels of TPTN and ALB, indicating parenchymal cell necrosis. Methanol extract (ME) at dose levels of 300 mg/kg and methanol fraction of methanol extract (MFME) at dose levels of 250 mg/kg has restored the altered parameters significantly as observed in case of silymarin treated group.

Conclusion: Methanol extract of *Acalypha indica* exhibited hepatoprotective activity and presence of flavonoids may be are responsible for the same.

Keywords: *Acalypha indica*, Thioacetamide, Hepatoprotective, Methanol extract, Silymarin.

INTRODUCTION

Acalypha indica Linn. belonging to the family Euphorbiaceae commonly known as haritha manjari is an erect annual herb with stem dark green, quadrangular and longitudinal furrows and wings [1]. It is used as expectorant, purgative, emetic, gastrointestinal irritant, diuretic, cathartic and anthelmintic. It is also used in constipation, skin diseases, ulcers bronchitis. The plant was found to contain alkaloids, flavonoids, glycosides, lactones, terpenoids, cyanogenetic glucosides and glucosinolates, phenantherenes, quinines, phenolic acids etc [2]. Stigmasterol, β-Sitosterol and its acetate derivatives have been isolated from *Acalypha indica* has been found to have Anti hepatotoxic action. Anti-implantation activity of the Petroleum ether extract of the *Acalypha indica* was reported by Hiremath et al 1999 [3]. Flavonoids such as Kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin, have been isolated from flower of *Acalypha indica* [4]. The leaves & twigs of *Acalypha indica* have been reported to contain acalyphamide, acalyphamide acetate, aurantimide, aurantimide acetate, succinamide , acalyphol acetate, 2-methylanthraquinone, acyclic amide have also been reported from the aerial parts [1]. Considering the role of flavonoids as anti oxidants in various liver disorders, an attempt was made to explore the hepatoprotective activity of aerial parts of *A. indica*. The hepatoprotective activity of methanol extract and its methanol fraction at various dose levels was assessed using thioacetamide as a hepatotoxin. Silymarin was used as positive control.

MATERIALS AND METHODS

Plant material

Entire plants of *Acalypha indica* were collected from college of premises and their entity was confirmed at The Botanical Survey of India, Southern circle, Coimbatore, India. The voucher specimen (COG/CESCOP /02/ SVSK/2009) has been deposited in the Herbarium of the Institute.

Preparation of extracts

Powdered (500 g) aerial parts of the plant was defatted with petroleum ether (60-80° C), and then completely extracted with methanol using soxhlet apparatus. The methanol extract concentrated in vacuum yielded solid mass (4.8%, W/W). About 50 g of methanol extract was adsorbed on silica gel (60-120 mesh) and fractionated using chloroform and methanol. The recovered fractions were finally evaporated to give yields of 2.46% and 71.78% respectively. Preliminary thin layer chromatographic studies [5] of methanol extract revealed the presence of flavonoids, phenolics, terpenoids and steroids; the chloroform fraction (CFME) showed the presence of steroids; while the methanol fraction (MFME) showed flavonoids, phenolics, and terpenoids. The ME was tested for hepatoprotective activity at dose levels of 100, 200 and 300 mg/kg po, where as MFME was tested at dose levels of 50, 150 and 250 mg/kg po. Chloroform fraction being steroids and less in quantity was not selected for activity. The results were compared with a standard hepatoprotective drug silymarin (100 mg/kg). All the test substances were suspended in vehicle (5% acacia mucilage).

Animals

Wistar albino rats of either sex weighing 175-225 g, maintained under standard husbandry conditions were used. Animals were allowed to take standard laboratory feed and water *ad libitum*. The experiments were performed after the experimental protocol was approved by the institutional animal ethics committee.

Estimation of phenolic Content

The total phenolic content of the methanol extract of *A. indica* was estimated by the method of Folin ciocalteu [6]. Stock solution (20mg/10ml) of the extract was prepared methanol. From the stock solution 1ml of the extract was taken into a 25 ml volumetric flask. To this added 10 ml of water and 1.5 ml of Folin ciocalteu reagent. The mixture was kept aside for 5 min and then 4 ml of 20% sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The mixture was kept aside for 30 min and absorbance of blue colour developed was recorded at 765 nm. For the preparation of calibration curve the solutions of standard gallic acid was prepared in concentration range of 50 to 250 µg/ml.

Estimation of total flavonoid content

The total flavonoid content of the methanol extract of *A. indica* was estimated by reported methods. The aluminium chloride colorimetric method was used for estimating Flavones, flavonols and isoflavones as reported by Chang et al [7]. Quercetin was used to make the calibration curve. From the stock solution of standard 0.1, 0.2, 0.3, 0.4 and 0.5 ml were taken which gave 10, 20, 30, 40 and 50 µg concentrations respectively. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of

10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm, after incubation at room temperature for 30 min. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly 0.5 ml of methanol extract of *A. aspera* (2 mg/ml) was reacted with aluminium chloride for determination of total flavonoid content.

The method reported by Chang et al was used for estimation of flavanones. Naringenin was used as a reference standard to prepare calibration curve. 20 mg of naringenin was dissolved in methanol and then diluted to give concentrations of 250, 500, 1000, 1500 and 2000 µg/ml. one millilitre of each of the diluted standard solutions was separately mixed with 2 ml of 1% 2, 4-dinitro phenyl hydrazine reagent and 2 ml of methanol at 50°C for 50 min. After cooling to room temperature the reaction mixture was mixed with 5 ml of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 min. Then 1 ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1000 rpm/min to remove the precipitate formed. The supernatant was collected and adjusted to 25 ml. the absorbance of the supernatant was measured at 495 nm. Similarly 5 ml each of methanol extract of *A. aspera* (5 mg/ml) were similarly treated with 2, 4-dinitro phenyl hydrazine reagent for determination of flavonoid content. The sum of the values obtained from these two methods was considered as total flavonoid content.

Toxicity studies

Acute toxicity studies were performed for ME and MFME according to the acute toxic classic method [8] described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. The rats were divided into two groups of 3 animals each. The groups of rats were administered orally with appropriate extracts of *A. indica* at a dose of 300 mg/kg. The animals were observed continuously after dosing during first 30 min, periodically for first 24 hr with special attention given during first 4 hr and daily thereafter, for a total of 14 days. As there was no mortality seen at this dose level, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

Hepatotoxin and test substances

Test substances including silymarin were suspended in vehicle i.e. 5 % acacia mucilage for administration. Thioacetamide at a dose level of 100 mg/kg in water for injection was administered intra peritoneally.

Thioacetamide-induced hepatotoxicity [9]

Rats were divided into 7 groups of 6 each, control, thioacetamide, silymarin and test groups. The rats of control group received a single daily dose of 5% acacia mucilage (1 ml/kg, p.o.). The rats of toxicant group received a single daily dose of vehicle (1 ml/kg, p.o.) for three days and a single intraperitoneal injection of thioacetamide (100 mg/kg) in water for injection, 30 min after the administration of the vehicle on the second day of treatment. The rats of silymarin group received silymarin (100 mg/kg p.o.) three times at 24 h intervals. Thioacetamide was administered 30 min after the second dose of silymarin while test groups were given orally a single daily dose of extracts in vehicle for three days and a single dose of thioacetamide (100 mg/kg i.p.) on the second day 30 min after the administration of respective test suspensions. After 48 h of thioacetamide administration i.e 4th day of the experiment the blood was collected and serum was used for determination of biochemical parameters.

Assessment of liver function

Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry [10]. Alkaline phosphatase (ALKP) [11], total bilirubin (TBL) [12], total cholesterol (CHL) [13], total protein (TPTN) [14], albumin (ALB) [15] was estimated. All the estimations were carried out using standard kits on semi auto analyser.

Histopathological studies

Animals were sacrificed to remove the liver. The liver was fixed in Bouin's solution for 12 h, and then embedded in paraffin using conventional methods [16], cut into 5 µm thick sections and stained using haematoxylin-eosin dye. The sections were then observed for histopathological changes.

Statistical Analysis

The mean values±SEM were calculated for each parameter. Percentage restoration against the hepatotoxin by the test samples was calculated by considering the difference between the hepatotoxin treated group and the control group as 100% restoration. For the determination of significant inter group difference each parameter was analyzed separately and one way analysis of variance (ANOVA) [17] was carried out. After that individual comparisons of group mean values were done using Dunnet's test [18].

RESULTS

The phenolic content of methanol extract of *A. indica* was found to be 10.89% w/w representing the presence of various phenolic compounds like poly phenols, flavonoids, phenolic acids etc. From the calibration curve of the quercetin, the concentrations of the flavonols and flavones in the methanol extract of *A. indica* was found to be 1.76% w/w. From the calibration curve of naringenin, the amount of flavanones in the methanol extract of *A. indica* was found to be 6.55% w/w.The sum of the values obtained from these two methods was considered as total flavonoid content and are found to be 8.31% w/w.

The ME and MFME did not cause any mortality up to 2000 mg/kg and were considered as safe.

Administration of thioacetamide (100 mg/kg i.p.) induced a marked increase in the serum levels of GOT, GPT, ALKP, TBL, and CHL; and decrease in the levels of TPTN and ALB, indicating parenchymal cell necrosis. Significant decrease ($p<0.05$) in all the elevated levels of biochemical parameters except TBL and significant ($p<0.05$) increase in depleted TPTN levels was observed with the groups of rats which received ME at dose levels of 300 mg/kg and MFME at dose levels of 250 mg/kg (except ALB levels), as observed in case of silymarin treated group. The activity exhibited by ME was statically similar to the activity exhibited by MFME. Results of thioacetamide-induced hepatotoxicity are shown in Table 1.

Table 1: Effect of ME and MFME of A. indica on thioacetamide-induced hepatotoxicity in rats

Group	GOT (IU/L)	GPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	121.83 ± 07.30	60.24 ± 09.01	360.82 ± 60.52	0.81 ± 0.14	49.12 ± 15.50	5.93 ± 0.33	2.37 ± 0.61
Thioacetamide	326.33 ± 28.38	199.68 ± 55.13	939.16 ± 78.54	2.58 ± 0.35	144.04 ± 29.76	2.70 ± 0.41	0.91 ± 0.29
Silymarin	152.67 ± 07.61*	77.71 ± 27.56*	385.92 ± 91.27*	0.86 ± 0.17*	64.16 ± 18.23*	5.41 ± 0.38**	2.36 ± 0.34**
ME 1	202.17 ± 56.75*	119.25 ± 25.92	673.82 ± 50.99	1.72 ± 0.48	86.64 ± 27.51	3.42 ± 0.36	1.36 ± 0.35
ME 2	140.17 ± 07.67*	84.31 ± 28.70*	427.48 ± 73.38*	1.58 ± 0.59	60.08 ± 15.72*	5.23 ± 1.17**	1.71 ± 0.11
MFME1	128.50 ± 09.29*	79.73 ± 20.75*	455.77 ± 73.14*	1.16 ± 0.15	64.67 ± 12.47*	5.63 ± 0.75**	2.28 ± 0.33 **
MFME2	70.76 ± 26.84*	68.29 ± 21.14*	402.39 ± 46.89*	1.76 ± 0.30*	57.71 ± 17.13*	5.42 ± 0.48**	2.09 ± 0.23
F Calculated	9.89	2.61	9.32	3.01	2.53	3.91	2.52
DV	97.44	111.88	259.69	1.34	76.35	2.34	1.33

Data represents the mean ± SEM of six animals. F theoretical = 2.42 ($p<0.05$).

ME 1 and ME 2: Methanol extracts 200 and 300 mg/kg; MFME 1, and MFME 2: Methanol fraction of methanol extract 150 and 250 mg/kg.; DV: Dunnett value.

* Significant reduction compared to thioacetamide ($p<0.05$). ** Significant increase compared to thioacetamide ($p<0.05$).

Normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Figure 1a) was observed with histological examination of liver sections of control group. The examination of liver sections of rats administered with TAA showed hepatic cells with severe toxicity characterised by centrilobular necrosis along with various gradation of fatty changes comprising of tiny to large sized vacuoles, disarrangement of hepatic cells with blood pooling in sinusoidal spaces (Figure 1b). The liver sections of the rats administered with 300 mg/kg p.o. of ME (Figure 1e) and 150 and 250 mg/kg of MFME (Figure 1f,g) followed by TAA intoxications, showed lesser degree of visible changes similar to that observed in case of silymarin treated rat liver sections (Figure 1c) there by suggesting the protective effect of the extracts.

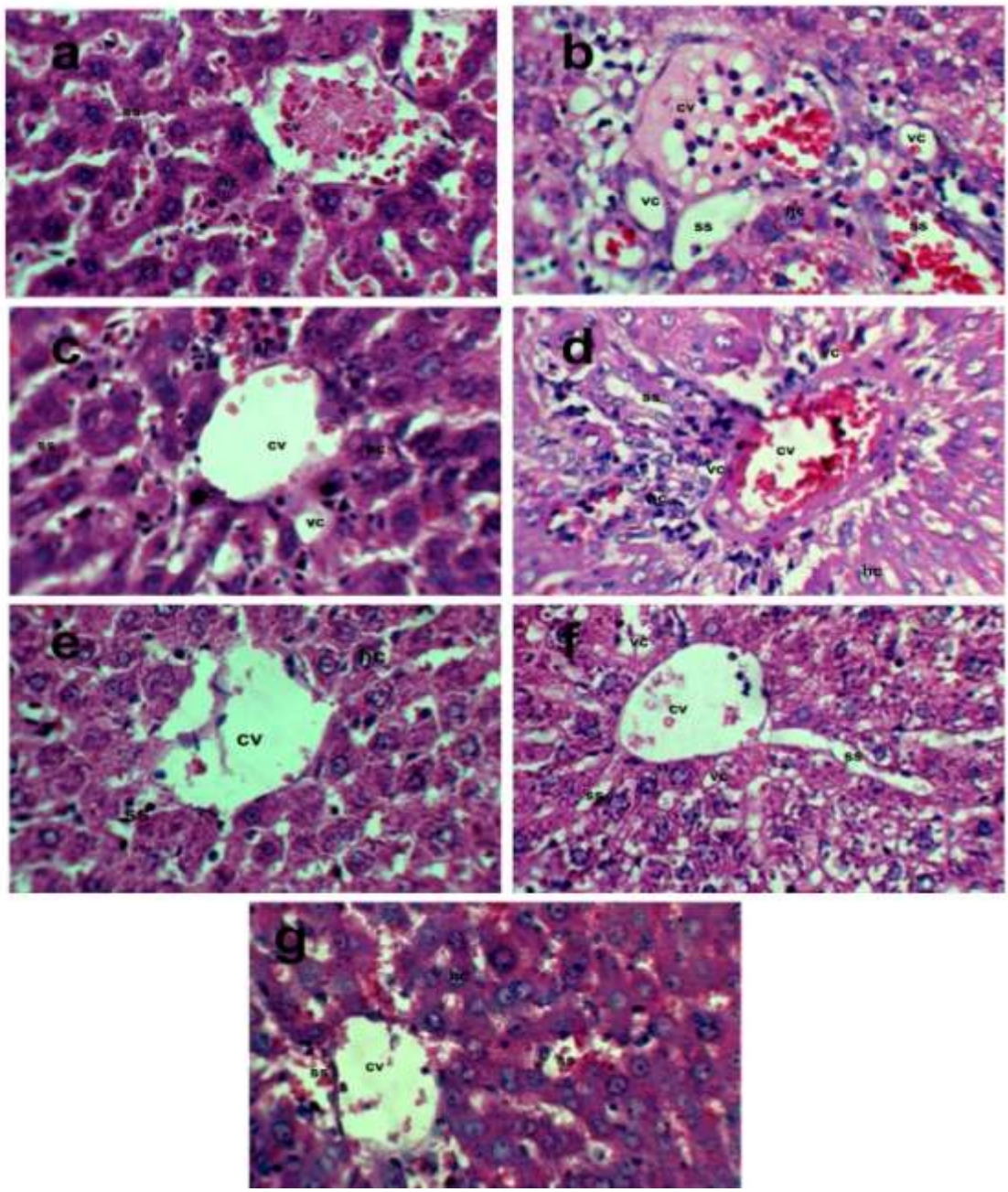

Fig. 1: Photomicrographs representing effect of ME and MFEE against thioacetamide -induced hepatotoxicity in rats.

a: Normal rat liver section; b: Liver section of the rat intoxicated with thioacetamide; c: Liver section of the rat treated with silymarin and intoxicated with thioacetamide; d: Liver section of the rat treated with ME 200 mg/kg and intoxicated with thioacetamide; e: Liver sections of the rat treated with ME 300 mg/kg and intoxicated with thioacetamide; f: Liver section of the rat treated with MFME 150 mg/kg and intoxicated with thioacetamide l; g: Liver section of the rat treated with MFME 250 mg/kg and intoxicated with thioacetamide l. Eosin-Haematoxylin stain. 400X. cv: central vein, vc: vacuole, ss: sinusoidal spaces, hc: hepatocytes

DISCUSSION

Administration of a single dose of thioacetamide in rats produces centrilobular hepatic necrosis. It get metabolises to a toxic metabolite thioacetamide-S-oxide, a direct hepatotoxin, which is further metabolised, at least in part, by cytochrome P_{450} monooxygenases. The subsequent product formed exerts hepatotoxicity by binding to hepatocyte macromolecules and causes centrilobular necrosis by generation of reactive oxygen species. The chronic thioacetamide exposure leads to cirrhosis in rats. It induces toxicity by altering semi permeable character of the cell membrane of hepatocytes resulting in an increased influx of calcium ions and

leading to necrosis and finally death [19]. Therefore extracts under study antagonise the effect of thioacetamide by inhibiting cytochrome P_{450} or by acting either as membrane stabiliser, thereby preventing the distortion of the cellular ionic environment associated with thioacetamide intoxication, or by preventing interaction of thioacetamide with the transcriptional machinery of the cells resulting in regeneration of hepatic cells.

Thus the hepatoprotective activity of these extracts and fractions may be due to their ability to affect the cytochrome P_{450} mediated functions or stabilisation of endoplasmic reticulum resulting in hepatic regeneration.

In literature many authors reported the hepatoprotective activity of flavonoid compounds. Galisteo et al [20] reported the hepatoprotective activity of flavonoids of *Rosmarinus tomentosus*. The hepatoprotective effect of quercetin was reported by Janbaz et al [21]. Protective effect of rutin was reported by Janbaz et al [22]. Silymarin obtained from *Silybum marianum* is a good hepatoprotective agent [23]. In accordance with these results, it may be hypothesized that flavonoids with their anti-oxidant properties, which are present in ME, and MFME of *A. indica*, are responsible for the hepatoprotective activity.

REFERENCES

1. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Poducts. Second supplements series Raw materials. New Delhi: Publication and Information Directorate NISCAIR; 2006. pp. 47.
2. Nadkarni KM. Indian Materia Medica. Mumbai: Popular Prakashan;2005. pp.17
3. Hiremath SP, Rudresh K, Badami S, Patil SR . Post-coital antifertility activity of Acalypha indica. J Ethnopharmacol 1999; 97(3): 253-258.
4. Nahrstedt A, Hungeling M, Petereit F. Flavonoids from Acalypha indica. Fitoterapia 2006; 77(6): 484-486.
5. Wagner G, Blatt S. Plant Drug Analysis. Berlin: Springer Verlag; 1998. pp. 352.
6. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphmolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic 1965; 16: 144-148.
7. Chang CC, Yang M, Wen H, Chern J. Estimation of flavonoid content in propolis by two complementary colorimetric methods. J Food drug Anal 2002; 10 (3):178-182.
8. OECD. Acute Oral Toxicity-Acute Toxic Class Method- Series on testing and assessment No. 423. Paris: Organisation for Economic Co-Operation and Development; 2001.
9. Handa SS and Singh S. Hepatorotective activity ofApium graveolens and Hygrophylla auriculata against paracetamol and thioacetamide oxicity in rats. J Ethnopharmacol 1995;49: 119-126.
10. Bergmeyer HU, Horder MR and Rej R. IFCC methods for the measurement of catalytic concentration of enzymes. J Clini Chem Clin Bio 1985; 23: 899-901.
11. Mac comb RB, Bowers GN. Alkaline phosphatase activity in serum. Clin Chem 1972; 18: 97.
12. Jendrassik L, Grof P. Quantitative determination of total and direct bilirubin in serum and plasma. Biochem Z 1938; 297: 81–89.
13. Richmond W. Preparation and properties of a cholesterol oxidase nocardia species and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 1973; 19: 1350-1356.
14. Peters T. Proposals for standardisation of total protein assays. Clin Chem 1968; 14: 1147-1159.
15. Webster D. Interaction of bromocresol green with isolated serum globulin fractions. Clin Chim Acta 1974; 53: 109-115.
16. Galighor AE, Kozloff EN. Essentials of practical micro technique, 2nd ed. New York: Lea and Febiger; 1976. pp. 210-214
17. Sanford B. In: Gennaro, A.R editor. Remington; The Science and Practice of Pharmacy. 19th ed. Easton PA: Mack publishing company; 1995. pp.111-113
18. Dunnet CW. New tables for multiple comparisons with a control. Biometrics 1964;20: 482-491.
19. Neal RA and Halpert J. Toxicology of thiosulfur compounds. Annu Rev Pharmacol 1982; 22:321-329.
20. Galisteo M, Suarez A, Montilla, Navarroa MC. Protective effects of *Rosmarinus tomentosus* ethanol extract on thioacetamide-induced liver cirrhosis in rats. Phytomedicine 2006; 13: 101-108.
21. Janbaz KA, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol and CCl_4 induced hepatotoxicity in rodents. Fitoterapia 2002; 73: 557-563.
22. Janbaz KA, Saeed SA, Gilani AH. Studies on the protective effects of caffeic acid and quercetin on chemical induced toxicity in rodents. Phytomedicine 2004; 11: 424-430.
23. Hiroshi H, Yoshinobu K. In: Wagner H, Hiroshi H, Farnswoth NR editors. Natural products for Liver Diseases. London: Academic Press; 1988. pp. 40-45.