Research Article

Hepatoprotective Potential of *Mimusopse lengi* L Leaf Extracts against Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT

Many traditional systems of medicines employ herbal drugs for the hepatoprotection. The aim of the study is to investigate the hepatoprotective activity of *Mimusopselengi* L leaf extracts extracts against paracetamol induced hepatotoxicity. However, herbal plants are the windfall for the humankind providing solution for most of the wellness breakdowns. *Mimusopselengi* L is one of such plants with enormous therapeutic and nutraceutical potencies. The main aspiration of the current investigation is to evaluate the hepatoprotective ability of methanolic and aqueous extract of *Mimusopselengi* L leaves against paracetamol induced hepatotoxicity using wistar rats through biochemical parameters and histopathological findings. The phytochemical screening was carried on the leaves extracts of *Mimusopselengi* L revealed the presence of some active ingredients such as Alkaloids, Tannins, Sponginess, Phenols, glycosides, steroids, terpenoids and flavonoids. Leaves of *Mimusopselengi* L was successively ethylacetate fraction with methanolic and aqueous extract against paracetamol (2 ml/kg.p.o) induced hepatotoxicity using Standard drug Liv 52 (5 ml/kg). There was a significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP), serum bilirubin in paracetamol treated rats, which were restored towards normalization in *Mimusopselengi* L methanolic and aqueous extract (200 mg/kg and 200 mg/kg) treated animals. Thus, the present study ascertains that the leaf extract of *Mimusopselengi* L possesses significant hepatoprotective activity.

Keywords: *Mimusopselengi* L, hepatoprotective activity, paracetamol, Liv 52, methanol extract and aqueous extract.

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INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction [1]. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate[2]. Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders . Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures [3-4].

Mimusopse lengi L[5-6] is an evergreen ornamental tree of the family *Sapotaceae* with pleasant fragrant flowers. It carries a variety of names such as Bakul (Hindi and Bengali), Spanish cherry, West Indian Medlar or Bullet wood tree (English), Bakula (Sanskrit) etc. in different languages. *M. elengi* is regarded as one of the best medicinal plants since each and every part of it is used in various ways to cure a variety of human diseases. The bark is used as a tonic, and in gargles to cure odontopathy, inflammation and bleeding of gums. It is also useful in urethrorrhoea, cystorrhoea, diarrhoea and dysentery. The bark and seed coat are used for strengthening the gum and are utilized along with tannin rich substances like catechu (Acacia catechu), pomegranate (Punica granatum) bark etc. in various herbal tooth powders, such as "Vajradanti". Further, it is one of the constituents in the preparation of "Mahakhadiradivati" prescribed for stomatitis, halitosis, appetizer, anorexia, spongy gums and pharyngeal problems. The bark is also useful in painful high fever. The bark of *M. elengi* produces a commercial dye. The chemical constituents of the color components responsible for dyeing have been identified. The dyeing behavior of these color components on wool has also been evaluated. The color components isolated from the bark mainly contain flavonoid moiety. The leaves have been considered as an antidote for snakebite. The flowers and unripe fruits are used as an ointment for treating wounds and ulcers. The powder from dried flowers is a brain tonic and relieves

from cephalalgia. The flowers are used as expectorant, to cure problems of liver, nose, and are smoked in asthma. Further, the flowers are used to make garlands and for stuffing pillows. The fruits are aphrodisiac, diuretic, astringent to the bowels and good in gonorrhoea. The pulp of the ripe fruits has been successfully used to cure chronic dysentery. The immature fruits are chewed to protect loose teeth. The ripe fruits are given orally to pregnant women to facilitate delivery. The hot aqueous extract of fruits is given orally to human as diuretic which also acts as antipyretic. The ripe fruits rich in carbohydrates are good source of food.

METHODS

Chemicals and instrumentation

All the chemicals used were of analytical grade, Liv 52 syrup (The Himalaya drug company, Makali Bangalore), paracetamol (SD fine chemical Ltd Mum-bai, India), ALT test kits (Span Diagnostics Ltd Surat), AST test kits(Span Diagnostics Ltd Surat), LDH (UV kinetics Accurex Biochemical Pvt Ltd., Mumbai), albumin test kit (Span Diagnostics Ltd Surat), bilirubin test kit (Span Diagnostics Ltd Surat), alkaline phosphatase test kits (Merk Specialities Ltd Mumbai), total protein test kit (Span Diagnostics Ltd Surat), mini centrifuge (Spinnwell), UV spectro-photometer (Pharma Spec UV-1700, Shimadzu).

Collection and authentication of the plant specimen

The leaf of *Mimusopselengi* L. were collected from the local areas of Sivakasi, Tamilnadu and were authenticated by DR.N.SENTHIL KUMAR Department of Botany, Ayya nadar janakiammal college, Sivakasi, Tamilnadu. A voucher specimen was deposited to authen-tication office for future reference.

Preparation of plant extract and evaluation of percentage yield

The dried leaves were subjected to size reduction to a coarse powder with the help of grinder and powdered material was then subjected to Soxhlet extraction employing ethanol as solvent. The extract was concentrated to dryness with the help of rotavapor and finally air dried thoroughly to remove all traces of the solvent. The obtained dried extracts were weighed and extractive value was calculated[7].

Experimental animals

The rats (150-200 g) were procured from Animal House and kept in departmental animal house. All the rats were housed separately in polypropylene cages at the 50-60% relative humidity and temperature of 23±2C with a12 h light/dark cycle for seven days before and during the commencement of the experiment. The rats were kept on standard pellet diet and drinking water throughout the housing period. All the experiments were conducted in accordance with the guidelines Committee for the Purpose of Control and Supervision'(CPCSEA)and ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Studies[8]

Albino mice of either sex weighing between 20-30gm were used during investigation. The animals were fasted over night. The OECD guideline no-423 fixed dose method was adopted and accordingly a dose of Total alcoholic extract was calculated. As per following the OECD guideline no-423 fixed dose method, the safest dose of the total alcoholic extract is 2000mg/kg body weight. The safe dose was found to be 2000mg/kg body weight; hence 1/10th of the dose was taken as effective dose which is found to be 200mg/kg body weight.

Experimental protocol

Group I: serve as normal control and received 1% Tween-80 (1ml/kg; p.o) daily for 7 days. Group II: serve as toxic control and received paracetamol : olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7 of the experiment. Group III: Animals treated with standard drug Liv-52 (5ml/kg; p.o) daily for 7 days and received paracetamol : olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7, 30 min after administration of Liv-52. Group IV: Animals treated with Successive Methanolic extract (200mg/kg b.w, p.o) daily for 7 days and received paracetamol : olive oil (1:1, 2ml/kg b.w, i.p.) on day 1 and day 7, 30 min after administration of extract. Group V: Animals treated with Successive Aqueous extract (200mg/kg b.w, p.o) daily for 7 days and received paracetamol: olive oil (1:1, 2ml/kg b.w, i.p.) on day 1 and day 7, 30 min after administration of extract. On the 8th day of the experiment, the animals were anaesthetized with mild ether and 1ml blood was collected into the Eppindrof tube by retro-orbital vein puncture. The blood collected in Eppindrof tube was centrifuged to separate serum and used for the estimation of various biochemical parameters like SGOT, SGPT, SALP and Bilirubin (total and direct). Livers were excised and fixed in formalin for assessment of Histopathological studies.

Assessment of liver function test

Serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, cholesterol, albumin (ALB) and total Protein (TP) were estimated by using standard kits from Span Diagnostic ltd Surat, India. Serum lactate dehydrogenase (LDH) was estimated by using standard kits from Accurex biochemical Pvt Ltd, Mumbai, India. All the enzymatic estimations were assessed as per standard kit methods using UV spectrophotometer and the standard kit methods were obtained in detail from the leaflets provided in the commercially kits.

Histopathological examinatio[9]

Slices of liver were stored in 10% neutral formalin solution to preserve them. The tissues were mounted by embedding in paraffin wax in the laboratory and sections of the size of 6mm were cut. The sections were stained with eosin and haemotoxylin dyes. The slides were observed under light microscope and photomicrographs

were captured by using camera. These were observed for fibrosis, fatty infiltration, centrilobular necrosis and lymphocyte infiltration.

Statistical analysis

The data was expressed as mean±standard error of mean (mean±SEM). Student's 't' test was followed by individual comparison by Newman-Keuls test using graph pad prism software for determination of level of significance. The value of probability less than 5% ($p<0.05$) was considered statistically significant.

RESULTS

The phytochemical screening of *Mimusopse lengi* L shows the presence of Alkaloids, Carbohydrates, Steroids, Tannins, Flavonoids and Glycosides. The effect methanolic and aqueous extract of *Mimusopse lengi* L serum transaminases, alkaline phosphates, bilirubin and total bilirubin level in paracetamol intoxicated rats are summarized in (Table 1- 5). There was a significant increase in bilirubin levels, SGOT, SGPT and ALP, in paracetamol intoxicated group compared to the normal control group. The total protein levels were significantly decreased to 3.42g/dl in paracetamol intoxicated rats from the level of 4.72 g/dl in normal group. On the other hand the groups with received both methanolic and aqueous leaf extract of *Mimusopse lengi* L (200mg/kg and 200mg/kg) + paracetamol (2ml/kg.p.o) (Group II) +Liv 52 syrup (5ml/kg, p.o) (Group III) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total billirubin to almost normal level (Table 5). The histopathological studies plate is shown in (Plates 1-5).

Table 1: Effect of Methanol and Aqueous extracts on SGPT levels in hepatotoxic rats.

Animals	Normal	Paracetamol	Standard	Methanol	Aqueous
1	47.43	164.41	68.16	78.66	125.44
2	50.11	154.35	84.35	94.17	122.42
3	53.45	146.33	61.34	80.46	112.41
4	48.14	156.34	60.45	74.32	126.48
5	54.67	134.33	57.65	62.46	135.31
6	55.13	164.55	75.11	58.46	125.24
Mean	51.49	153.3	67.84***	74.75***	124.5**
SEM	3.369	11.57	10.26	12.97	7.385
SD	1.376	4.725	4.190	5.295	3.015

***P < 0.0001, **P < 0.001, when compared to Paracetamol control.

Table 2: Effect of Methanol and Aqueous extracts on SGOT levels in hepatotoxic rats.

Animals	Normal	Paracetamol	Standard	Methanol	Aqueous
1	64.65	165.31	87.23	106.35	124.15
2	55.57	176.26	91.63	85.64	134.56
3	57.77	174.25	86.14	98.68	104.22
4	62.17	184.22	78.65	101.45	126.25
5	58.42	176.23	82.13	112.82	117.31
6	56.12	165.18	94.58	88.46	121.23
Mean	59.11	173.6	86.74 ***	98.90 ***	121.3***
SEM	3.582	7.313	5.868	10.40	10.15
SD	1.462	2.986	2.396	4.243	4.146

***P < 0.0001, when compared to Paracetamol control.

Table 3: Effect of Methanol and Aqueous extracts on SALP levels in hepatotoxic rats.

Animals	Normal	Paracetamol	Standard	Methanol	Aqueous
1	146.14	346.14	167.01	198.16	214.63
2	138.22	421.34	156.45	194.34	248.34
3	158.13	384.68	178.15	187.64	201.44
4	162.14	425.45	164.55	201.65	224.62
5	159.46	412.06	182.15	227.15	253.18
6	147.24	304.31	177.13	175.13	204.12
Mean	151.9	382.3	170.9***	197.4***	224.4**
SEM	9.412	48.33	9.816	17.35	22.05
SD	3.842	19.72	4.008	7.081	9.007

***P < 0.0001, **P < 0.001 when compared to Paracetamol control.

Table 4: Effect of Methanol and Aqueous extracts on Total Bilirubin levels in hepatotoxic rats.

Animals	Normal	Paracetamol	Standard	Methanol	Aqueous
1	0.07	3.12	1.12	1.45	2.45
2	0.13	3.45	0.77	2.01	3.01
3	0.21	4.63	0.84	2.12	2.14
4	0.15	5.31	1.04	0.83	2.16
5	0.16	3.41	1.13	1.56	3.05
6	0.17	4.72	1.42	2.13	3.42
Mean	0.1517	4.110	1.053	1.683	2.703
SEM	0.04708	0.8899	0.2295***	0.5041***	0.5273***
SD	0.01922	0.3633	0.09369	0.2058	0.2153

***P < 0.0001, when compared to Paracetamol control.

Table 5: Effect of Methanol and Aqueous extracts on Direct Bilirubin levels in hepatotoxic rats.

Animals	Normal	Paracetamol	Standard	Methanol	Aqueous
1	0.084	0.41	0.05	0.16	0.27
2	0.052	0.74	0.10	0.24	0.34
3	0.073	0.63	0.13	0.34	0.35
4	0.064	0.34	0.08	0.42	0.37
5	0.056	0.42	0.22	0.31	0.21
6	0.071	0.57	0.23	0.17	0.32
Mean	0.0664	0.5216	0.1367***	0.2733***	0.3117**
SEM	0.01210	0.1525	0.07004	0.1018	0.06113
SD	0.004938	0.06226	0.0286	0.04161	0.02495

***P < 0.0001, **P < 0.001 when compared to Paracetamol control.

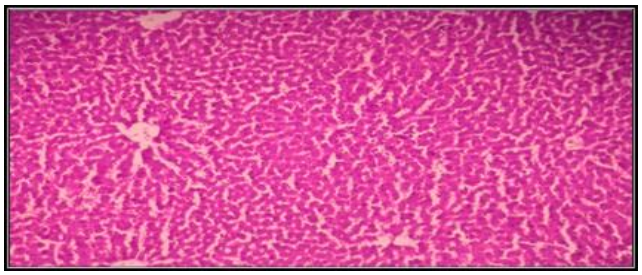

Plate No 01: Photograph of normal liver biopsy (Normal group).

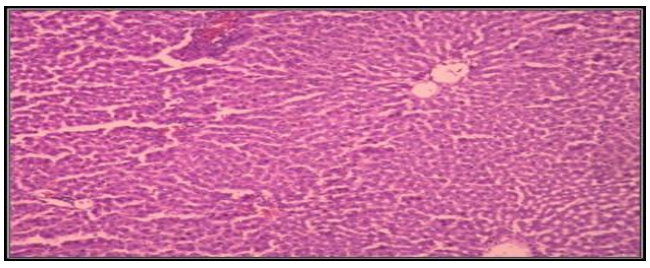

Plate No 02: Photograph showing necrosis of hepatocytes (Paracetamol)

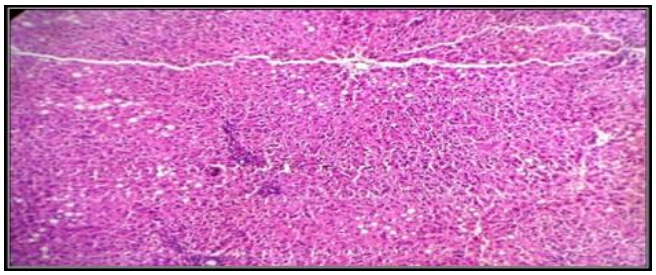

Plate No 03: Photograph showing effect of liv-52 on regeneration of Hepatocytes

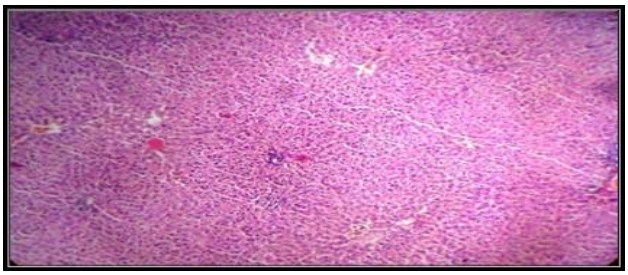

Plate No 04: Photograph showing effect of Successive Methanol extract on regeneration of Hepatocytes.

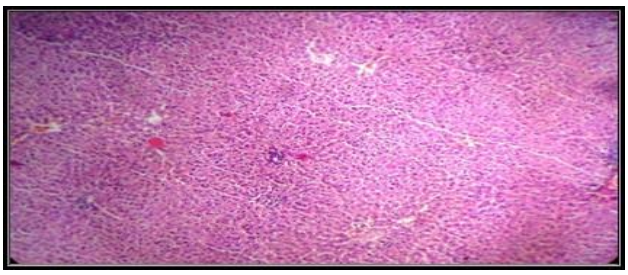

Plate No 05: Photograph showing effect of Successive Aqueous extract on regeneration of Hepatocytes.

DISCUSSION

Paracetamol (PCM) is an analgesic and antipyretic drug which, when taken in at toxic doses, becomes a potent hepatotoxic sub-stance liberating fulminated renal tubular and hepatic necrosis lethal to experimental animals and humans [10-11].Its overdose can cause liver function failure, centrilobular hepatic necrosis and even death in experimental animals as well as human.[12]The laboratory features of PCM induced hepatotoxicity is similar to other

kinds of acute inflammation and liver ailment with major increase of SGOT, SGPT, Total bilirubin and decrease of direct billirubin[12-13]. Results of the study clearly demonstrated that the serum level of hepatic enzymes SGOT, SGPT, Total bilirubin and decrease of direct billirubin were increased in the present study, reflecting the hepatocellular damage in the PCM induced hepato-toxicity in animal model.

The methanolic and aqueous extract of *Mimusopse lengi* L screened for acute toxicity according to OECD (Organization for Economic Co-operation and Development) 423 guidelines upto the dose of 2000 mg/kg b. wt. was found to be non-toxic and safe[14]. Hence, dose levels of 200 mg/kg of methanolic and aqueous extract of *Mimusopse lengi* L were selected here for the experiment. Methanolic and aqueous extract of *Mimusopse lengi* L at the doses of 200 mg/kg significantly ($p<0.0001$ top<0.001) lowered the SGOT, SGPT, Total bilirubin and decrease of direct billirubin in these PCM intoxicated rats. The results of liver function tests were correlated to the histopathological changes from photomicrographs taken. The centrilobular hepatic necrosis, cell degeneration and infiltrating lymphocytes were well displayed in PCM intoxicated group II. Treatment with methanolic and aqueous extract of *Mimusopse lengi* L prevented these PCM induced histopathological changes. These results suggested that the inhibition of elevated hepatic damage and hepatic function markers may participate in the protective effect of the methanolic and aqueous extract of *Mimusopse lengi* L against PCM induced hepatotoxicity. The hepatoprotection by methanolic and aqueous extract of *Mimusopse lengi* L may be due to antioxidant property of the phytochemicals present in methanolic and aqueous extract of *Mimusopse lengi* L which reduce the oxidative stress imposed by PCM and other like anti-inflammatory and analgesic properties preventing the inflammatory hepatic damage[15-16].

CONCLUSION

Methanolic and aqueous extract of *Mimusopse lengi* L possesses hepatoprotective effect as it exhibited protective effect against paracetamol induced hepatotoxicity in rats demonstrated by significant decrease in AST, ALT, ALP, LDH, cholesterol, bilirubin and increase in ALB, TP concentration, and prevention of PCM induced histopathological changes in liver.

Abbreviations

$CHCl_3$=Chloroform, MeOH = Methanol, AQ = Aqueous, EA=Ethylacetate, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, cholesterol, albumin(ALB) and total Protein (TP).

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