



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.1174176>Available online at: <http://www.iajps.com>

Research Article

**HEPATOPROTECTIVE AND TOXICOLOGICAL STUDIES ON
MICROCEPHALA LAMELLATA, PERIPLUCA APHYLLA AND
ALHAJI MOURARROUM**Rahiya Gul¹, Muhammad Younis¹, Shafi Muhammad^{2*}, Abdul Jabbar² and Gul Dana¹¹Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan²Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan**Abstract:**

Balochistan is native home of many medicinal plants. Peoples living in rural areas mostly rely on these medicinal plants to cure diseases. Microcephala lamellata, Peripluca aphylla and Alhaji mourarroum are important medicinal plants used for cure of various diseases. Current study was carried to explore hepatoprotective and toxicological profile of these plants. Hepatoprotective activity was carried out by CCl₄ induced liver damage in rabbits. Chronic toxicity test was carried out on rabbits. Microcephala lamellata, Peripluca aphylla and Alhaji mourarroum produced significant hepatoprotective activity as there was marked decrease in serum hepatic parameters. Methanolic extracts of these three plants did not produced any significant toxic effects.

Key Words: *hepatoprotective, Microcephala lamellata, Peripluca aphylla, Alhaji mourarroum*

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Please cite this article in press as Shafi Muhammad *et al.*, *Hepatoprotective and Toxicological Studies on Microcephala Lamellata, Peripluca Aphylla and Alhaji Mourarroum*, *Indo Am. J. P. Sci*, 2018; 05(01).

INTRODUCTION:

In human body liver is the largest organ and chief site for excretion and intense metabolism. So it play surprising role in regulating homeostasis, performance and maintenance of the body. Hence involved with nutrient supply, fight against disease, provision, almost all the biochemical pathways to growth and reproduction [1].

Liver diseases which are still a global health problem may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis). Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects [2,3].

Herbal drugs are utilized world wide as an orthodox to alternative drugs [4] and these medicinal plants have immensely contributed to the development of human health and welfare [5]. Previous studies on several folklore herbs showed that plant extracts contain many compounds with chemoprotective potentials that may prevent the attack of carcinogens [6,7].

For liver and biliary tract diseases a natural remedy which has been use for centuries is Silymarin, derived from the milk thistle plant, *Silybum marianum*. In United States, patient with little understanding about purported properties regarding Silymari, gastroenterologist have encounter increase number of patient taking that herbal drug. Active constituent (silybin) of Silymarin and drug itself have been shown to work as scavenging free radicals, inhibiting lipid peroxidation and anti oxidants. Its studies also propose that they protect against tumor promotion, stabilize mast cells, genomic injury, increase hepatocyte protein synthesis, slow calcium metabolism and chelate iron [8].

Modern medicines have slight scope mitigation of diseases of liver and it is primarily the products and preparations of plants which are used for the liver disorders treatment. And the treatment presently is insufficient for the liver disorders [1], Therefore, 3 medicinal plants *Microcephala lamellata*, *Periploca aphylla* and *Alhaji mourarroum* used traditionally in Balochistan were studied for its potential hepatoprotective activity against CCl_4 induced liver damage in experimental animal model and their toxicological studies were carried out.

MATERIAL AND METHODS:

Plant material

Plant material was collocated from Quetta, Kalat and Jhal magsi, were identified and dried under the shade, grinded and macerated with methanol by using rotary evaporator.

Experimental animals

Healthy adult local breed both male and female rabbits were used (each weighing about 1.0-1.5 kg). Animals were held in cages at room temperature ($23 \pm 12^\circ\text{C}$).

Hepatoprotective activity

Study design

Rabbits of body weight 1.5-2.5 kg were selected. The total study population of thirty-two (32) rabbits was placed within five (5) groups, six (6) animals each. The different groups were treated as follows:

- Group I: Control (Non treated)
- Group II: CCl_4 treated group
- Group III: CCl_4 + *M. lamellata* Crude extract (300 mg/kg orally)
- Group IV: CCl_4 + *P. aphylla* Crude extract (300 mg/kg orally)
- Group IV: CCl_4 + *A. mourarroum* Crude extract (300 mg/kg orally)

Liver function tests

In anti-coagulant containing tubes, blood was collected for liver profile tests. Following tests are for determination of liver function tests i.e. Albumin, Alkaline Phosphatase, total Bilirubin, and SGPT were determined on automatic analyzer at 37°C by using standard reagent kits (Merck Germany) [9].

Chronic Toxicity test

The chronic toxicity test was performed following the protocol described by the OECD guideline 408 for testing chemicals [10]. Rabbits of both sexes were randomly assigned into 4 groups: a control group and drug 300mg/kg treatment groups (For Each Plant). methanol extracts were dissolved in 10% Tween 20 and administered orally on daily basis for 90 days at single doses of 300 mg/kg, while the control group received only 10% Tween 20 in distilled water.

The extract was freshly prepared with vehicle on daily basis. The rats were weighed and visual observations for mortality, behavioral pattern (Salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain and signs of illness were conducted once daily during that period [9].

Hematology

At the end of the dosage period blood was drawn through cardiac puncture, in test tubes containing EDTA (anticoagulant) that prevent coagulation. Parameters were Total white blood cells counts, hemoglobin, platelets count, Red blood cells count and Hematocrit (HCT/PCV), MCHC, MCH, and MCV, were determined by using automatic analyzer (using Beckman Coulter HMX analyzer, USA)[11].

Biochemical tests

In anti-coagulant containing tubes, blood was collected for serum biochemistry. Kidney Function test i.e. Urea & creatinine, Lipid profile i.e. Cholesterol, Triglycerides, Calcium serum, Uric acid and Blood glucose was determined by using automatic analyzer at 37°C by using standard reagent kits (Merck Germany) [9,12].

Statistical analysis

The statistical analysis was performed using SPSS (16 version). The significance level when testing the statistical hypotheses was $p < 0.05$.

RESULTS:**Hepatoprotective activity**

Liver function test

Total Bilirubin (mg/dL) was 0.52 ± 0.058 for control, 0.13 ± 0.11 for CCl_4 treated group and 0.68 ± 0.0375 for *M. lamellata* treated group, 0.42 ± 0.146 for *P. aphylla* treated group and 0.64 ± 0.040 *A. mourorum* treated group. Alkaline Phosphatase (U/L) was 159.4 ± 2.58 for control, 24.4 ± 20.706 for CCl_4 treated group and 200 ± 0.9721 for *M. lamellata* treated group, and 29.2 ± 2.42 for *P. aphylla* treated group and 200 ± 0.682 for *A. mourorum* treated group. SGPT (U/L) was 35 ± 0.354 for control, 26.9 ± 23.485 for CCl_4 treated group and 45 ± 0.44 for *M. lamellata* treated group, 39.6 ± 5.65 for *P. aphylla* treated group and 31 ± 0.782 for *A. mourorum* treated group. Albumin (g/dl) was 6.44 ± 0.44 for control, 10.54 ± 9.30 for CCl_4 treated and 7.04 ± 0.59 for *M. lamellata* treated group, and 9.9 ± 0.532 for *P. aphylla* treated group and 8.32 ± 0.264 for *A. mourorum* treated group Results shown in Table 1.

Table 1: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on Liver function test.

S NO.	Test,	Control, (Mean+, SEM)	CCl4 treated (Mean±SEM)	<i>M. lamellata</i> (Mean,+SEM)	<i>P. aphylla</i> (Mean,+SEM)	<i>A. mourorum</i> (Mean,+SEM)
1	Total, Bilirubin (mg/dL)	0.52+0.058	0.13±0.11	0.68+0.037	0.42+0.146	0.64+0.040
2	Albumin(g/dl)	6.44+0.244	10.54±9.304	7.04+0.590	9.9+0.532	8.32+0.264
4	Alkaline, Phosphatase(U/L)	159.4+2.58	24.4±20.70	200+0.9721	29.2+2.42	200+0.862
6	SGPT,(U/L)	35+0.354	26.9±23.48	41+0.44	39.6+5.65	31+2.78

All values, are mean \pm SEM; n=5; * = Significant, ($P < 0.05$), ** =, highly significant, ($P < 0.01$).

Table 2: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on kidney function test

S ,NO.	Test,	Control (Mean+ ,SEM)	<i>M. lamellata</i> (Mean+SEM)	<i>P. aphylla</i> (Mean,+SEM)	<i>A. mourorum</i> (Mean,+SEM)
1	Creatinine,	0.826+0.0268	2.34±0.129	2.14±0.17	2.74±0.44

All values, are mean \pm SEM; n=5; * = Significant, ($P < 0.05$), ** =, highly significant, ($P < 0.01$).

Table 3: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on Blood Glucose.

S, NO.	Test	Control (Mean±SEM)	<i>M. Lamellata</i> (Mean±SEM)	<i>P. aphylla</i> (Mean±SEM)	<i>A. Maurorum</i> (Mean+SEM)
1	Blood, Glucose Random	47.8+ 1.127	104.9+1.456	81.4+4.030	81+7.831

All values, are mean \pm SEM; n=5; * = Significant, ($P < 0.05$), ** =, highly significant, ($P < 0.01$).

Table 4: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on serum Calcium and Uric acid.

S NO.	Test	Control (Mean,+ SEM)	<i>M. lamellata</i> (Mean+SEM)	<i>P. aphylla</i> (Mean,+SEM)	<i>A. Maurorum</i> (Mean,+SEM)
1	Calcium-Serum, (mg/dL)	8.06+0.37	7.64±0.103	8.5±0.281	9.46±0.136
2.	Uric ,acid (mg/dL)	4.9+0.203	3.66±0.150	5.3±0.387	4.56±0.140

All values, are mean ,± SEM; n=5; * = Significant , ($P<0.05$), ** =, highly significant, ($P<0.01$).

Table5: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on Hematological profile

S NO.	Test	Control (Mean+SEM)	<i>M. lamellata</i> (Mean±SEM)	<i>P. aphylla</i> (Mean+SEM)	<i>A. mourorum</i> (Mean,+SEM)
1	Hb (g/dl)	12.7+0.371	11.78±0.363	11.84±0.340	11.68±0.537
2	RBC Count (million/ul)	5.164±0.191	6.104±0.327	5.39±0.470	6.216±0.290
3	Hematocrit (HCT/PCV) %	32.38+0.340	34.39±0.556	34.33±0.754	35.15±2.305
4	MCV (fl)	52.81+6.74	54.8±0.375	54.6±0.68	61.4±1.695
5	MCH (pg)	19.54+0.625	17.56±0.503	18.6±0.136	19.5±0.148
6	MCHC (g/l)	32.69+0.955	34.14±0.438	34.±0.764	33.82±0.387
7	Total WBC Count (×10 ⁹ /L)	9.94+0.243	6.234±0.3301	6.662±0.252	9.08±0.885
8	Platelet Count (×10 ⁹ /L)	191.5±1.862	74.4±0.7503	73.4±1.169	203.2±44.52

All values, are mean ,± SEM; n=5; * = Significant , ($P<0.05$), ** =, highly significant, ($P<0.01$).

Table 6: Effect of *M. lamellata*, *P. aphylla* and *A. maurorum* on, lipid profile

S.NO	Test,	Control (Mean+ SEM)	<i>M. lamellata</i> (Mean±SEM)	<i>P. aphylla</i> (Mean±SEM)	<i>A. maurorum</i> (Mean±SEM)
1.	Cholesterol, (mg/dL)	83.2+5.927	105.4±0.511	81.4±2.093	76±1.186
2.	Triglycerides, (mg/dL)	57.6+50.83	54.6±0.98	80.8±1.659	75.6±5.497

All values, are mean ,± SEM; n=5; * = Significant , ($P<0.05$), ** =, highly significant, ($P<0.01$).

Chronic toxicity test

Kidney Function Test

Level of creatinine was 0.826±0.026 for control, 2.34±0.129 for *M. lamellata* treated group, 2.14±0.17 for *P. aphylla* treated group and 2.74±0.44 for *A. murorum* treated group.

Blood Glucose (Random) Results shown in Table 2.

Level of blood Glucose (random) was 47.8±1.127 for control, 81.2±0.802 for *M. lamellata* treated rabbit, 81.4±4.030 for *P. aphylla* treated group and 81±7.831 for *A. mourorum* treated group.

Serum Calcium and Uric acid. Results shown in Table 3.

Level of Calcium-Serum (mg/dL) 8.06±0.37 was for control, 7.64±0.103 was for *M. lamellata* treated rabbit, 8.5±0.281 for *P. aphylla* treated group and 9.46±0.136 for *A. mourorum* treated group.

Uric acid (mg/dL) level was 4.9+0.203 for control, 3.66±0.150 was for *M. lamellata* treated group, 5.3±0.387 for *P. aphylla* treated group and 4.56±0.140 for *A. mourorum* treated group.

Hematological profile. Results shown in Table 4.

Hb (g/dl) was 12.7+0.37 for control, 11.78±0.363 for *M. lamellata* treated group, 11.84±0.340 for *P. aphylla* and 11.68±0.53 for *A. mourorum* treated

group. RBC Count (million/ul) was 5.164 +0.191 for control, 5.72+0.102 6.10±0.32 for *M. lamellata* treated rabbit, 11.84±0.340 for *P. aphylla* treated group and 11.68±0.537 for *A. mourorum* treated group. Hematocrit (HCT/PCV) % was 32.388+0.340 for control, 34.39±0.556 for *M. lamellata* treated group, 34.33±0.754 for *P. aphylla* treated group and 35.14±2.305 for *A. mourorum* treated group. MCV (fl) 52.81±0.743 for control, 54.4±0.375 for *M. lamellata* treated group, 54.6±0.68 for *P. aphylla* treated group and 61.4±1.695 for *A. mourorum* treated group. MCH (pg) was 19.54+0.625 for control, 17.56±0.503 for *M. lamellata* treated rabbit, 18.6±0.136 for *P. aphylla* treated group and 19.5±0.148 for *A. mourorum* treated group. MCHC (g/l) was 32.69+0.955 for control, 34.14±0.438 for *M. lamellata* treated group, 34±0.764 for *P. aphylla* treated group and 33.82±0.387 for *A. mourorum* treated group. Total WBC Count (×10⁹/L) was 9.94+0.242 for control, 6.234±0.3301 for *M. lamellata* treated group, 6.662±0.252 for *P. aphylla* treated group and 9.08±0.885 for *A. mourorum* treated group. Platelet Count (×10⁹/L) was 191.5+1.862 for control, 74.04±0.7503 for *M. lamellata* treated group, 73.4±1.169 for *P. aphylla* treated group and 203.2±44.52 for *A. mourorum* treated group. Lipid Profile. Results shown in Table 5.

Cholesterol (mg/dl) 83.2±5.927 for control, 105.4±0.511 for *M. lamellata* treated group, 81.4±2.093 for *P. aphylla* treated group and 76±1.186 for *A. mourorum* treated group. Triglyceride (mg/dl) 57.6±50.83 for control, 54.6±0.98 for *M. lamellata* treated rabbit, 80.8±1.659 for *P. aphylla* treated group and 75.6±5.497 for *A. mourorum* treated group. Results shown in Table 6.

DISCUSSION:

CCl₄ induced liver damaged for hepatoprotective drug screening is most commonly used model. Damaged structural integrity of the liver shows increase in serum levels of cholesterol ALT and AST, because they release in circulation after cellular damages as they are located in cytoplasm. Carbon tetrachloride treated mice induces hepatotoxicity by metabolic activation that is why they selectively causes toxicity in liver cells but maintaining semi-normal metabolic function. Trichloromethyl free radical (CCl₃) is from in endoplasmic reticulum by metabolically activation of Carbon tetrachloride which is done by cytochrome P-450 dependent mixed oxidase, than this free radical in the presence of oxygen combined with cellular protein and lipid to induce lipid peroxidation. All these results in structural changes of membranes and endoplasmic reticulum, loss of glucose 6--phosphate activation, protein synthesis

reduction and loss of metabolic enzyme activation resulting in liver injury [13,14]. There was non significant change as compared with control animals. Observed result shows that there was no toxicological effect.

Kidney Function Test

There was significant increase in creatinine for *M. lamellata*, *P. aphylla* and *A. mourorum* in experimental group were observed as compare to control group, which produce toxicological effect in experimental group.

Blood glucose

There was significant increase in blood glucose levels in drug treated groups were observed as compare to control group which produce toxicological effect in experimental group

Serum Calcium and Uric acid

There was non significant change in serum calcium and uric acid values in drug treated group was observed as compare to control group.

Lipid Profile

There was significant increase in cholesterol values of *M. lamellata* treated groups as compare to controls group. were as non significant changes in cholesterol values are noticed in other drug treated group for *P. aphylla* and *A. maurorum* as compare to control group.

There was non significant change in triglyceride values of drug treated group which are treated with *M. lamellata* as compare to control group were as there is significant increase in triglycerids values of drug treated group those who were treated with *P. aphylla* and *A. maurorum* as compare to control group.

CONCLUSION:

Microcephala lamellata, *Periploca aphylla* and *Alhaji mourarroum* crude methanolic extracts produced significant hepatoprotective activity with non toxic profile, however further studies are required to isolate the compounds responsible for activity.

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