INTERNATIONAL JOURNAL OF SCIENCE AND NATURE
© 2004 - 2017 Society For Science and Nature(SFSN). All Rights Reserved
www.scienceandnature.org

HEPATO-RENAL TOXICITY STUDIES OF THE CRUDE AQUEOUS LEAF EXTRACT OF *MEMECYLON MALABARICUM* Cogn. IN MALE WISTAR RATS

Bharathi, T.R., Noor Mohamed Jameel and *Prakash, H.S.
Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore– 570 006,
*Corresponding author email: jameel.nm@gmail.com

ABSTRACT
Memecylon malabaricum Cogn., is an indigenous medicinal plant used in ethno medicine including ayurveda. However, toxicity potential of this plant has not been evaluated so far. The present study evaluates the LD_{50} and hepato-renal toxicity of *M. malabaricum* which is used traditionally to treat herpes, anthelmintic and skin allergies. LD_{50} value was determined by feeding male Wistar rats with the single oral dose of *M. malabaricum* aqueous extract ranging from 300 to 2000 mg/kg body weight (BW) against control. Hepato-renal toxicity was analyzed by assay of functional markers of liver and kidney and histopathological and hematological studies were carried out by oral treatment of extract at the range of 0-2000 mg/kg BW for four weeks. Signs of toxicity like behavior, heartbeat, diarrhea, depression, and body weight loss were observed. Treatment of animals with leaf extract of *M. malabarium* does not show any mortality upto the dose of 2000 mg/kg BW. In lower concentrations, no hepato-renal toxicity, behavioral changes and hematological parameters was noted but at the higher concentration (1500 and 2000 mg/kg BW) slight increase in the liver functionality markers was observed after three weeks of treatment. It is therefore concluded that the dose of *M. malabaricum* aqueous extract below 2000 mg/kg BW may safely be used for therapeutic purposes in long term treatments.

KEYWORDS: Biochemical parameters, Histopathology and LD_{50}.

INTRODUCTION

Memecylon malabaricum Cogn. (Melastomataceae) the synonyms include *Memecylon* amplexicaule var. malabarica C.B. Clarke. Their common names include Volle kudi, Dodda nekkare, Hulli soppu, Locundi, Limbtoli, used in the treatment of herpes. Tender or mature leaves of Volle kudi along with caraway fruits, cow's milk or preferably cow's urine are ground to a syrupy paste and taken internally twice a day depending on the severity of herpes and also applied externally any number of times. In Ayurveda 'Volle kudi' is considered as pithahara and the leaves taken with water stops vomiting due to pitha. Paste of roots with lime juice is also applied to boils or wounds, and also as a bitter tonic, anthelmintic, female sterility cases, skin allergies and stomach disorders (Iyengar *et al.,* 1994; Prakasha *et al.*, 2010; Bharathi *et al.,* 2014). Several other biological properties have been reported such as anti-inflammatory, anti-diabetic, antioxidant and anti-microbial activity (Bharathi *et al.,* 2015; Bharathi *et al.,* 2016a; Bharathi *et al.*, 2016b; Ramasetty *et al.*, 2016). 4,9,14, 19-tetramethyl-1, 6, 11, 16-tetraoxacycloeicos-3, 8, 13, 19-tetraene (memecylaene) is a phytoconstituent which has been reported from *Memecylon malabaricum* (Rekha *et al.*, 2014). Several other phytoconstituents such as Isophthalic acid, Epigallocatechin, Myricetin, isorhamnetin 3-glucoside, tiliroside Spiraeoside etc., were characterised. Since various pharmacological studies of *M. malabaricum* has been carried out earlier, the toxicity profile, especially of their extracts, has not been yet explored. The present investigation is therefore carried out to study the lethal toxicity and hepato-renal toxicity of aqueous extract of *M. malabaricum*.

EXPIERMENT

Plant source

The entire plant of *M. malabaricum* was collected from the Kigga region of the Western Ghats, Karnataka during June 2015 and authenticated by plant taxonomist Prof. Sampath Kumara K.K. and herbarium specimens have been deposited in the herbarium (*M. malabaricum* # IOE LP0003) at the Department of studies in Biotechnology, University of Mysore, Mysore.

Preparation of plant extract

Fresh leaves were washed and dried under shade. They were ground to a powder. 25 g of ground material was suspended in 100mL of distilled water. The suspension was shaken and sonicated for 1h at room temperature (RT) and strained. The extraction process was repeated thrice and the extracts were filtered, evaporated in speed vac (Savant SPD 2010, Thermo Scientific) under vacuum and freeze dried and stored at 4°C. The yield was 3.37g from 25 g dry weight of leaf.

Experimental animals

Male Wistar rats weighing 180 ±20 g were used in the experiment and maintained on standard pellet diet and water *ad libitum*. Rats were maintained under standard rat house conditions for 20 days before the trial was initiated. The temperature of housing environment was maintained at 26 ±2ºC. The study was approved by the Institutional Animal Ethics Committee. The animal care and experimental procedures performed were in compliance with the Regulations for Animal Research and Animal

Ethical Committee of the UOM (Animal Order No: UOM/IAEC/07/2013).

Experimental plan

Animals were divided into two major groups. Group I are used for LD_{50} studies and Group II for hepato-renal toxicity studies. Group II rats were divided into 5 groups (5 rats in each group). The aqueous extract of *M. malabaricum* was selected by grading the doses as 300, 900, 1500 and 2000 mg/kg body weight (BW) along with control. Animals were treated orally with a special syringe that has needle equipped with a ball tip for four weeks with above doses and normal control rats received similar amount of distilled water.

Lethal dose (LD_{50})

Acute toxicity of *M. malabaricum* to male rats was determined. Rats were sub-grouped into five groups (3 rats in each group). Animals were treated orally with *M. malabaricum* leaf extract as a single dose. The first group was considered as a control. The second, third, fourth and fifth groups were treated with 300, 900, 1500 and 2000 mg/kg BW of *M. malabaricum* aqueous extract, respectively.

Animals were observed upto 72 h to check the mortality. The LD_{50} was calculated using the formulae,

$$\mathbf{LD_{50} = LD_{100} - \sum \times (a \times b)/n}$$

n = total number of animals in a group.

a=difference between two successive doses of administered extract/substance.

b = average number of dead animals in two successive doses.

LD_{100} = Lethal dose causing 100% death of all test animals.

Experimental design for hepato-renal toxicity studies

Rats used in this study were divided randomly into five groups, each of five rats wherein first group was considered as a control. The second, third, fourth and fifth groups were treated with 300, 900, 1500 and 2000 mg/kg BW of *M. malabaricum* aqueous extract, respectively.

Behavioural and wellness studies

The behavioral and wellness parameters such as mucous membrane, eyes, salivation, skin, movement, fur, sleep, tremors, lethargy, coma and diarrhea was evaluated in the treated as well as the control animals were analyzed.

Hematological studies

For hematological analysis, blood was collected and hematological parameters such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV), haemoglobin (Hb) concentration, total erythrocyte (Red Blood Cell, RBC's), absolute and differential leukocyte (White Blood Cell, WBC's) counts were evaluated according to the method described by (Rodak 1995; Vancampen *et al.,* 1996; Ambali *et al.,* 2007).

Biochemical study

The influences of aqueous extract of *M. malabaricum* on liver and kidney function markers were assessed by the estimation of alanine aminotransferase (ALT), aspartate aminotranferase (AST), (kits obtained from Beacon Diagnostics Pvt. Ltd., Navsari, India). Alkaline phosphatase (ALP) (kit obtained from Agappe Diagnostics Pvt. Ltd, Ernakulam, Kerala, India). Urea, uric acid and creatinine levels in plasma samples of normal control and plant extracts treated rats (kits obtained by Bio Direct Laboratories, La villeneuve-france and Elitect Laboratories, SEPPIMS A. France). All examinations were performed by standard methods using commercially available kits (as mentioned above) according to manufactures instructions.

Histopathological study

For the histopathological studies, liver and kidney tissue samples were collected and fixed in 10% buffered formalin, embeded in paraffin wax. Paraffin embedded tissue sections (4µm each) were deparaffinized, rehydrated and subjected to hematoxylin and eosin staining and examined microscopically at 1×400 magnification and results were recorded (Ghaffari *et al.,* 2013).

Statistical Analysis

Data are presented as a mean ± SEM. Comparisons were made between the treated groups by the use of single way analysis of variance (ANOVA). All the data were analysed using sigmastat version 3.1. $P< 0.05$ was considered as the level of statistical significance.

RESULTS

LD_{50} determination

This study was carried out as per the OECD guidelines 423. The LD_{50} values of *M. malabaricum* were determined at the doses of 300 mg/kg BW to 2000 mg/kg BW along with appropriate control and no mortalities in rats up to dose of 2000 mg/kg were observed. The oral LD_{50} was indeterminable being in excess of 2000 mg/kg BW. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-lethal.

General Sign and Behavioural Analysis

No significant changes were observed at oral doses of 0-2000 mg/kg body weight, except one hour after the administration of 1500 and 2000 mg/kg aqueous extracts, the rats becoming less active for 30 min. The behavioural and wellness parameters such as mucous membrane, eyes, salivation, skin, movement, fur, sleep of the treated as well as the control animals were analysed and used for the evaluation of toxicity which was found to be normal. Lethargy, tremors, coma and diarrhoea did not occur in any of the animal. After the administration of oral doses, in first 6 h rapid heartbeat was observed in the group treated with higher doses, it becomes normal after few min and this observation may be due to the stress while receiving the extract orally or immediate action of extract on animals. Furthermore, food intake and water consumption is determined, which is found to be normal (Table 1).

TABLE 1: Experimental observations of rats upto 2000 mg/kg dose of *M. malabaricum* methanol extract

Signs	Control	300 mg/kg	900 mg/kg	1500 mg/kg	2000 mg/kg
Behaviour	Normal	Normal	Normal	Normal	Normal
Skin and Fur	Normal	Normal	Normal	Normal	Normal
Eyes and mucous membranes	Normal	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Normal	Normal	Normal	Normal	Normal
Salivation	Absent	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent	Absent
Death	No	No	No	No	No
Other symptoms	Nill	Nill	Nill	Nill	Nill

Body weight

Changes in the weight of individual animals were calculated and compared with that control animals as stated in paragraph 26 of OECD guidelines 423. The weights of the animals were monitored every day after oral administration of extract. On the second day, little Weight loss was observed, but the weight increased again in the following days. However, there is no significant weight difference between treated and untreated animals. The results on animal weight, pre and post administration of *M. malabaricum* aqueous extract is shown in Table 2.

TABLE 2: Effect of *M. malabaricum* extract on body weight in rats

		Weeks			
Group	Initial weight	1	2	3	4
Control	184.12 ± 20.04	185.00 ± 7.38	186.75 ± 10.09	186.25 ± 0.96	190.09 ± 4.62
300 mg/kg	188.10 ± 22.02	180.45 ± 19.48	183.10 ± 13.29	190.20 ± 34.25	192.15 ± 15.26
900 mg/kg	180.60 ± 30.20	182.02 ± 41.22	184.25 ± 12.15	186.15 ± 12.23	190.15 ± 2.02
1500 mg/kg	181.20 ± 5.40	182.60 ± 21.23	185.20 ± 12.19	187.14 ± 10.22	190.40 ± 10.19
2000 mg/kg	182.02 ± 26.70	183.15 ± 16.23	184.10 ± 14.25	187.10 ± 14.07	191.25 ± 17.25

The mean body weight of rats treated with *M. malabaricum* leaf aqueous extract at different weeks and different doses. Values are expressed as mean ± SEM, $p<0.05$

Biochemical Analysis

Table 3 shows the changes of biochemical parameters in the rat serum in the experimental animals. In the treated group after the third week of oral administration of *M. malabaricum* extract there is an increase in liver parameters such as ALP, ALT and AST with higher doses (1500 and 2000 mg/kg BW). But there are no significant changes for the serum levels of TBIL, total protein and kidney function parameters at any dose level.

TABLE 3: Effect of *M. malabaricum* extract on biochemical parameters in toxicity study

Parameters	300 mg/kg	900 mg/kg	1500 mg/kg	2000 mg/kg	Control
Urea mg/dL	16.31 ± 0.91	16.34±0.86	16.39 ± 0.12	17.09 ± 1.8	16.2 ± 1.26
CRT mg/dL	0.61 ± 0.6	0.62 ± 0.8	0.62 ± 0.4	0.61 ± 0.5	0.62 ± 0.6
Alb g/dL	4.06 ± 0.12	4.11 ± 0.20	4.18 ± 0.12	4.23 ± 0.8	4.51±0.3
ALT U.I/I	22.31 ± 0.14	22.42 ± 0.23	23.06±0.59	28.26 ± 0.22	21.4 ± 2.2
AST U.I/I	72.6 ± 5.10	74.31 ± 1.53	92.61 ± 1.22	94.56 ± 5.23	69.5 ± 2.07
ALP U.I/I	81.14 ± 1.32	81.27 ± 2.12	90.55 ± 2.3	94.86±2.21	80.6 ± 6.14
HDL mg/dL	41.43 ± 4.3	42.10 ± 1.21	42.52 ± 1.23	43.28 ± 0.91	42.6 ± 6.21
LDL mg/dL	80.35 ± 1.6	83.20 ± 1.52	84.16 ± 2.9	85.24 ± 1.26	84.9 ± 3.29
Glu mg/dL	69.21 ± 2.1	69.60 ± 1.23	69.77 ± 1.23	69.81 ± 4.1	70.6 ± 6.06
Chol mg/dL	94.09±1.8	94.16 ± 1.71	94.52 ± 2.20	95.65 ± 2.4	96.6 ± 5.18
TG mg/dL	69.24 ± 2.9	69.81 ± 3.3	69.82 ± 2.12	69.99 ± 3.5	70.0 ± 4.12
Bil mg/dL	0.30 ± 0.21	0.32 ± 0.2	0.33 ± 0.04	0.34 ± 0.06	0.34 ± 0.04
T.P g/dL	7.35 ± 0.57	7.42 ± 0.24	7.53 ± 0.16	7.68 ± 0.32	7.69 ± 0.11

Note: CRT - Creatinine, Alb – Albumin, ALT - Alanine Transaminase, AST - Aspartate Transaminase, ALP - Alkaline phosphatase, HDL - High Density Lipoprotein, LDL - Low Density Lipoprotein, Glu – Glucose, Chol - Cholesterol, TG - Triglyceride, Bil - Bilirubin, T.P - Total protein. Values are expressed as mean ± SEM, $p<0.05$

Hematological Analysis

As shown in Table 4, repeated oral administration of *M. malabaricum* extract of different concentrations to the rats caused no significant effects on RBC, Hb, PCV and WBC's, lymphocyte and neutrophil counts.

TABLE 4: Effect of *M. malabaricum* extract on haematological parameters in toxicity study

Parameters	300mg/kg	900mg/kg	1500mg/kg	2000mg/kg	Control
Hb g/dL	14.02 ± 0.33	14.23 ± 0.20	14.16 ± 0.23	14.40 ± 0.12	14.75 ± 0.2
RBC 10^6/µL	7.02 ± 0.33	7.11 ± 0.10	7.27 ± 0.81	7.29 ± 0.31	7.62 ± 0.11
PCV %	44.60 ± 0.56	46.12 ± 0.79	46.70 ± 1.2	46.80 ± 0.56	47.92 ± 0.5
MCV fl	92.32 ± 19.19	92.42 ± 3.12	93.44 ± 3.8	94.43 ± 2.41	94.0 ± 4.01
MCH pg	19.36 ± 5.06	19.70 ± 1.24	19.88 ± 1.6	19.91 ± 0.29	19.93 ± 0.25
MCHC g/dL	43.23 ± 0.03	43.23 ± 0.03	43.33 ± 0.03	43.32 ± 0.03	42.6 ± 0.55
WBCx10^3/µL	7.36 ± 1.68	7.38 ± 1.86	7.69 ± 0.59	7.84 ± 1.18	7.89 ± 0.05
Neut x10^3/µL	35.00 ± 3.28	35.10 ± 3.32	35.20 ± 1.88	35.40 ± 1.17	35.44 ± 0.08
Lympx10^3/µL	81.20 ± 3.32	81.40 ± 2.32	81.50 ± 1.11	82.50 ± 2.49	82.65 ± 0.04
Monox10^3/µL	3.40 ± 0.61	3.46 ± 0.50	3.50 ± 0.41	3.60 ± 0.15	3.91 ± 0.02
Eosinx10^3/µL	2.20 ± 0.39	2.30 ± 0.62	2.40 ± 0.61	2.60 ± 0.16	2.75 ± 0.02

Note: HB - Haemoglobin, RBC- Red Blood Cell, PCV - Packed Cell Volume, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, WBC - White Blood Cell, Neut - Neutrophil, Lymp - Lymphocyte, Mono - Monocytes, Eosin – Eosinophil. Values are expressed as mean ± SEM, $p<0.05$.

Histopathological study

Histopathological studies of the liver and kidney tissues of both normal control rat as well as extracts treated rats did not revealed any major anatomical changes (Figure 1).

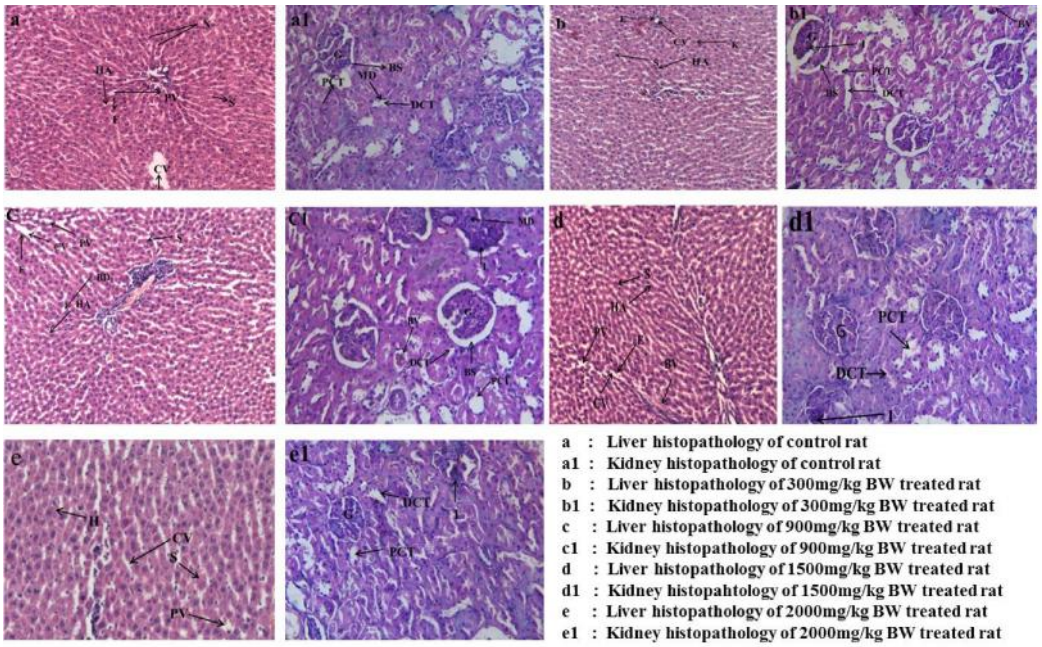

FIGURE 1: Histopathological observartions of both liver and kidney, a and a1: liver and kidney of normal control rats; b and b1: liver and kidney of 300mg/kg BW treated rats; c and c1: liver and kidney of 900 mg/kg BW treated rats; d and d1: liver and kidney of 1500 mg/kg BW treated rats; e and e1: liver and kidney of 2000 mg/kg BW treated rats, H – Hepatocytes, S – Sinusoids, HA=Hepatic artery, CV=Central vein, E=Endothelial cells, PV=Portal vein, BD=Bile duct, G=Glomeruli, BS=Bowman's space, DCT=Distal convoluted tubule, PCT=proximal convoluted tubule, I=Infiltration, MD=Macula densa, BV=Blood vessel.

DISCUSSION

Plants are directly used as medicines in a majority of cultures around the world for the treatment of numerous diseases since these plants contain numerous exclusive compounds that are used as drugs targeting specific ailments (Rahman *et al.*, 2007). The safety study is

accomplished by the application of common pre-clinical toxicity tests to reveal potential poisonous effects of many drugs mainly in liver and kidney of animals. A key point in confirming the welfare of drugs is to conduct toxicity tests in suitable animal models which will help in potential health beneficial properties for future studies (Nordeng *et al.,* 2013; Debelo *et al.,* 2016). The term acute oral toxicity is most frequently used in linking to lethality and lethal dose determinations (Gatne *et al.,* 2015). The plants for which toxicity studies is carried out and used safely to treat numerous diseases which include *Panax ginseng* C.A. Meyer. (Araliaceae), *Withania somnifera* Dunal. (Solanaceae), *Catharanthus roseus* Don. (Apocynaceae), *Phragmanthera incana* Schum. (Loranthaceae), *Phyllanthus fraternus* G.L.Webster. (Phyllanthaceae) and *Citrus hystrix* DC. (Rutaceae) (Aphale *et al.,* 1998; Kevin *et al.,* 2012; Ogunmefun *et al.,* 2013; Singh *et al.,* 2014; Abirami *et al.,* 2015). The present investigation was aimed to study the lethal dose, hepato-renal toxicity of aqueous extract of *Memecylon malabaricum*. The observed values in toxicity study of *M. malabaricum* aqueous extract exhibited low toxicity and definitely safe to use as traditional medicine. No lethality in LD_{50} study of the crude aqueous extract of the plant was found upto the dose of 2000 mg/kg BW. According to OECD guideline the drug is accepted as safe if the LD_{50} values are greater than 3000 mg/kg (OECD 2001; OECD 2002). This study is supported by several researchers who reported different LD_{50} values for different plant extracts. LD_{50} of *Vitex leucoxylon* Roxb. (Lamiaceae) leaf ethanol extract (>3000 mg/kg), *Ailanthus excels* Roxb. (Simaroubaceae) (1000 mg/kg), *Toddalia asiatica* Lam. (Rutaceae) (350 mg/kg), *Araucaria bidwilli* Hook. (Araucariaceae) (250 mg/kg) (Dahanukar *et al.,* 2000), *Boerhavia diffusa* L. (Nyctaginaceae) (>2000 mg/kg) body weight in both mice and rats (Orisakwe *et al.,* 2003) and *Albizzia chevalieri* Harms**. (**Leguminosae) leaf extract was also reported to be greater than 3000 mg/kg in rats (Saidu *et al.,* 2007). In the present observation plant treated rats did not show any sign of behavioural changes such as heartbeat (except at 6 h), skin and fur, convulsions, salivation, diarrhoea, death and other symptoms compare to control rats upto the dose of 2000 mg/kg BW that reveal its safety to use therapeutically. However slight increase in bodyweight is observed after three weeks and the similar observations were made in *Boerhaavia diffusa* treated rats (Orisakwe *et al.,* 2003). This study is important because safety of a product with therapeutic purpose, as proper intake of nutrients is essential. In this study, the food intake and water consumption was not affected and it did not induce appetite suppression and had no deleterious effects by the administration of *M. malabaricum* extract. Thus, this may indicate that the drug does not affect the feed utilisation ratio of the animals with no disturbance in carbohydrate, protein or fat metabolism. Abnormalities in body metabolic processes can be revealed by studying the hematological parameters and blood profile generally provides important information on the response of the body to injury, deprivation and stress (Mbaka *et al.,* 2010). In the present study the effect of aqueous extract of *M. malabaricum* on mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were insignificant in treated group compared to the control. These observations demonstrate that the aqueous extract of the leaves in this study did not cause significant toxic effect on the levels of calculated red blood cell (RBC) indices at different doses. Similarly white blood cell (WBC) count was also performed where no significant changes in total WBC count were observed after treatment of plant extract at different doses compared to control. Similar observations were made by (Mbaka *et al.,* 2010) while treating animals with *Sphenocentrum Jollyanum* Pierre (Menispermaceae) root ethanol extract.

The enzymes, ALT, ALP and AST are liver function markers, elevation of these markers may indicate hepatocellular damage (Woodman *et al.,* 1988; Saidu *et al.,* 2010). In the present study there is no much difference in plasma ALT, ALP and ASP enzymes activity of *M. malabaricum* treated rats at lower concentrations compared to control group. However there is a slight increase in these enzyme levels at the concentrations of 1500 and 2000 mg/kg BW after three weeks of treatment. This indicates, the prolonged use of *M. malabaricum* extract may cause damage to the liver above these concentrations. Similarly uplift of kidney function markers such as creatinine, urea and uric acid indicate the malfunctioning of the kidney Saidu *et al.,* 2010). However in the present investigation the plant extracts treated rats did not show significant difference in renal function markers against normal control group. It can also be supported by histopathological observation of renal tissues which showed no cellular damage in all groups of rats (Figure 1). The histopathological studies indicate there is no liver damage is observed as shown in figure 1. Similar observations were made by Saidu et al. Saidu *et al.,* 2010 while treating rats with the root extract of *Albizzia chevalieri*. This toxicity study may further be mechanistically evaluated in human being by incorporating long term drug toxicity, drug metabolism and toxic kinetic studies. The compounds responsible for the hepatotoxic effect may be elucidated further.

CONCLUSION

Prolonged treatment of repeating doses of 1500 mg/kg and 2000 mg/kg BW of *M. malabaricum* leaf extract increases the liver functionality markers without causing any tissue damage and no mortality. Only below 1500 mg/kg BW dose of *M. malabaricum* leaf extract may be safe for long term treatment.

Hence once should be careful while selecting the dose levels for treatment of herpes and other ailments.

Conflict of interest

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors acknowledge the recognition of University of Mysore as an Institution of Excellence and financial support from the Ministry of Human Resource Development, Govt. of India through UGC under UOM/IOE/RESEARCH/1/2010-11, dt 22-04-2010 project and UGC fellowship scheme (Or. No. DV9/192/NON-NETFS/2013-14 dated: 11-11-2013).

REFERENCES

Abirami, A., Nagarani, G., Siddhuraju, P. (2015) Hepatoprotective effect of leaf extracts from Citrus hystrix and C. maxima against paracetamol induced liver injury in rats, Food Sci Human Wellness. 4, 35-41.

Ambali, S., Akanbi, D., Igbokwe, N., Shittu, M., Kawu, M., Ayo, J. (2007) Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. J toxicol sci. 32. 111-120.

Aphale, A.A., Chibba, A.D., Kumbhakarna, N.R., Mohd, M., Dahat, S.H. (1998) Subacute toxicity study of the combination of ginseng (Panax ginseng) and ashwagandha (Withania somnifera) in rats: a safety assessment. Ind J Phys & Pharmacol. 42, 299-302.

Bharathi, T.R., Madhusudan, M.C., Pradeep kumar, P.M., Chandranayaka, S., Prakash, H.S. (2015) Antimicrobial Potential of Memecylon L. species from Western Ghats against clinical isolates of pathogenic bacteria. Res J Pharm & Biol Chem Sci. 6, 1280-1287.

Bharathi, T.R., Nadafi, R., Prakash, H.S. (2014) Invitro antioxidant and anti-inflammatory properties of different Solvent Extracts of Memecylon talbotianum Brandis. Int J Phytopharmacy. 4, 148-152.

Bharathi, T.R., Sampath kumara K.K., Prakash, H.S. (2016a) *Memecylon* species: A review of Traditional information and taxonomic description. Int J Phar Pharm Sci. 8, 1-9.

Bharathi, T.R., Shailasree, S., Sampath Kumara K.K., Madhusudan, M.C., Prakash, H.S. (2016b) Metabolite profiling by UPLC-PDA-ESI/HDMS and antibacterial activity of *Memecylon talbotianum* Brandis. Pharmacogn. Commn. 6, 1-10.

Dahanukar, S.A., Kulkarni, R.A., Rege, N.N. (2000). Pharmacology of medicinal plants and natural products. Ind J pharmacol. 32, S81-S118.

Debelo, N., Afework, M., Debella, A., Makonnen, E., Ergete, W., Geleta, B. (2016) Assessment of Hematological, Biochemical and Histopathological Effects of Acute and Sub-chronic Administration of the Aqueous Leaves Extract of *Thymus schimperi* in Rats. J Clin Toxicol. 6, 286.

Gatne, M.M., Adarsh, A., Ravikanth, K. (2015) Acute oral toxicity study of polyherbal formulation AV/KPC/10. Int J Biom and Adv Res. 6, 281-283.

Ghaffari, H., Venkataramana, M., Nayaka, S.C., Ghassam, B.J., Angaswamy, N., Shekar, S., Kumara, K.S., Prakash, H.S. (2013) Hepatoprotective action of Orthosiphon diffusus (Benth.) methanol active fraction through antioxidant mechanisms: An in vivo and in vitro evaluation. J ethnopharmacol. 149, 737-744.

Iyengar, M.A., Nayak, S.G.K., Singh, R. (1994) Folkloric uses of Memecyclon malabaricum (CB cl.) cogn. Anc sci life. 13, 242.

Kevin, L.Y.W., Hussin, A.H., Zhari, I., Chin, J.H. (2012) Sub–acute oral toxicity study of methanol leaves extract of Catharanthus roseus in rats. J Acu Dise. 1, 38-41.

Mbaka, G.O., Adeyemi, O.O. (2010). Toxicity study of ethanol root extract of Sphenocentrum Jollyanum (Menispermaceae) Pierre. Asian J Experi Biolo Scien. 14, 869-874.

Nordeng, H., Al-Zayadi, W., Diallo, D., Ballo, N., Paulsen, B.S. (2013) Traditional medicine practitioners' knowledge and views on treatment of pregnant women in three regions of Mali. J ethnobiol and ethnomed. 9, 1.

OECD (2002) *Test No. 423: Acute Oral toxicity - Acute Toxic Class Method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Ogunmefun, O.T., Fasola, T.R., Saba, A.B., Oridupa, O.A. (2013) The Toxicity Evaluation of Phragmanthera incana (Klotzsch) Growing on Two Plant Hosts and Its Effect on Wistar Rats' Haematology and Serum Biochemistry. Acad J Plant Sci. 6, 92-98.

Organization of Economic Co-operation Development (OECD) (2001) The OECD Guideline for Testing of Chemicals: 420 Acute Oral Toxicity-Fixed Dose Procedure, OECD, Paris, France.

Orisakwe, O.E., Afonne, O.J., Chude, M.A., Obi, E., Dioka, C.E. (2003) Sub-chronic toxicity studies of the aqueous extract of Boerhavia diffusa leaves. J heal sci. 49, 444-447.

Prakasha, H.M., Krishnappa, M., Krishnamurthy, Y.L., Poornima, S.V. (2010) Folk medicine of NR Pura taluk in Chikmagalur district of Karnataka. Ind J Tradi Know 9 55-60.

Rahman, M.S., Anwar, M.N. (2007) Antimicrobial activity of crude extract obtained from the root of Plumbago zeylanica. Bang J Micro. 24, 73-75.

Ramasetty, B.T., Bajape, S.N., Kadappa, S.K.K., Saini, R.K., Basavaraju, S.B., Ramachandra, K.K., Sripathy, P.H. (2016) Identification and genetic diversity analysis of Memecylon species using ISSR, RAPD and gene based DNA barcoding tools. Electron J Biotechnol. 24, 1-8.

Raza, M., Al-Shabanah, O.A., El-Hadiyah, T.M., Al-Majed, A.A. (2002) Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Pharmace Scien. 70, 135-145.

Rekha, N.D., Gowda, T.V., Aradhya, S.M., Suresha, R.N., Jayashree, K. (2014) Anti-Inflammatory Properties of Memecylaene: A Novel Compound Isolated from

Memecylon malabaricum. Res J Pharm & Biol Chem Sci. 5, 1645-1654.

Rodak, L.C. (1995) Routing testing in haematology, Dign Haematol. 128-144.

Saidu, Y., Bilbis, L.S., Lawal, M., Isezuo, S.A., Hassan, S.W., Abbas, A.Y. (2007) Acute and sub-chronic toxicity studies of crude aqueous extract of Albizzia chevalieri harms (Leguminosae). Asian J Biochem. 2, 224-36.

Saidu, Y., Nwachukwu, F.C., Bilbis, L.S., Faruk, U.Z., Abbas, A.Y. (2010) Toxicity Studies of the Crude Aqueous Root Extract of Albizzia chevalieri Harms in Albino Rats. Niger J Basic and Appl Sci. 18.

Singh, S.K., Prakash, V. (2014) Toxicity assessment of *Oxalis corniculata* and *Phyllanthus fraternus* plants. Int J Pharm pharm Sci. 6, 388-392.

Van campen, J.E., Zistra, W.G. (1961) Standardization of haemoglobinometry-hemoglobincyanide method. Clin Chem Acta. 6, 538-544.

Woodman, D.D. (1988) Assessment of hepatic function and damage in animal species. A review of the current approach of the academic, governmental and industrial institutions represented by the Animal Clinical Chemistry Association. J Appl Toxicol. 8, 249-254.