Hepatoprotective potential of polyphenol rich extract of *Murraya koenigii* L.: An *in vivo* study

Swati N. Desai, Dipak K. Patel, Ranjitsinh V. Devkar, Prabhudas V. Patel, A.V. Ramachandran *

Division of Phytotherapeutics and Metabolic Endocrinology, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India

ARTICLE INFO

ABSTRACT

The present study investigates hepatoprotective effects of polyphenol rich *Murraya koenigii* L. (MK) hydro-ethanolic leaf extract in CCl_4 treated hepatotoxic rats. Plasma markers of hepatic damage, lipid peroxidation levels, enzymatic, and non-enzymatic antioxidants in liver and histopathological changes were investigated in control and treated rats. MK pretreated rats with different doses (200, 400 and 600 mg/kg body weight) showed significant decrement in activity levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, and bilirubin. Also, MK treated rats recorded a dose dependent increment in hepatic super oxide dismutase, catalase, reduced glutathione and ascorbic acid and, a decrement in lipid peroxidation. Microscopic evaluations of liver revealed CCl_4-induced lesions and related toxic manifestations that were minimal in liver of rats pretreated with MK extract. These results demonstrate that hydro-ethanolic leaf extract of MK possesses hepatoprotective potentials.

1. Introduction

Continuous exposure to various xenobiotics like pesticides, food additives, industrial chemicals and consumer products seriously compromise human health causing inflammation, tissue damages and cancers (Hasegawa et al., 1995). Carbon tetrachloride (CCl_4), an extensively used lipid soluble industrial solvent reportedly stimulate production of free radicals resulting in damage to tissues such as brain, lung, heart, liver, kidney, testis and blood (Recknagel et al., 1989; Kumar et al., 2005; Khan and Ahmed, 2009; Khan et al., 2009). This is the best-characterized chemical to study xenobiotic-induced free radical mediated acute liver injury in rats (Recknagel and Glende, 1973). Since, the pathological lesions developed in CCl_4 treated animals closely resemble the symptoms of cirrhosis in human, it serves as an excellent model to assess the efficacy of hepatoprotectants (Ivor and Schneider, 2005). The initial step involves covalent binding of the metabolic intermediates of CCl_4 (CCl_3^- and/or $CCl_3OO^{\cdot}$; trichloromethyl free radicals) with cellular proteins that triggers a cascade of events culminating in cellular necrosis due to peroxidation of membrane lipids (Recknagel et al., 1989). Reactive oxygen species (ROS) stand implicated in induction of liver damage and subsequent CCl_4-induced hepatotoxicity. Extensive membrane damage causes oozing of cellular enzymes into blood resulting in tissue malfunction (Obi et al., 2001). Further, trichloromethyl free radicals interact with the sulfhydryl group of glutathione (GSH) and protein thiols rendering the

cellular antioxidant system vulnerable to further damage by superoxide and hydroxyl radicals (Vitaglione et al., 2004; Jadeja et al., 2011). Amelioration of hepatotoxicity using synthetic drugs causes undesirable side effects (Rao et al., 2006) and may even be inadequate in curing the damage. Hence, an herbal alternative would be invaluable especially in view of their minimal side effects.

Murraya koenigii L. (MK), popularly known as curry leaves (family Rutaceae) are native to India, Sri Lanka and other South Asian countries (Rastogi and Mehrotra, 1998). They are extensively used in Indian food preparations to add characteristic flavor and aroma to the conventional Indian cuisines (Birari et al., 2010). In Ayurveda, MK leaves find mention for their anti-diabetic properties (Satyavati, 1987) while, traditionally they are used for curing ailments like stomachache, constipation, diarrhea, nausea, vomiting etc. Fresh raw leaves are consumed to cure dysentery while, freshly ground paste is applied on eruptions, bruises and bites of venomous organisms (Chakrabarty et al., 1997). Various extracts of MK leaves have been reported to exhibit anti-tumor (Fiebig et al., 1985), anti-oxidant (Das et al., 1965), chemo-modulatory (Dasgupta et al., 2003), immuno-modulatory (Shah et al., 2008), anti-diabetic (Naraya and Sastry, 1975), anti-hypertensive (Bhakuni et al., 1969), anti-obesity and lipid lowering (Birari et al., 2010) properties. Further, a recent report on ethanol induced toxicity in HepG2 cells has highlighted the antioxidant and hepatoprotective properties of a crude extract of MK leaves (Sathaye et al., 2011).

Ningappa et al. (2008) have shown the hydro-ethanolic extract (ethanol:water; 1:1) of MK leaves to be richer in polyphenols and more efficient in scavenging free radicals compared to other

* Corresponding author. Mobile: +91 9824365335; fax: +91 0265 2342109.
E-mail address: phyto_met@yahoo.com (A.V. Ramachandran).

solvent extracts (aqueous, ethanolic, hexane or chloroform). In this context, the present study attempts to assess the possible hepato-protective potential of the polyphenol rich hydro-ethanolic MK leaf extract in CCl$_4$-induced hepatotoxicity in rats.

2. Materials and methods

2.1. Collection of leaves and preparation of MK leaf extract

Fresh MK leaves obtained locally and authenticated by Dr. Padmanabhi S. Nagar, Assistant Professor, Department of Botany, The M.S. University of Baroda, Vadodara were rinsed in water and shade dried. Ten grams of dried leaves were soaked in 100 ml of ethanol:water (1:1) for 3 days and filtered using a sterilized muslin cloth. Resultant extract was concentrated in a rotary evaporator under reduced pressure to obtain a thick semisolid paste. The final yield obtained from 10 gm dried leaves was 20% W/W. Known amounts (200, 400 and 600 mg) of the extract were weighed and freshly reconstituted in 1 ml of 0.5% carboxymethyl cellulose (CMC; viscosity 0.5) every day before administration.

2.2. Experimental animals

Adult Male Wistar rats (175 ± 25 gm) obtained from Zydus Cadila Research Centre, Ahmedabad, Gujarat, India were housed in clean polypropylene cages and maintained in the animal house under controlled conditions of temperature (23 ± 2 °C), humidity (45–50%) and photoperiod (LD 12:12). The rats were fed with standard pellet diet (M/S Pranav agro, Ltd., Baroda, India) and provided with water *ad libitum* throughout the period of study. The experimental protocol was reviewed, discussed and approved by the animal ethical committee of the Department of Zoology, The M.S. University of Baroda, Vadodara (Approval No. 827/ac/04/CPCSEA) and carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.3. Experiment design

A total of 36 rats were equally divided into six groups (*n* = 6). Group I served as normal control (NC) and received 0.5% carboxymethyl cellulose (CMC) solution orally daily for 15 days. Group II (CCl$_4$) was given 0.5% CMC solution daily for 15 days. Group III (CCl$_4$ + MK200), IV (CCl$_4$ + MK400) and V (CCl$_4$ + MK600) were orally given MK extract (200, 400 and 600 mg/kg) daily for 15 days. Based upon the results obtained in dose response studies, it was inferred that dosages less than 100 mg/kg bodyweight did not record any favorable response whereas, doses above 600 mg/kg bodyweight showed identical and comparable results. Hence, 200, 400 and 600 mg doses were chosen for the present study. Group VI (CCl$_4$ + SYL) served as reference control and were orally given commercially available Silymarin (Silybon-70, Microlab Ltd., Dist. Solan HP; SYL; 25 mg/kg) daily for 15 days (Manokaran et al., 2008). Groups II, III, IV, V and VI were injected with a single dose of CCl$_4$ (0.5 ml/kg intraperitoneally) on 15th day of the study (Arhoghro et al., 2009; Jadeja et al., 2011).

2.4. Collection of blood and tissues

On the 16th day, blood was collected in EDTA coated vials from over-night fasted animals by retro orbital sinus puncture under mild ether anesthesia. Plasma was obtained by centrifugation of the vials at 3000 rpm for 10 min at 4 °C. Blood was also collected in uncoated vials and serum was obtained by a similar process. Animals were later sacrificed by cervical dislocation under mild ether anesthesia for autopsy and liver was excised and rinsed in 0.9% saline.

2.5. Estimation of plasma markers of hepatic damage

Liver damage was assessed by estimating the plasma levels of ALT, AST, total protein, and bilirubin using commercially available test kits from Recon Diagnostics Ltd., Vadodara, India. Activity levels of serum ALP were assessed by using the test kits of the same make.

2.6. Methodology for estimation of plasma and serum markers for hepatic damage

2.6.1. Assay for ALP

1.0 ml buffered substrate was added to 0.02 ml sample, mixed and the absor-bance was read at 30, 60, 90 and 120 s at 405 nm. The final values were expressed in U/L.

2.6.2. Assay for AST and ALT

1.0 ml working reagent was added to 0.05 ml sample, mixed, incubated (37 °C for 60 s) and read at 340 nm continuously for 2 min at an interval of 30 s. The values were expressed in U/L.

2.6.3. Assay for total protein

1.0 ml reagent was added in 0.02 ml sample, mixed well and incubated at room temperature for 10 min. Identical amount of aliquots for standard and distilled water blank were used and absorbance was read at 540 nm against reagent blank. The values were expressed in g/dL.

2.6.4. Assay of bilirubin

A set of working solutions 1, 2 and 3 were used for the assay of plasma bilirubin by monochromatic method. 50 µl aliquot was added in sample blank and test sam-ple tubes followed by 100 µl of solutions 2 + 3 in sample blank and, solutions 1 + 3 in test sample respectively. The contents were mixed and incubated for 5 min at room temperature and read against sample blank at 546 nm and the values were expressed in mg/dl.

2.7. Estimation of lipid peroxidation and enzymatic and non-enzymatic anti-oxidants

Enzymatic anti-oxidants such as super oxide dismutase (SOD: Kakkar et al., 1984) and catalase (CAT: Aebi, 1983) were assayed in liver homogenate of control and treated rats. The levels of lipid peroxidation (LPO: Buege and Aust, 1978) and contents of non-enzymatic anti-oxidants, reduced glutathione (GSH: Beutler et al., 1963) and ascorbic acid (AA: Roe and Küether, 1943) and, protein (Lowry et al., 1951) were estimated in the liver homogenate of control and treated rats. Tis-sue homogenates were prepared in phosphate buffer saline (pH 7.4) using a homog-enizer and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was used immediately for assay of all parameters. Homogenate for ascorbic acid was pre-pared in 6% trichloro acetic acid.

2.8. Histology of liver

The liver samples of control and treated animals were collected and fixed in neutral buffer formalin, processed and paraffin embedded as per the standard pro-tocol. Seven micron thick sections were cut and stained with hematoxylin and eosin and, observed for possible histopathological changes. The stained sections were ob-served under Leica microscope and photographed using a Canon power shot S57 digital camera (400×) for documenting evidence for possible hepatic damage.

2.9. Statistical analysis

Statistical evaluation of the data was done by one-way ANOVA followed by Bon-ferroni's multiple comparison tests. The results were expressed as mean ± SE using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA.

3. Results

3.1. Plasma markers of hepatotoxicity

Significant increase ($p < 0.001$) in the plasma levels of AST, ALT, ALP and bilirubin were observed in CCl$_4$ treated group as compared to the NC group. However, the MK treated groups showed significantly lesser increase in a dose dependent manner, with the highest dose of MK (CCl$_4$ + 600) and SYL (CCl$_4$ + SYL) recording comparable levels of protection. Total hepatic protein content was significantly ($p < 0.001$) lowered after CCl$_4$ treatment while, CCl$_4$ + MK and SYL treated groups recorded significantly ($p < 0.001$) min-imal decrease (Table 1).

3.2. Enzymatic and non-enzymatic antioxidants and lipid peroxidation

Animals exposed to CCl$_4$ showed significant ($p < 0.001$) decrease in the levels of both enzymatic (SOD and CAT) and non-enzymatic (GSH and AA) antioxidants and increase in LPO compared to NC animals. However, CCl$_4$ + MK treated groups depicted significantly lesser decrease in antioxidants and significantly lesser increase in LPO with a clear dose dependency. The levels of antioxidants and indices of LPO in CCl$_4$ + MK600 group were very much comparable to those in the CCl$_4$ + SYL group (Table 2).

3.3. Histological observations

Sections of liver (HXE stained) of NC rats showed intact central vein and hepatic cords with healthy hepatocytes and thin

Table 1
Effect of *Murraya koenigii* L. leaves extract on plasma hepatic markers.

	NC	CCl$_4$	CCl$_4$ + MK200	CCl$_4$ + MK400	CCl$_4$ + MK600	CCl$_4$ + SYL
ALT[†]	50.25 ± 3.68	165.8 ± 10.70[C]	79.50 ± 5.05[c]	68.00 ± 4.91[c]	64.00 ± 3.48[c]	61.00 ± 3.74[c]
AST[†]	94.25 ± 9.37	372.3 ± 19.51[C]	150.8 ± 16.63[c]	145.8 ± 14.41[c]	127.5 ± 13.03[c]	116.6 ± 10.49[c]
ALP[†]	104.8 ± 5.74	357.0 ± 6.46[C]	289.8 ± 8.27[c]	217.8 ± 8.54[c]	160.9 ± 10.98[c]	141.7 ± 7.14[c]
Total protein[§]	7.33 ± 0.17	4.23 ± 0.26[C]	6.19 ± 0.21[c]	6.37 ± 0.33[c]	6.56 ± 0.36[c]	6.68 ± 0.42[c]
Bilirubin[ϵ]	0.08 ± 0.01	0.44 ± 0.05[C]	0.19 ± 0.08[a]	0.14 ± 0.04[c]	0.10 ± 0.04[c]	0.09 ± 0.03[c]

Where † = Unit/L, § = g/dl, ϵ = mg/dl. Data are expressed as the mean ± SE. Where (C) $p < 0.001$ and when, NC v/s CCl$_4$, and (a) $p < 0.05$, (c) $p < 0.001$, when, CCl$_4$ v/s CCl$_4$ + MK200, CCl$_4$ + MK400, CCl$_4$ + MK600 and CCl$_4$ + SYL.

Table 2
Effect of *Murraya koenigii* L. leaves extract on hepatic enzymatic and non-enzymatic antioxidants.

	NC	CCl$_4$	CCl$_4$ + MK200	CCl$_4$ + MK400	CCl$_4$ + MK600	CCl$_4$ + SYL
SOD[λ]	26.57 ± 0.99	13.90 ± 1.07[C]	16.63 ± 0.38[a]	21.16 ± 1.05[c]	22.62 ± 1.75[c]	24.61 ± 1.56[c]
Catalase[λ]	24.26 ± 0.64	7.67 ± 0.62[C]	10.27 ± 0.74[a]	12.38 ± 0.30[c]	15.31 ± 0.61[c]	21.26 ± 1.46[c]
GSH[δ]	6.17 ± 0.14	2.30 ± 0.42[C]	3.34 ± 0.33[ns]	4.06 ± 0.22[b]	5.28 ± 0.29[c]	5.66 ± 0.25[c]
LPO[η]	3.07 ± 0.17	8.14 ± 0.67[C]	5.87 ± 0.34[a]	5.01 ± 0.18[b]	3.50 ± 0.30[c]	3.21 ± 0.32[c]
Ascorbic acid[δ]	4.04 ± 0.19	2.46 ± 0.27[C]	2.94 ± 0.18[ns]	3.15 ± 0.16[ns]	3.48 ± 0.12[b]	3.63 ± 0.14[b]

Where λ = Unit/min/mg protein, δ = mg/g, η = nmols MDA/mg protein. Data are expressed as the mean ± SE. Where (C) $p < 0.001$ and when, NC v/s CCl$_4$, and (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.001$, ns: non-significance when, CCl$_4$ v/s CCl$_4$ + MK200, CCl$_4$ + MK400, CCl$_4$ + MK600 and CCl$_4$ + SYL.

sinusoidal spaces. However, CCl$_4$ treated rats showed central vein disruption, ballooned lipid laden hepatocytes and dilated sinusoidal spaces. However, CCl$_4$ + MK200 treated rats showed moderate hypertrophy of hepatocytes with relatively intact central vein and marginal distortions of sinusoids. CCl$_4$ + MK (400 or 600) showed intact central vein and well-marked hepatic cords, features very much comparable those seen in CCl$_4$ + SYL treated liver (Fig. 1).

4. Discussion

Polyphenols exhibit antibacterial, antifungal, anti-viral, anti-inflammatory, anti-carcinogenic, immunomodulatory, cardioprotective and hepatoprotective effects. These pharmacological effects are attributable to their potent antioxidant and free radical scavenging property. Maximal polyphenol content and superior free radical scavenging ability stand attributed to hydro-ethanolic extract of MK leaves (Ningappa et al., 2008). The present study designed to evaluate the competency of hydro-ethanolic extract of MK leaves in preventing CCl$_4$-induced hepatic damage reveals a dose dependent favorable effect. The extract has been also given for 28 days in normal animals as a part of toxicological evaluation and it was found to be non-toxic up to 3000 mg/kg body weight (unpublished observation).

Metabolic activation of CCl$_4$ by cytochrome P$_{450}$ dependent monooxygenase and consequent formation of trichloromethyl radical (CCl$_3^{\cdot}$) is well established. The CCl$_3^{\cdot}$ radicals attack polyunsaturated fatty acids in presence of oxygen to produce lipid peroxides contributing to liver damage (Bishayee et al., 1995). The resultant hepatic damage is assessable by the serum levels of marker enzymes (ALT, AST and ALP) and bilirubin as they leach out of hepatocytes into circulation denoting hepatotoxicity. The
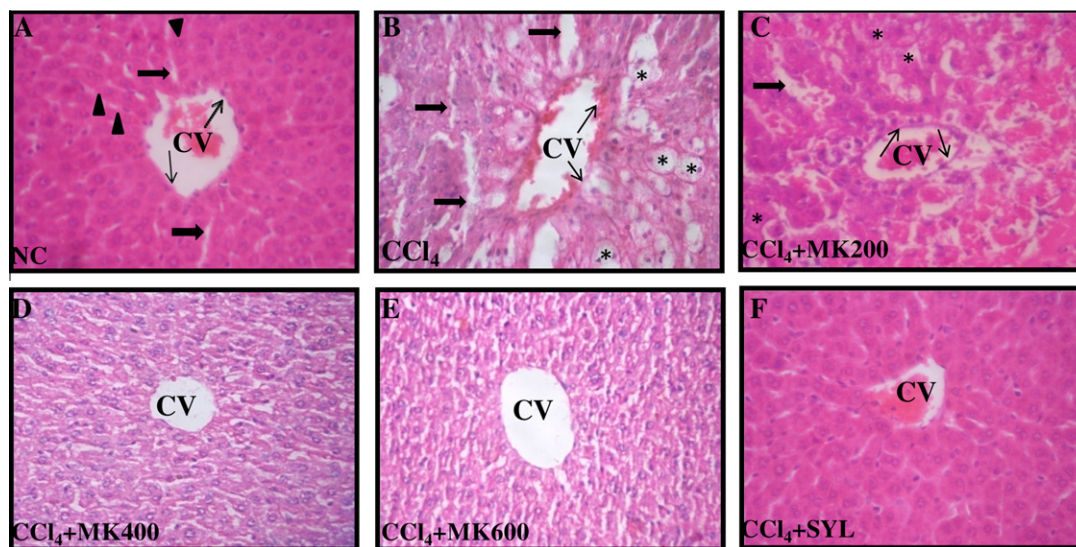

Fig. 1. Photomicrographs of sections of liver section of control and experimental rats. (A) Control liver showing intact central vein (thin arrows), narrow sinusoidal spaces (thick arrows) and well formed hepatic cords (arrow head). (B) CCl$_4$ treated liver showing disruptions in central vein (thin arrows), distended sinusoidal spaces (thick arrows) and lipid laden ballooned hepatocytes (asterisk). (C) MK extract (200 mg) supplemented liver showing more or less intact central vein (thin arrows), reduced though still distended sinusoidal spaces (thick arrows) and hypertrophied but lipid free hepatocytes (asterisk). (D) and (E) MK extract (400 and 600 mg) supplemented liver showing near normal hepatic architecture and (F) Sylimarin administered liver showing almost normal histological features.

elevated levels of these serum markers in CCl$_4$ treated animals herein clearly indicate hepatotoxicity. Several studies accredit the hepatoprotective effect of drugs and herbal agents to their antioxidant and free radical scavenging abilities (Naik and Panda, 2007; Kodai et al., 2007). Pre-treatment with MK extract prevents significantly the increase in the levels of serum markers induced by CCl$_4$ indicating the potent hepatoprotective effect by way of its free radical scavenging role.

CAT, a hemoprotein present in all aerobic cells, metabolizes H$_2$O$_2$ to oxygen and water while SOD, an exceedingly effective defense enzyme catalyzes the dismutation of superoxide anions to hydrogen peroxide (H$_2$O$_2$). Several studies identify them as a pair of powerful protective enzyme battery against oxidative damage (Halliwell and Gutteridge, 1990; Reiter et al., 2000; Wang et al., 2004). The non-enzymatic antioxidants, GSH and AA protect against xenobiotic toxicity by scavenging free radicals and getting themselves oxidized in the process (Kadiska et al., 2000) and as such, CCl$_4$-induced hepatotoxicity is known to be induced by the free radicals formed from it (CCl$_3^-$ and/or CCl$_3$OO$^{\cdot}$). However, feeding of rats with MK leaf extract prior to CCl$_4$ treatment successfully prevents the depletion in non-enzymatic and enzymatic antioxidants in a dose dependent manner. Other hepatoprotective agents such as Sylimarin (Manokaran et al., 2008), vitamin E (Sodergren et al., 2001), *Hippophae rhamnoides* seed oil (Hsu et al., 2009), Zizyphus spina-christi (Amin and Mahmoud-Ghoneim, 2009), *Platycodon grandiflorum* (Lee et al., 2008), *Commiphora berryi* (Gowri Shankar et al., 2008) and *Phyllanthus niruri* (Bhattacharjee and Sil, 2007) have all been reported to prevent ROS mediated lipid peroxidation by their free radical scavenging ability. A previous report on the phytochemical composition of MK leaf extract revels the hydro-ethanolic extract to have maximal content of polyphenols (12%) compared to other solvent extracts (Ningappa et al., 2008). In the present study, the 'near normal' levels of enzymatic and non-enzymatic antioxidants recorded in CCl$_4$ + MK treated groups further underlines the importance of high content of polyphenols in safe guarding the endogenous cellular antioxidant machinery.

Elevated indices of LPO observed herein are indicative of CCl$_4$-induced hepatotoxicity and cellular damage has also been reported by Castro (1974). However, lowered indices of hepatic LPO in CCl$_4$ + MK treated groups indicate the protective effect of MK and the resultant minimal hepatocyte membrane damage. These observations stand further validated by the histologically visible disruption of central vein, centrilobular necrosis, hepatocyte ballooning, infiltration and hepatic lesion in CCl$_4$ treated rats, as also reported by Plaa and Charbonneau (1989), and the prevention of these effects in MK400 and MK600 groups of rats. These histological observations are in agreement with the observed effects on the levels of enzymatic and non-enzymatic antioxidants, LPO and plasma markers of liver function. The observations together provide substantial evidence for the protective effect of MK extract against CCl$_4$-induced hepatic damage, essentially by preventing the formation of superoxide anion and H$_2$O$_2$ and/or by exerting a sparing effect on the antioxidant enzymes.

5. Conclusion

It can be concluded from the present study that the high content of polyphenols in hydro-ethanolic extract of MK leaves has hepatoprotective potential by effectively countering the CCl$_4$-induced oxidative damage of hepatocytes by conserving the endogenous antioxidant machinery and scavenging free radicals.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Aebi, H., 1983. Catalase. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis, vol. 3. Academic Press, New York, pp. 276–286.

Amin, A., Mahmoud-Ghoneim, D., 2009. Zizyphus spina-christi protects against carbon tetrachloride-induced liver fibrosis in rats. Food Chem. Toxicol. 47, 2111–2119.

Arhoghro, E.M., Ekpo, K.E., Anosike, E.O., Ibeh, G.O., 2009. Effect of aqueous extract of bitter leaf (Vernonia Amygdalina Del) on carbon tetrachloride (CCl$_4$) induced liver damage in albino Wistar rats. Eur. J. Sci. Res. 26, 122–130.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.

Bhakuni, D.S., Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N., 1969. Screening of Indian plants for biological activities. Part II. Indian J. Exp. Biol. 7, 250–262.

Bhattacharjee, R., Sil, P.C., 2007. Protein isolate from the herb, *Phyllanthus niruri* L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. Food Chem. Toxicol. 45, 817–826.

Birari, R., Javia, V., Bhutani, K.K., 2010. Antiobesity and lipid lowering effects of *Murraya koenigii* (L.) spreng leaves extracts and mahanimbine on high fat diet induced obese rats. Fitoterapia 81, 1129–1133.

Bishayee, A., Sarkar, A., Chaterjee, M., 1995. Hepatoprotective activity of carrot against carbon tetrachloride intoxication in mouse liver. J. Ethanopharmacol. 47, 69–74.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Castro, J.E., 1974. Antitumour effects of *Corynebacterium parvum* in Mice. Eur. J. Cancer. 10, 121.

Chakrabarty, M., Nath, A., Khasnobis, S., 1997. Carbazole alkaloids from *Murraya koenigii*. Phytochemistry 46, 751–755.

Das, K.C., Chakraborty, D.P., Bose, P.K., 1965. Antifungal activity of some constituents of *Murraya koenigii* Spreng. Experentia 6, 340.

Dasgupta, T., Rao, A.R., Yadava, P.K., 2003. Chemomodulatory action of curry leaf (*Murraya koenigii*) extract on hepatic and extrahepatic xenobiotic metabolising enzymes, antioxidant levels, lipid peroxidation, skin and forestomach papillomagenesis. Nut. Res. 23, 1427–1446.

Fiebig, M., Pezzuto, J.M., Soejarto, D.D., Kinghorn, A.D., 1985. Koenoline, a further cytotoxic carbazole alkaloid from *Murraya koenigii*. Phytochemistry 24, 3041–3043.

Gowri Shankar, N.L., Manavalan, R., Venkappayya, D., David Raj, C., 2008. Hepatoprotective and antioxidant effects of *Commiphora berryi* (Arn) Engl barkextract against CCl$_4$-induced oxidative damage in rats. Food Chem. Toxicol. 46, 3182–3185.

Halliwell, B., Gutteridge, J.M., 1990. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 186, 1–85.

Hasegawa, R., Chujo, T., Sai-kato, K., Umemura, T., Tanimura, A., Kurokawa, Y., 1995. Preventive effects of Green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food. Chem. Toxicol. 33, 961–965.

Hsu, Y.W., Tsai, C.F., Chen, W.K., Lu, F.J., 2009. Protective effects of seabuckthorn (*Hippophae rhamnoides* L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem. Toxicol. 47, 2281–2288.

Ivor, J.B., Schneider, M.D., 2005. Learning from failure: congestive heart failure in the postge- nomic age. Review series introduction. J. Clin. Invest. 115, 495–499.

Jadeja, R.N., Thounaojam, M.C., Ansarullah, Jadav, S.V., Patel, M.D., Patel, D.K., Salunke, S.P., Padate, G.S., Devkar, R.V., Ramachandran, A.V., 2011. Toxicological evaluation and hepatoprotective potential of *Clerodendron glandulosum* Coleb leaf extract. Hum. Exp. Toxicol. 30, 63–70.

Kadiska, M.B., Gladen, B.C., Baird, D.D., Dikalov, A.E., Sohal, R.S., Hatch, G.B., Jones, D.P., Mason, R.P., Barret, J.C., 2000. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl$_4$ poisoning? J. Free. Rad. Biol. Med. 28, 838–845.

Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of SOD. Indian J. Biochem. Biophys. 21, 130–132.

Khan, M.R., Ahmed, D., 2009. Protective effects of *Digera muricata* (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. Food. Chem. Toxicol. 47, 1393–1399.

Khan, M.R., Rizvi, W., Khan, G.N., Khan, R.A., Shaheen, S., 2009. Carbon tetrachloride induced nephrotoxicity in rat: protective role of *Digera muricata*. J. Ethnopharmacol. 122, 91–99.

Kodai, S., Takemura, S., Minamiyama, Y., Hai, S., Yamamoto, S., Kubo, S., Yoshida, Y., Niki, E., Okada, S., Hirohashi, K., Suehiro, S., 2007. S-allyl cysteine prevents CCl$_4$-induced acute liver injury in rats. Free Radic. Res. 41, 489–497.

Kumar, G., Banu, G.S., Pandian, M.R., 2005. Evaluation of the antioxidant activity of Trianthema portulacastrum L. Ind. J. Pharmacol. 37, 331–333.

Lee, K.J., Choi, J.H., Kim, H.G., Han, E.H., Hwang, Y.P., Lee, Y.C., Chung, Y.C., Jeong, H.G., 2008. Protective effect of saponins derived from the roots of *Platycodon grandiflorum* against carbon tetrachloride induced hepatotoxicity in mice. Food Chem. Toxicol. 46, 1778–1785.

Lowry, O.H., Rosenbrough, N.J., Farr, A.I., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Manokaran, S., Jaswanth, A., Sengottuvelu, S., Nandhakumar, J., Duraisamy, R., Karthikeyan, D., 2008. Hepatoprotective activity of *Aerva lanata* Linn. against paracetamol induced hepatotoxicity in rats. Res. J. Pharm. Tech. 1, 398–400.

Naik, S.R., Panda, V.S., 2007. Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int. 27, 393–399.

Naraya, K., Sastry, K.N.V., 1975. The hypoglycaemic effects of *Murraya koenigii* (Spreng.) in normal and alloxan diabetic dogs. Mysore. J. Agric. Sci. 9, 132–136.

Ningappa, M.B., Dinesha, R., Srinivas, L., 2008. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. Food Chem. 106, 720–728.

Obi, F.O., Omogbai, L.A., Oriafo, S.J., Ovat, O.D., 2001. Effect of a short time post carbon tetrachloride treatment interval on rat plasma enzyme levels and percentage mortality. J. Appl. Sci. 5, 5–8.

Plaa, G.L., Charbonneau, M., 1989. Detection and evaluation of chemically induced liver injury. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology. Raven Press, New York, pp. 399–628.

Rao, Y.K., Lu, S.C., Liu, B.L., Tzeng, Y.M., 2006. Enhanced production of an extracellular protease from Beauveria bassiana by optimization of cultivation processes. Biochem. Eng. J. 28, s57–s66.

Rastogi, R.P., Mehrotra, B.N., 1998. Compendium of Indian medicinal plant. Central Drug Research Institute of Lucknow, India 5, 629–630.

Recknagel, R.O., Glende, I.A., 1973. Carbon tetrachloride hepatotoxicity. An example of lethal cleavage. CRC Crit. Rev. Toxicol. 2, 263.

Recknagel, R.O., Glende, E.A., Dolak, J.A., Waller, R.L., 1989. Mechanisms of carbon tetrachloride toxicity. Pharmacol. Ther. 43, 139–154.

Reiter, R.J., Tan, D., Osuna, C., Gitto, E., 2000. Actions of melatonin in the reduction of oxidative stress. J. Biomed. Sci. 7, 444–458.

Roe, J.H., Küether, C.A., 1943. The determination of ascorbic acid in whole blood and urine through 2–4 dinitrophenyl hydrazine derivative of dihydro ascorbic acid. J. Biol. Chem. 147, 399–407.

Sathaye, S., Bagul, Y., Gupta, S., Kaur, H., Redkar, R., 2011. Hepatoprotective effects of aqueous leaf extract and crude isolates of *Murraya koenigii* against *in vitro* ethanol-induced hepatotoxicity model. Exp. Toxicol. Pathol. 63, 587–591.

Satyavati, G.V., 1987. In: Gupta, A.K., Tandon, N. (Eds.), Medicinal Plants of India, vol. 2. Cambridge Printing works, India, pp. 347–351.

Shah, A.S., Wakade, A.S., Juvkar, A.R., 2008. Immunomodulatory activity of methanolic extract of *Murraya koenigii* (L.) spreng leaves. Indian J. Exp. Biol. 46, 505–509.

Sodergren, E., Cederberg, J., Vessby, B., Basu, S., 2001. Vitamin E reduces lipid peroxidation in experimental hepatotoxicity in rats. Eur. J. Nutr. 40, 10–16.

Vitaglione, P., Morisco, F., Caporaso, N., Fogliano, V., 2004. Dietary antioxidant compounds and liver health. Crit. Rev. Food. Sci. Nutr. 44, 575–586.

Wang, B.J., Lien, Y.H., Yu, Z.R., 2004. Supercritical fluid extractive fractionation: study of the antioxidant activities of propolis. Food Chem. 86, 237–243.

Research Paper

ACUTE AND SUB-CHRONIC TOXICOLOGICAL EVALUATION OF *Anethum graveolens* L. SEED EXTRACT

Swati N. Desai[1] and Dipak K. Patel[2]

[1]Division of Phytotherapeutics and Metabolic Endocrinology, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390002, Gujarat, India,
[2]Section Ecotoxicology, Department of Chemistry, Jai Research Foundation, Next to Daman Ganga Bridge, N.H. No. 8 Valvada, Dist- Valsad - 396 108.

Abstract

Anethum graveolens L. belonging to *Apiaceae* (*Umbelliferae*) family, is an annual aromatic herb known for culinary and medicinal use since ancient times. The well-known properties of *Anethum graveolens* L. from the traditional medicine are carminative, stomachic, diuretic, antibacterial, antioxidant, antisecretory, antispasmodic, insecticidal, anti-diabetic, anti-cancer, Hypolipidemic and antihypercholesterolaemic. This study was aimed to assess the toxicity profile of seed extract of *Anethum graveolens* L. by determining its effects after administration of acute and subcronic doses in swiss albino mice. Swiss albino mice were divided into four groups of six animals in each group. In acute toxicity study the mice were administered single oral doses of 1000, 2000, 3000 and 5000mg/kg body weight and observed general behavior, adverse effect and possible mortality for 24 hours after administration. In the chronic dose study, the extract was administered orally at the doses of 1000, 2000 and 3000mg/kg body weight and control group was administered vehicle Carboxy Methyl Cellulose (0.5%) for 28 days. There was neither adverse effect nor death in treated groups. There were no alteration in organ weight, hematological profile and biochemical parameters The overall finding of this study indicates that this extract is absolutely non-toxic and considered as safe.
Key words: Acute toxicity, *Anethum graveolens* L., Seeds, Sub-chronic toxicity.

INTRODUCTION

Medicinal plants are widely used in traditional medicine and play an important role in the development of novel pharmacological agents [1, 2]. In recent times, herbal medicine has been gaining wide acceptance. One reason for this trend is the cost of orthodox medicines which put them beyond the reach of many people particularly in resource poor countries [3, 4]. World Health Organization (WHO) stressed its commitment in encouraging the development of public policies with the objective of inculcating herbal medicine in national health system of its member states in its global strategy on traditional, complementary and alter-native medicine, for the period 2014–2023 [5].

The one of the Indian traditional medicinal system is Ayurveda and it has been mentioned even in the ancient Vedas and other scriptures. It has been evolved between 2500 and 500 BC in India and it is based on the use of plants for the remediation [6]. Approximately 70 percent of rural population has been using traditional Ayurvedic system of medicine. The Ayurvedic practitioners of the traditional systems of medicine prepare formulations by their own recipes and used for healing ailments. Approximately 40 per cent of people are using the herbal medicine for the treatment of various diseases in western countries [6].

The herbal drugs, tinctures, extracts, resin, and latex are derived from the plants; they are derived from natural sources and have been used considered as non toxic. However, evidences on the toxicity risks associated with a wide variety of such remedies have emerged in the last few years [2, 7]. Hence, it is important that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated [8, 9].

Despite the interesting results obtained from the pharmacological studies and their potential therapeutic usefulness, no pharmacological study or toxicological investigation of AG has been reported. In this study, we have evaluated acute and sub-chronic toxicity of hydro-ethanolic extract of AG seeds.

MATERIALS AND METHODS
Collection of Plant
The seeds of AG were purchased locally from the market sown and allowed to grow. Fully grown plants were harvested, identified and authenticated at the Department of Botany, Faculty of Science, The M. S. University of Baroda, Vadodara.

Preparation of Plant Extract
100 gm of dried seeds were soaked in 100 ml ethyl alcohol: water (1:1) for 3 days. The filtrate kept in rotatory evaporator for evaporate ethanol and water and obtained semisolid paste was stored at -20ºC. The yield was 20% W/W. Known amount of the extract was weighed and reconstituted in appropriate volume of 0.5% carboxy methyl cellulose (CMC).

Experimental Animals
Adult swiss albino mice weighing between 20-25 g were obtained from Zydus Cadilla Research Centre, Ahmedabad, Gujarat, India. They were housed and maintained in clean poly-propylene cages placed in animal house conditions (temperature: 23 ± 2°C; photoperiod: 12 h light and 12 h dark; humidity: 45-50%). They were fed with standard laboratory pellets (M/S Pranav agro, Ltd., Baroda, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India and approved by the animal ethical committee of Department of Zoology, The M.S. University of Baroda, Vadodara (Approval No.827/ac/04/CPCSEA).

Experimental Design
Acute Oral Toxicity
The acute oral toxicity study was conducted according to the guidelines of Organization for Economic Cooperation and Development (OECD) test 420. Twenty four mice were randomly divided into four groups of six animals in each group. Various doses (1000 mg/Kg, 2000 mg/Kg, and 3000 mg/kg and 5000 mg/kg body weight, respectively) of seed extract of AG were administered orally to respective groups of mice. Mice were

observed closely for toxic symptoms and behavioral changes for two hours after administration and possible mortality was recorded within twenty four hours.

Sub-chronic Oral Toxicity

The sub chronic oral toxicity study was conducted according to the guidelines of OECD Test 407. The mice were divided into four groups of six mice per group. Groups I served as normal control (NC) and animals were administered vehicle (0.5% CMC) via gastric incubation. Groups II, III and IV were administered 1000 mg/kg, 2000 mg/kg and 3000 mg/kg doses of hydro ethanolic extract of AG respectively via gastric incubation for 28 days consequently. Food and water intake of each group were monitored regularly.

Body Weight

The body weight of each mouse was assessed once before commencement of dosing, once every 7 days during the dosing period and once on the day of sacrifice. The relative body weight (RBW) of each animal was then calculated using the formula RBW= [absolute body weight of one time interval(g)/body weight of mouse on commencement of dosing day (g)]×100.

Collection of Blood and Autopsy of Tissues

At the end of 28 days, over night fasted animals were given mild ether anesthesia and blood was collected into ethylene diamine tetra acetate (EDTA) coated vials by retro orbital sinus puncture. The vials were centrifuged at 3000 rpm for 10 minutes at 4°C and plasma was collected and stored at -80 ºC for biochemical analysis. Animals were sacrificed by cervical dislocation under mild ether anesthesia and dissected. Liver, kidney, heart, lungs and spleen were excised and rinsed with 0.9% saline. All tissues were blotted dried and weighted.

Biochemical Parameters

Plasma levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Acid phosphatase (ACP), Alkaline phosphatase (ALP), Blood glucose, Urea, Creatinine, Total protein, Bilirubin, Lipid profile [Triglyceride (TG), Total cholesterol (TC) and HDL-C] were analyzed using commercially available kits (Recon diagnostic, Vadodara). Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL) were calculated by Friedewald's formula [10].

Hematology

At the end of 28 days, over night fasted animals were given mild ether anesthesia and blood was collected into EDTA coated vials by retro orbital sinus puncture for hematological analysis. White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Red cell distribution width (RCDW) estimations were carried out using an automated globular counter (BC 2300 Hematology Analyzer, Mindray).

Statistical Analysis

Statistical analysis of the data was done by one way ANOVA followed by Bonferroni's multiple comparison tests. The results were expressed as mean ± S.E.M using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA.

RESULTS

Acute Oral Toxicity

The mice treated with the dose 1000, 2000, 3000 and 5000mg/kg body weight did not show any behavioral changes and mortality for 24 after administration.

Sub-chronic Oral Toxicity

No remarkable changes in general behavior or other activities during 28 days study.

Relative Body Weight and Food Intake
No significant alterations were recorded in body weight and food intake (Table 1).
Relative Organ Weight
There were no significant changes in the relative weight of Heart, Lungs, Liver, Spleen, Kidney and adrenal gland (Table 2).
Hematology
The hematological parameters of experimental and control groups are presented in Table 3. The results suggest that all parameters (RBC, WBC, HB, MCV, MCH, MCHC, RCDW, monocytes, lymphocytes, eosinophil and platelets) remained within normal physiological rang throughout 28 days.
Biochemical Parameters
The values of different biochemical parameters are showed in Table 4. The activities of marker enzymes AST, ALT, ACP and ALP also did not show any significant changes. The chronic oral administration of AG extract did not alter the levels of creatinine, blood glucose, total protein and bilirubin. There were no noticeable changes in lipid profile (TC, TG, HDL, LDL and VLDL).

Table 1: Effect of *Anethum graveolens* L. Seed Extract on Body Weight, Food Intake and Water Intake

Groups	Body Weight (g)		Weight Gain (g)	Food Intake (g/day)	Water Intake (ml/day)
	Initial	Final			
NC	21.60 ± 0.72	25.32 ± 0.17	3.669 ± 0.76	2.88 ± 0.66	8.42 ± 0.17
AG1000	20.36 ± 0.19^{ns}	24.27 ± 0.13^{ns}	3.863 ± 0.21^{ns}	2.71 ± 0.18^{ns}	8.18 ± 0.10^{ns}
AG2000	20.53 ± 0.28^{ns}	23.38 ± 0.18^{ns}	2.798 ± 0.46^{ns}	2.47 ± 0.32^{ns}	8.65 ± 0.21^{ns}
AG3000	20.62 ± 0.20^{ns}	22.75 ± 0.20^{ns}	2.260 ± 0.28^{ns}	2.21 ± 0.42^{ns}	8.72 ± 0.20^{ns}

Where, n=6. Data were expressed as mean ± SE, A (p<0.05), B (p<0.01) and C (p<0.001) when NC v/s AG1000, AG2000 and AG3000

Table 2: Effect of *Anethum graveolens* L. Seed Extract on Relative Organ Weights

Organs (g)	NC	AG1000	AG2000	AG3000
Liver	4.59 ± 0.01	4.12 ± 0.20^{ns}	4.10 ± 0.10^{ns}	4.05 ± 0.08^{ns}
Kidney	1.47 ± 0.01	1.38 ± 0.05^{ns}	1.29 ± 0.03^{ns}	1.08 ± 0.07^{ns}
Heart	0.63 ± 0.01	0.60 ± 0.02^{ns}	0.54 ± 0.01^{ns}	0.49 ± 0.02^{ns}
Adrenal	0.05 ± 0.003	0.05 ± 0.004^{ns}	0.04 ± 0.007^{ns}	0.04 ± 0.005^{ns}
Lungs	0.63 ± 0.07	0.72 ± 0.06^{ns}	0.70 ± 0.07^{ns}	0.65 ± 0.10^{ns}
Spleen	0.45 ± 0.03	0.73 ± 0.10^{ns}	0.58 ± 0.04^{ns}	0.54 ± 0.03^{ns}

Where, n=6. Data expressed in mean ± SE, A (p<0.05), B (p<0.01) and C (p<0.001) when NC v/s AG1000, AG2000 and AG3000

Table 3: Effect of *Anethum graveolens* L. Seed Extract on Haematological Parameters

Parameters	NC	AG1000	AG2000	AG3000
RBC@	8.71 ± 0.65	8.97 ± 0.39[ns]	8.94 ± 0.27[ns]	8.77 ± 0.27[ns]
Hb§	14.78 ± 0.96	14.87 ± 0.95[ns]	14.70 ± 0.40[ns]	13.98 ± 0.34[ns]
MCV£	42.83 ± 1.34	41.43 ± 0.53[ns]	41.27 ± 0.32[ns]	41.47 ± 0.13[ns]
MCH¥	16.87 ± 0.45	16.90 ± 0.46[ns]	16.65 ± 0.52[ns]	16.56 ± 0.44[ns]
MCHC§	39.76 ± 0.49	39.53 ± 0.82[ns]	38.50 ± 0.47[ns]	38.30 ± 0.40[ns]
RCDWψ	17.83 ± 0.83	17.60 ± 0.42[ns]	16.70 ± 0.40[ns]	16.30 ± 0.40[ns]
WBCθ	6.03 ± 1.21	6.12 ± 0.64[ns]	4.76 ± 0.57[ns]	4.82 ± 1.07[ns]
Monocytesψ	1.76 ± 0.34	2.10 ± 1.00[ns]	1.56 ± 0.33[ns]	1.42 ± 0.37[ns]
Lymphocytesψ	22.77 ± 4.81	27.67 ± 1.55[ns]	32.00 ± 1.42[ns]	37.33 ± 3.80[ns]
Eosinophilsψ	2.30 ± 0.34	2.36 ± 0.33[ns]	3.60 ± 0.57[ns]	3.30 ± 0.57[ns]
Plateletθ	580.6 ± 26.49	612.6 ± 51.77[ns]	631.6 ± 27.76[ns]	645.4 ± 28.62[ns]

Where, @ = x 10^{12}/l, § = g/dl, £ = fl, ¥ = pg, ψ = %, θ = x 10^3/µl
RBC, red blood cell; Hb, Haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RCDW, red cell distribution width; WBC, white blood cell. Where, n=6. Data expressed in mean ± SE, A ($p<0.05$), B ($p<0.01$) and C ($p<0.001$) when NC v/s AG1000, AG2000 and AG3000.

Table 4: Effect of *Anethum graveolens* L. Seed Extract on Biochemical Parameters

Parameters	NC	AG1000	AG2000	AG3000
AST†	80.27 ± 1.72	80.00 ± 1.24[ns]	80.07 ± 1.59[ns]	80.60 ± 2.91[ns]
ALT†	33.27 ± 1.78	42.00 ± 4.15[ns]	44.87 ± 4.65[ns]	39.66 ± 2.31[ns]
ACP†	0.30 ± 0.02	0.33 ± 0.03[ns]	0.37 ± 0.04[ns]	0.33 ± 0.09[ns]
ALP†	18.06 ± 0.84	18.2 ± 0.92[ns]	19.3 ± 1.19[ns]	19.8 ± 0.76[ns]
Urea€	71.48 ± 5.56	80.90 ± 2.82[ns]	84.41 ± 2.43[ns]	86.35 ± 5.94[ns]
Creatinine€	0.48 ± 0.02	0.50 ± 0.02[ns]	0.51 ± 0.02[ns]	0.63 ± 0.03[B]
Blood glucose€	169.3 ± 6.54	157.7 ± 7.65[ns]	165.2 ± 5.72[ns]	178.50 ± 4.10[ns]
Total protein§	4.65 ± 0.20	4.62 ± 0.22[ns]	4.85 ± 0.26[ns]	4.71 ± 0.26[ns]
Bilirubin€	0.36 ± 0.05	0.30 ± 0.03[ns]	0.37 ± 0.05[ns]	0.32 ± 0.03[ns]
TC€	66.83 ± 2.93	59.00 ± 5.23[ns]	60.170 ± 3.99[ns]	63.00 ± 2.38[ns]
TG€	103.7 ± 3.70	97.00 ± 5.7[ns]	109.8 ± 3.48[ns]	95.50 ± 4.86[ns]
HDL€	20.75 ± 1.04	24.77 ± 1.48[ns]	21.65 ± 0.97[ns]	20.19 ± 1.30[ns]

VLDL$^{€}$	20.75 ± 1.04	24.77 ± 1.48[ns]	21.65 ± 0.97[ns]	20.19 ± 1.30[ns]
LDL$^{€}$	20.73 ± 0.74	19.40 ± 1.14[ns]	21.97 ± 0.69[ns]	19.10 ± 0.97[ns]

Where, † = Unit/L, €= mg/dl, § = g/dl

Where, n=6. Data expressed in mean ± SE, A ($p<0.05$), B ($p<0.01$) and C ($p<0.001$) and ns: non significance when NC v/s AG1000, AG2000 and AG3000

DISCUSSION

Since ancient times plants have been exemplary sources of medicine. 25 percent of modern medicines are derived from plants and the global market for herbal medicines currently stands over US$ 60 billion annually and growing steadily [11]. Increased popularity of herbal medicines might be due to easy access, relative low cost and lesser side effects than synthetic drugs. In present study, we evaluated the possible toxicity of hydro ethanolic extract of *Anethum graveolens* L. seeds. This type of study is needed before a phytotherapeutic agent can be generally recommended for use [12].

Acute toxicity evaluation reveals neither behavioral alterations nor mortality. Since AG extract is non-toxic even at a dose of 5000 mg/kg body weight, its LD_{50} value can be predicted to be more than 5000 mg/kg body weight. Hence, AG extract can be considered non-toxic and fit for human consumption [13]. Decrement in body and organ weights is co-related with adverse effect of drugs [14, 15, 16]. In the present study, AG extract fed mice did not record any significant alterations in body or organ weights, food and water intake. Hematology is one of the important indexes of physiological and pathological status in man and animal [17]. In our finding, there are no significant changes in hematological parameters like (White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width (RCDW) and platelet counts as compared to control group suggesting non-toxic effect of this plant extract.

AST and ALT are well known enzymes for indication of liver function [14, 18] and biomarkers for predicting possible toxicity [19]. Generally, the elevation of AST and ALT in serum indicates liver damage [20]. As the activity of both enzymes increases the liver damage also increases. In present study the AST and ALT levels are normal as compared to control group. It shows that given doses are non-toxic.

Creatinine is good indicator of renal function [14]. The rise in Creatinine level indicates kidney damage [21, 22]. There are not significant changes in Urea and Creatinine levels in this study. A rise in blood total Bilirubin level can be cause by liver cell damage [23]. In our finding, there is no significant change in bilirubin level. Decrease in total protein is related to high tissue demands due to liver disease associated with a reduction of protein synthesis [23]. Increase in plasma total protein indicates tissue damage [24]. Here, neither increase nor decrease in total protein level. There is no AG extract related changes in histopathology. Hence, the sub chronic toxicity study provides persuasive evidence for non-toxicity of AG leaf extract even up to 3000 mg/kg body weight.

In conclusion, this study does not show any significant alteration in hematological, biochemical and histological parameters. The acute and subcronic toxicity profiles are considered as non-toxic. This finding is useful for further *in vivo* and *in vitro* clinical study.

CONCLUSION

In conclusion, this study does not show any significant alteration in hematological, biochemical and histological parameters. The acute and subcronic toxicity profiles are considered as non-toxic.

REFERENCES

1   Munari, C.C., Alves, J.M., Bastos, J.K., Tavares, D.C., 2010, Evaluation of the genotoxic and antigenotoxic potential of *Baccharisdra cunculifolia* extract on V79 cells by the comet assay, J Appl Toxicol., 30: 22–28.

2   de Azevedo Neta Mahon, R.C.P.C., Colodel, E.M., Balogun, S.O., Oliveira, R.G., de Oliveira Martins, D.T., 2014, Toxicological evaluation of the hydroethanolic extract of *Dilodendron bipinnatum*, J Ethnopharmacol, 55: 665–671.

3   Osujih, M., 1993, Exploration of the frontiers of tradomedical practices: Basis for development of alternative medical healthcare services in developing countries, J R Soc Health, 113: 190-194.

4   Cohen-Kohler, J.C., 2007, The morally uncomfortable global drug gap, Clin Pharmacol Ther, 84: 610-614.

5   WHO, 2013.WorldHealthOrganization traditional medicine strategy: 2014–2023.

6   Pandey, M.M., Rastogi, S., Rawatm, A.K.S., 2013, Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation, eCAM, 1-12 .

7   Jesus, N.Z.T., Júnior, I.F.S., Lima, J.C.S., Colodel, E.M., Domingos, T.O., Martins, D.T.O, 2012, Hippocratic screening and subchronic oral toxicity assessments of the methanol extract of *Vataireama crocarpa* heart wood in rodents, Braz J Pharmacog, 22: 1308–1314.

8   Ernst, E., 2005, The efficacy of herbal medicine-an overview, Fund Clin Pharmacol, 19: 405-409.

9   Saad, B., Azaizeh, H., Abu-Hijleh, G., Said, O., 2006. Safety of traditional Arab herbal medicine, Evid Based Comp Alternat Med, 3: 433-439.

10   Friedewald , W.T., Levy, R.I., Fredrickson, D.S., 1972, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18: 499-502.

11   WHO, 2009. Traditional Medicine, Available at http://www. who.int/mediacentre/factsheets/fs134/en/ (Accessed 1.17.2009).

12   Abdel-Daim, M.M., Abuzead, S.M., Halawa, S.M., 2013, Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats, PLoS One, 8: e72991.

13   Diezi, J., 1989, Toxicologie: Principes de base etrépercussionscliniques*dans* Pharmacologie: des concepts fondamentaux aux applications thérapeutiques, Frisson-Roche., 33-43.

14   El-Hilaly, J., Israili, Z.H., Lyoussi, B., 2004, Acute and chronic toxicological studies of *Ajuva Iva* in experimental animals. J Ethnopharmacol, 91: 43-50.

15   Bakoma, B, Eklu-.Gadegbeku, K., Agbonon, A., Aklikokou, K., Bassene, E., Gbeassor, M., 2011, Preventive effect of *Bridelia ferruginea* Benth against high-fructose diet induced glucose intolerance, oxidative stress and hyperlipidemia in male *Wistar rats* J Pharmacol Toxicol, 3: 249–257.

16   Tahraoui, A., Israili, Z.H., Lyoussi, B., 2010, Acute and sub-chronic toxity of a lyophilised hydro ethanolic extract of *Centaurium erythraea* in rodents, J Ethnopharmacol, 132: 48–55.

17   Mukinda, J.T., Syce, J.A., 2007, Acute and chronic toxity of the hydro ethanolic extract of *Artemisia afra* in rodents, J Ethnopharmacol, 112: 138–144.

18   Ferreira, S.A., Guimarães, A.G., Ferrari, F.C., Carneiro, C.M., Paiva, N.C.N.D., Guimarães, D.A.S., 2014, Assessment of acute toxicity of the ethanolic extract of *Lychnophora pinaster* (Brazilian arnica), Rev Bras Farmacogn, 24: 553–560.

19   Rahman, S., Poulton, J., Marchington, D., Suomalainen, A., 2001, Decrease of 3243 A>G mtDNA mutation from blood in MELAS syndrome: a longitudinal study, Am J Hum Genet, 68: 238–40.

20   Dhluli, M., 2001, Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (Chlorocebusaethiops). Discipline of Physiological Sciences, University of the Western Cape, Cape Town, South Africa.

21   Lameire, N., Jager, K., Biesen, W.V., Bacquer, D.D., Vanholder, R.,2005, Chronic kidney disease: A European perspective, Kidney Int, 68: S30–S38.

22   Palm, M., Lundblad, A., 2005, Creatinine concentration in plasma from dog, rat, and mouse: A comparison of 3 different methods, Vet Clin Pathol, 34: 232–236.

23   Cheesbrough, M., 1991, Medical Laboratory Manual Countries. vol. 2: 479.

24   Emerson, F.S., Shadara, A.C., Devi, P.U., 1993, Toxic effects of Crude extract of *Plumbago rosea* (Rokta chitraka), J Ethnopharmacol, 38: 79-84.