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ORIGINAL ARTICLE

Evaluation of Hepatoprotective and Anti-Inflammatory Activities of Aqueous Leaf Extract of *Blumea mollis* in Rats

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ABSTRACT

Antioxidant,The antioxidant, hepatoprotective, and anti-inflammatory activities of the traditional medicinal hepatoprotective, and anti-inflammatory activities of the traditional medicinal plant *Blumea mollis* have been studied. The aim of this study was to evaluate its pharmacological properties and safety profile. In vitro antioxidant activity was estimated using superoxide, hydroxyl radical scavenging, and lipid peroxidation assays. Acute toxicity was tested orally at doses up to 2000 mg/kg. The in vivo hepatoprotective activity was screened using carbon tetrachloride (CCl_4) and paracetamol-induced liver damage in Wistar rats. The anti-inflammatory activity was assayed using carrageenan- and formalin-induced paw oedema models. *Blumea mollis* exhibited dose-dependent antioxidant activity, with considerable inhibition of oxidative markers on par with ascorbic acid (***p<0.001). A significant hepatoprotective effect was observed at 200 mg/kg, with decreased SGOT, SGPT, ALP, and bilirubin levels in both hepatotoxic models (***p<0.001). Histopathological evidence confirmed the preservation of liver tissue. Anti-inflammatory activity was also dose-dependent, showing oedema inhibition at 200 mg/kg compared to diclofenac (***p<0.001). This study offers the first in-depth assessment of *Blumea mollis* in various pharmacological models, confirming its safety and authenticating its folk use as a natural drug agent.

Keywords: Blumea mollis; Antioxidant; Hepatoprotective; Anti-inflammatory

INTRODUCTION

Medicinal plants have been the mainstay of indigenous systems of medicine for centuries, providing a rich source of bioactive compounds with therapeutic value. Of these, plant-derived antioxidant and hepatoprotective agents have become increasingly prominent owing to their effectiveness and low incidence of adverse effects compared to chemically derived drugs. Oxidative stress due to an imbalance between ROS and antioxidant defenses is a major contributor to the development of many chronic diseases, such as liver ailments and inflammatory diseases.[1] The liver is a major organ involved in the metabolism of xenobiotics and is particularly vulnerable to oxidative damage. As a result, there is a greater need for natural products with the capacity to reduce oxidative damage and enhance liver function.[2]

Blumea mollis (*B. mollis*) belongs to the Asteraceae family, is widely used in Indian traditional medicine, and is one such herb considered to have pharmacological properties, especially in the treatment of hepatic and inflammatory diseases. Despite its ethnobotanical significance, extensive scientific evidence of its pharmacological activity is lacking. Species in the Asteraceae family have been reported to be rich in phenolics, flavonoids, and terpenoids, which scavenge free radicals, inhibit lipid peroxidation, and modulate inflammatory pathways.[3] The protective potential of such compounds against chemically-induced liver injury and inflammation has been established in various studies with standard hepatotoxins such as carbon tetrachloride (CCl_4) and paracetamol.[4]

In addition, anti-inflammatory activity is frequently assayed using carrageenan- and formalin-induced paw oedema models, which closely resemble the cellular and molecular processes of inflammation. Those plant extracts exhibiting marked inhibition in these models are considered to be potential anti-inflammatory compounds.[5] As the demand for safe and effective natural products increases, screening *B. mollis* for its antioxidant, hepatoprotective, and anti-inflammatory potential is timely and justified. This study aimed to evaluate the in vitro antioxidant potential and

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in vivo hepatoprotective and anti-inflammatory activities of *B. mollis* against its efficacy as a reference drug.

MATERIALS AND METHODS

B. Mollis extract preparation

The plant material was taxonomically confirmed and authenticated by Dr. K. Madhava Shetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. Aqueous extracts of *B. mollis* leaves were prepared by harvesting fresh leaves, which were then dried in the shade at room temperature. The dried leaves were powdered and stored in airtight cellophane bags to shield them from environmental conditions. The powdered material was then extracted using distilled water. The extract was suspended in a 2.5% gum acacia solution for oral administration at different doses.

In vitro Antioxidant Activity of B. mollis leaf extract

The antioxidant activity of the aqueous extract of *B. mollis* was evaluated using three in vitro models: superoxide radical scavenging activity, hydroxyl radical scavenging activity, and lipid peroxidation inhibition.

Superoxide radical scavenging activity was assessed using the riboflavin photoreduction assay, in which different concentrations of the extract (50–300 μg/ml) were incubated with riboflavin, EDTA, and NBT, and the absorbance was recorded at 560 nm after illumination. Hydroxyl radical scavenging activity was assessed using the Fenton reaction, wherein hydroxyl radicals produced from an Fe^{3+}/ascorbate/EDTA/H_2O_2 system were allowed to react with deoxyribose. TBARS formation was measured at 560 nm wavelength. Lipid peroxidation was catalysed by Fe^{2+}/ascorbate in rat liver homogenate, and inhibition was measured using the thiobarbituric acid method with absorbance at 532 nm. The percentage inhibition was determined by measuring the antioxidant potential of the extract.

In vivo hepatoprotective activity of B. mollis leaf extract and anti-inflammatory Activity

Wistar albino rats of either sex weighing 175–250 g was used. The animals were maintained under normal laboratory conditions, and all experiments were approved by the Institutional Animal Ethics Committee (KCP/IAEC-27/2008-09), to the CPCSEA guidelines. Acute toxicity was studied according to OECD guidelines No.423. Rats were orally administered the aqueous extract of *B. mollis* in the range of 300 to 2000 mg/kg and observed for 24 h for death and behavioural changes.

The hepatoprotective effect was assessed using two experimental models: paracetamol-induced hepatotoxicity and carbon tetrachloride (CCl_4)-induced hepatotoxicity. The rats were equally divided into five groups (n = 6 in each group). Group I was used as the control and treated with normal saline. Group II was treated with the hepatotoxin (paracetamol 750 mg/kg or CCl_4 0.7 ml/kg, respectively) every 72 hours for 10 days. Group III was treated with silymarin (50 mg/kg) as the reference hepatoprotective agent. Groups IV and V were administered the aqueous extract of *B. mollis* at doses of 50 mg/kg and 200 mg/kg, respectively, with hepatotoxins. Blood samples were collected through retro-orbital puncture on the 11th day for biochemical estimation, and the animals were sacrificed for liver collection and histopathological examination. Biochemical parameters of serum bilirubin, SGOT (AST), SGPT (ALT), and alkaline phosphatase (ALP) were analysed using standard photometric and UV kinetic techniques.

Bilirubin was estimated by the method of modified Jendrassik and Grof's, the absorbance was taken at 540 nm.[6] SGOT and SGPT activities were assayed using optimised UV methods of NADH consumption and absorbance at 340 nm. ALP activity was measured using the DGKC method following the hydrolysis of p-nitrophenyl phosphate to a yellow-coloured product read at 405 nm. Histopathology was carried out by fixation of the liver tissues in 10% formalin, paraffin embedding, sectioning at 4–6 μm thickness, haematoxylin and eosin staining, and examination under a microscope.

In vivo anti-inflammatory activity of B. mollis leaf extract

The anti-inflammatory activity was determined using carrageenan- and formalin-induced paw oedema models. In both models, rats were allocated to five groups (n = 5 per group): a control vehicle group, an inflammation control group (receiving carrageenan or formalin), a control drug group (receiving diclofenac potassium 10 mg/kg), and two treatment groups receiving *B. mollis* extract at 50 mg/kg and 200 mg/kg. Paw volume was determined by plethysmography at baseline and 3 and 6 h after injection.

Statistical analysis

Statistical comparisons were performed using one-way ANOVA with Tukey's multiple comparison test. Data are reported as the mean $\pm$ SEM, and statistically significant results are those with p values <0.05.

RESULTS

In vitro Antioxidant Activity

Superoxide Radical Scavenging Activity

B. mollis exhibited dose-dependent superoxide radical scavenging activity from 26$\pm$1.52 at 50 μg/ml to 73.66$\pm$0.88

at 200 μg/ml (***p<0.001). Ascorbic acid was slightly more active, reaching 83.3±1.528 at 200 μg/ml. The findings validated the strong antioxidant activity of *B. mollis* compared to the control, with all values being strongly significant at 50 μg/ml (Table 1).

Hydroxyl Radical Scavenging Activity

B. mollis presented a significant dose-dependent hydroxyl radical scavenging activity, rising from 13±1.15 at 50 μg/ml to 58±1.15 at 200 μg/ml (***p<0.001). Ascorbic acid had slightly higher activity with a value of 61.6±0.88 at the same concentration. All values were statistically significant relative to 50 μg/ml, indicating the good antioxidant potential of *B. mollis* in this test (Table 1).

Lipid Peroxidation Inhibition Activity

B. mollis also showed notable, dose-dependent inhibition of lipid peroxidation, from 11.6±0.88 at 75 μg/ml to 58±1.15 at 350 μg/ml (***p<0.001). Ascorbic acid was inhibited to a greater extent at the respective doses, to the level of 52±1.1 at 350 μg/ml. All values were significantly different from 50 μg/ml, indicating the antioxidant activity of *B. mollis* against lipid peroxidation (Table 1).

Acute Toxicity Studies

The acute toxicity of *B. mollis* was evaluated by oral administration at doses of 300, 500, 1000, and 2000 mg/kg body weight in male albino Wistar rats. No mortality was observed in any of the groups. Therefore, doses of 50 mg/kg and 200 mg/kg (1/10th and 1/40th of the maximum safe dose) were chosen for further study.

Carbon Tetrachloride Induced Hepatotoxicity

Serum Biochemical Markers

Carbon tetrachloride greatly increased liver enzyme and bilirubin levels. *B. mollis* at 50 mg/kg did not show noteworthy protection. However, 200 mg/kg significantly lowered SGOT, SGPT, ALP, and bilirubin levels (***p<0.001), which is a sign of hepatoprotection. Silymarin also exhibited significant protective actions with readings near normal controls, reaffirming its status as a standard hepatoprotective agent (Table 2).

Paracetamol Induced Hepatotoxicity

Administration of paracetamol increased Serum Biochemical Markers - SGOT, SGPT, ALP, and bilirubin levels. *B. mollis* at 50 mg/kg indicated no considerable protection. However, at 200 mg/kg, it significantly decreased all markers (***p<0.001), which signifies hepatoprotection. Silymarin gave similar reductions, which confirms its standard role. These findings validated the dose-dependent hepatoprotective activity of *B. mollis* against CCl_4-evoked liver injury.

Anti-Inflammatory Activity - Carrageenan Induced Paw Oedema and Formalin Induced Paw Oedema

The table lists the anti-inflammatory activity of *B. mollis* at doses of 50 mg/kg and 200 mg/kg in carrageenan- and formalin-induced paw oedema models against normal, negative control, and the standard drug diclofenac (10 mg/kg). In both models, the negative control group exhibited significant oedema. *B. mollis* at 50 mg/kg expressed moderate inhibition, significant inhibition at 6 hours in both the models (**p<0.01, ***p<0.001). *B. mollis* at 200 mg/kg caused significant oedema reduction at both 3 and 6 hours (***p<0.001), demonstrating dose-dependent effect. Diclofenac elicited the greatest inhibition at all the time points (***p<0.001). These results indicate that *B. mollis* has strong anti-inflammatory activity, particularly at high doses, similar to that of the control drug. (Table 4)

Histopathological Studies

Normal liver histology revealed a preserved sinusoidal architecture. CCl_4 caused mild and moderate liver injury in the form of necrosis, fatty changes, and inflammation. *B. mollis* (200 mg/kg) greatly restored liver architecture, comparable to silymarin, while in the 50 mg/kg dose, it exhibited reduced protection with increased apoptosis. Histology attested to the dose-dependent hepatoprotection of *B. mollis*, validating biochemical observations (Figure 1).

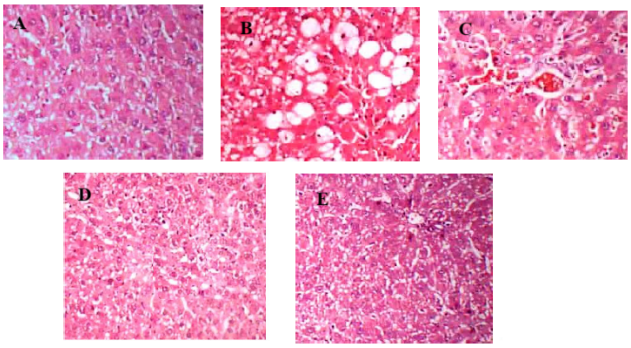

Fig. 1: Histopathological analysis of liver tissues under various treatments (H&E Staining) showingA-Normal liver with preserved tissue (saline-treated control), B-CCl_4-treated liver showing severe damage, fatty changes, and necrosis, C- *B. mollis* (30 mg/kg) showing partial hepatoprotection, D-*B. mollis* (200 mg/kg) showing improved liver structure, E- Silymarin (200 mg/kg) showing near-normal histology and strong protection

Control liver tissue revealed central veins and radial hepatocytes. Paracetamol induces centrilobular necrosis, fatty changes, and sinusoid congestion. Silymarin maintained liver architecture and inhibited oxidative damage. *B. mollis* (200 mg/kg) revealed protection similar to that of control, whereas the 50 mg/kg dose was not as effective. Apoptosis was greater at a lower dose, representing a dose-dependent

Table 1: *In vitro* Antioxidant Activities of *B. mollis* and Ascorbic Acid at Various Concentrations

Superoxide Radical Scavenging							
Extract	**50 μg/ml**	**75 μg/ml**	**100 μg/ml**	**150 μg/ml**	**200 μg/ml**	**250 μg/ml**	**350 μg/ml**
B. mollis	26±1.52	41.3±1.202***	53±1.732***	62.3±1.45 ***	73.66±0.88***	–	–
Ascorbic acid	31±0.57	44±0.57 ***	61.3±0.88***	68±1.15 ***	83.3±1.528***	–	–
Hydroxyl Radical Scavenging							
B. mollis	13±1.15	22±1.15 ***	33.6±1.202 ***	45.6±0.88***	58±1.15 ***	–	–
Ascorbic acid	14.3±0.88	24±1.15 ***	35±0.57 ***	51±1.15 ***	61.6±0.88 ***	–	–
Lipid Peroxidation Inhibition							
B. mollis	–	11.6±0.88	24±1.15 ***	32±1.15 ***	–	34.3±0.88 ***	58±1.15 ***
Ascorbic acid	–	16.6±0.88	26.6±0.8***	37.6±2 ***	–	46.3±0.8 ***	52±1.1 ***

Values are Mean ± SEM. ***p<0.001 v/s 50μg/ml

Table 2: Effect of Silymarin and *B. mollis* on liver enzymes in CCl_4 -induced hepatotoxicity.

Treatment	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**Bilirubin (mg/dL)**
Control	82±5.32	59.4±4.34	144.4±7.08	0.26±0.01
CCl_4	469.6±27.15	285.2±12.61	323.8±15.50	1.35±0.03
B. mollis 50 mg/kg	433.2 ± 15.73	238.2±14.59*	274.2±18.14	1.16±0.02**
B. mollis 200 mg/kg	213±11.47***	199.2±5.74 ***	252±11.79 **	1.08±0.03 ***
Silymarin	151±9.95 ***	159.6±7.80 ***	202.6±6.43 ***	0.75±0.05 ***

All values are Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 vs CCl_4

Table 3: Effect of *B. mollis* on liver enzymes in paracetamol-induced hepatotoxicity

Treatment	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**Bilirubin (mg/dL)**
Control	84.60±5.817	48.60±3.31	229±16.75	1.14±0.169
CCl_4	231.6±19.21	415.4±36.44	553±36.52	2.8±0.216
B. mollis 50 mg/kg	189.9±8.430 (ns)	405.2±21.29 (ns)	542.2±28.62 (ns)	2.42±0.193 (ns)
B. mollis 200 mg/kg	122±12.47***	263.0±24.79***	276.0±18.60***	1.54±0.174***
Silymarin	101±8.44***	134.4±10.48***	265.0±8.60***	1.28±0.153

Values are Mean ± SEM. ***p<0.001 vs Paracetamol control

Table 4: Anti-inflammatory activity of *B. mollis* in Carrageenan and formalin-induced rat paw oedema

Treatment	**Carrageenan 3 hr**	**Carrageenan 6 hr**	**Formalin 3 hr**	**Formalin 6 hr**
Normal Control	0.6±0.054	0.6±0.054	0.796±0.005	0.796±0.005
Negative Control	2.24±0.120	2.32±0.106	1.414±0.010	1.652±0.010
B. mollis – 50 mg/kg	1.9±0.173^{ns}	1.74±0.153**	1.394±0.007^{ns}	1.376±0.006***
B. mollis – 200 mg/kg	1.64±0.103**	1.5±0.089***	1.196±0.005***	1.186±0.007***
Diclofenac (10 mg/kg)	1.08±0.153***	0.9±0.044***	1.26±0.004***	1.098±0.003***

Values are expressed as Mean ± SEM, **p<0.01, ***p<0.001 vs respective negative control

hepatoprotective activity of *B. mollis* (Figure 2).

DISCUSSION

In the present study, the antioxidant and protective effects of *B. mollis* on the liver were thoroughly investigated using different *in vitro* and in vivo models and were similar to the existing literature that has shown the therapeutic efficacy of plants with similar phytoconstituents. In the superoxide radical scavenging assay, activity of *B. mollis* increased dose dependently from 26±1.52 at 50 μg/ml to 73.66±0.88 at 200 μg/ml, which, though less than ascorbic acid (83.3±1.528 at 200 μg/ml), reflected good antioxidant potential. These findings were consistent with those by Lobo et al., who highlighted the ability of plant flavonoids and polyphenols to scavenge superoxide radicals.[1] Likewise, in the hydroxyl radical scavenging assay, *B. mollis* showed an increasing activity with increasing concentration, reaching 58±1.15 at 200 μg/ml compared to 61.6±0.88 for ascorbic acid, consistent with previous research indicating phenolic-rich extracts' hydroxyl radical inhibitory activity.[3]

In addition, *B. mollis* has a strong dose-dependent inhibitory effect on lipid peroxidation, an action commonly

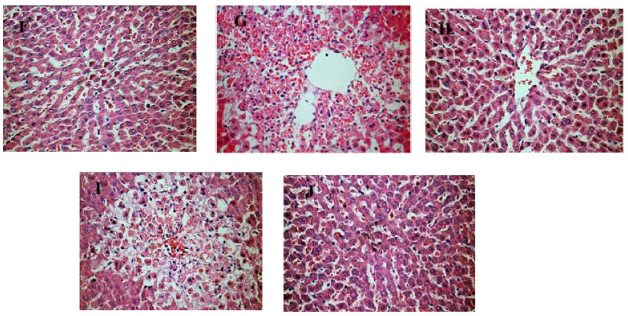

Fig. 2: Histopathological Analysis of Liver Tissues Following Paracetamol-Induced Hepatotoxicity and Treatment (H&E Staining) showing F-Normal liver with preserved tissue (saline-treated), G-Paracetamol-treated liver showing significant hepatocellular damage, H-*B. mollis* (30 mg/kg) showing mild protection, I-*B. mollis* (200 mg/kg) showing improved liver structure and J-Silymarin (200 mg/kg) showing nearly normal liver histology

associated with the prevention of oxidative stress-mediated membrane damage. The highest inhibition achieved was 58±1.15 at 350 μg/ml, being very near the activity of ascorbic acid (52±1.1), supporting its lipid peroxidation inhibitory activity, as also indicated by Halliwell and Gutteridge.[7] The acute toxicity test also validated the safety profile of *B. mollis* since mortality was not noted at doses of up to 2000 mg/kg, as supported by OECD guidelines and plant safety investigations.[8]

In hepatoprotective experiments, *B. mollis* at 200 mg/kg markedly lowered serum biochemical markers (SGOT, SGPT, ALP, and bilirubin) in both carbon tetrachloride- and paracetamol-induced hepatotoxicity models, indicating excellent hepatoprotective potential, comparable to that of silymarin. These outcomes concurred with previous results wherein polyphenolic-rich extracts lowered liver enzyme levels and normalized hepatic function.[2] Histopathological assessments further supported these biochemical findings as *B. mollis* maintained liver structure and counteracted necrotic and inflammatory alterations in a dose-dependent manner, a pattern similarly documented in experiments on hepatoprotective plants such as Phyllanthus niruri.[4]

The anti-inflammatory activity of carrageenan and formalin-induced paw oedema model asserted the dose-dependent efficacy of *B. mollis*, where inhibition by the higher dose (200 mg/kg) was comparable to that of diclofenac, a reference NSAID. This is consistent with research which has shown flavonoids to inhibit pro-inflammatory mediators and suppress oedema.[5] Thus, the present study demonstrated the traditional use of *B. mollis* in the management of oxidative stress and inflammation and presented a scientific rationale for its therapeutic uses.

CONCLUSION

In conclusion, *B. mollis* showed dose-dependent antioxidant, hepatoprotective, and anti-inflammatory activity. Its activity was comparable to that of reference drugs, such as ascorbic acid, silymarin, and diclofenac, especially at higher concentrations. The absence of toxicity at high doses further supports this safety profile. These results confirmed the traditional use of *B. mollis* and highlighted it as a potential candidate for development as a natural therapeutic agent.

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