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Original Research Article

Evaluation of anti-nociceptive, anti-inflammatory and hepatoprotective effects of methanol extract of *Mazus pumilus* (Burm. f.) Steenis (Mazaceae) herb

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Abstract

Purpose: *This study was designed to investigate the anti-nociceptive, anti-inflammatory and hepatoprotective activities of the methanol extract of Mazus pumilus (Mazaceae) herb.*
Methods: *Anti-nociceptive activity was determined using hot plate, tail flick and acetic acid-induced writing methods. Carrageenan-induced rat paw edema (0.1 mL of 1 %) model was used for the assessment of anti-inflammatory activity. The methanol extract was administered orally at three different doses (150, 300 and 600 mg/kg) to three separate groups in all the experiments. Diclofenac sodium (50 mg/kg) was used as standard drug while control group received DMSO (1 %, 10 mL/kg). The hepatocurative effect of methanol extract of M. pumilus (400 mg/kg) was determined in isoniazid (50 mg/kg) and rifampicin (100 mg/kg) induced liver injury. Silymarin (100 mg/kg) was used as standard drug for comparison. The control group received distilled water (10 mL/kg). Preliminary phytochemical screening was also carried out.*
Results: *The methanol extract of M. pumilus significantly ($p < 0.05$) augmented latency time and reduced the number of writhes in the pain models at all doses used for the assessment of anti-nociceptive actions. The anti-inflammatory activity of different doses of extract was evaluated by measuring the reduction in the size of the paw. A significant ($p < 0.05$) hepatocurative effect was observed when administered after anti-tuberculosis drugs. Histopathological analysis of the liver tissues also revealed restored hepatocellular architecture.*
Conclusion: *The results demonstrate the anti-nociceptive, anti-inflammatory and hepatoprotective effects of the methanol extract of M. pumilus, thus substantiating the ethnomedical claims associated with the herb.*

Keywords: *Mazus pumilus, Tong quan cao, Asian Mazus, Anti-nociceptive, Anti-inflammatory, Hepatoprotective*

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INTRODUCTION

The search for new anti-inflammatory and analgesic compounds has been a priority for researchers in the past few years due to increased burden of inflammatory diseases [1]. According to the WHO survey in various developing countries, 40 to 90 % of the population relies on traditional system of medicines to meet primary healthcare needs [2]. Because of the undesirable impacts of the allopathic medications on liver, the use of natural pharmaceuticals and phytopharmaceuticals has expanded for liver diseases. Another factor for their greater worthiness, is their simple accessibility, monetary and less untoward impacts on human health. However herbal medicines lack evidenced based therapeutic uses [3].

Mazus is a low–growing perennial plant consisting of 30 species. *Mazus pumilus,* an annual herb belongs to family Mazaceae*,* also called Asian mazus or Japanese Mazus. It is generally found in damp habitats in lowland or mountain regions of China, Japan, South East Asia, Australia, New Zealand and Punjab region of Pakistan [4]. In Chinese medicine, it is known as *"Tong quan cao"*. The herb is of great significance owing to its diverse therapeutic benefits. As an ethnomedicine, the leaves of herb have been used in epileptic seizures [5]. The herb possesses antimicrobial activity against certain bacteria and fungi [6]. The anticancer activity by the leaf extract of *M. pumilus* on human cell lines have been reported by Priya *et al* [7]. The herb is likewise accounted to relieve constipation, a stimulator to menstrual flow, a vigor tonic and antipyretic agent. The juice of the herb is used as a remedy for typhoid fever. In an ethnobotanical review, the herb was discovered as fodder for the livestock [8]. The plant extract possess appreciable antioxidant activity, because of which the herb is also mentioned as cardioprotective [9]. Because of the anti-oxidative properties of the herb's extracts, the present investigation was brought up with a target to assess methanol extract of *M. pumilus* herb for the anti-nociceptive, anti-inflammatory and hepatoprotective activities.

EXPERIMENTAL

Chemicals

Methanol, Dimethyl sulfoxide (DMSO), Diclofenac Na., distilled water, acetic acid and carrageen, isoniazid (INH), rifampicin (RMP) were procured from Pacific Pharmaceuticals Ltd. (Lahore, Pakistan). Diagnostic kits of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and total protein was obtained from Global (London) and silymarin was acquired from Wilson's Healthcare Pakistan. All other reagents were of analytical grade. The reagents used were freshly prepared in Postgraduate Research laboratory of Pharmacognosy, Punjab University College of Pharmacy, University of the Punjab.

Plant material

The plant was collected from botanical garden of Government College University Lahore and was authenticated by Dr. Uzma Hanif, Assistant Professor, Department of Botany, Government College University Lahore, Pakistan. A specimen of the plant was deposited in herbarium of Government College University Lahore, under voucher No: GC. Herb. Bot. 2270. All plant parts were dried under shade, pulverized and stored in hermetic containers in a dry place.

Animals

Swiss albino mice and Wistar rats of either sex were purchased from the University of Veterinary and Animal Sciences, Lahore Pakistan. The animals were kept at room temperature 25 ºC under 12 h of dark and light cycles, and fed with standard pelleted diet with water *ad libitum*. All the protocols of the study were approved by the Animal Ethical Committee of the Punjab University College of Pharmacy, University of the Punjab. A reference number was issued by the departmental ethical committee (no. AEC/PUCP/1042/4313). The Animal Ethical Committee follows the international guidelines of National Institute of Health [10].

Preparation of methanol extract

The extract was prepared by macerating 500 g of dried powder of *M. pumilus* in 1500 mL of methanol for three days in round bottom flask. The extract was filtered using *Whatman* No. 1 filter paper. The filtrate was evaporated in rotary evaporator and dried below 40 ºC.

PHYTOCHEMICAL SCREENING

The phytochemical analysis was performed according to standard procedures [11].

Study design

Analgesic activity

The tests were performed on male mice having weight between 22 – 35 g. Total twenty five

animals were utilized for experimentation, partitioned into 5 groups (n = 5). All treatments were administered orally and plant extract was dissolved in 1 % DMSO. Group I was administered 1 % DMSO (10 mL/kg, po) as control. Group II was given standard drug, diclofenac sodium (50 mg/kg, po). Groups III, IV and V were administered 150, 300 and 600 mg/kg (po) of methanol extract of *M. pumilus* dissolved in 1 % DMSO, respectively.

Anti-inflammatory activity

The anti-inflammatory test was carried out on rats of either sex. Twenty five rats weighing between 200 – 335 g, were incorporated in this activity. The animals were divided into five groups (n = 5). Plant extract was dissolved in 1 % DMSO. Group I was labeled as control and administered 1 % DMSO (10 mL/kg, po). Group II was given standard drug diclofenac sodium (50 mg/kg, po). Groups III, IV and V were administered 150, 300 and 600 mg/kg (p.o.) of methanol extract of *M. pumilus* dissolved in 1 % DMSO, respectively. All treatments were administered orally.

Hepatoprotective activity

Hepatoprotective activity was carried out on Wistar rats of either sex. The study was conducted for twenty one days. The weight of the rats included in the study was in the range of 150 – 200 g. Twenty animals were divided into four groups (n = 5). Group I was given distilled water (10 mL/kg) and labeled as Control. Group II was administered Isoniazid INH (50 mg/kg) and Rifampicin RMP (100 mg/kg). Group III was treated with extract of silymarin (100 mg/kg), INH (50 mg/kg) and RMP (100 mg/kg). Group IV was treated with *M. pumilus* (400 mg/kg), INH (50 mg/kg) and RMP (100 mg/kg). All treatments were administered orally dissolved in distilled water.

Assessment of anti-nociceptive activity

The anti-nociceptive activity of the methanol extract of *M. pumilus* was determined by three different methods.

Hot plate method

Animals were subjected to pre-testing on hot plate (Havard apparatus). The temperature of hot plate was set at 55 ± 0.1 °C. After 30 min of treatment, the animals were exposed to hot plate and latency time was recorded in seconds. Latency time is the time for which mouse remains on the hot plate without licking or flicking of hind limb or jumping. The animals showing latency time greater than fifteen seconds were not included in the study. To avoid the tissue damage, 30 sec cut-off time was selected for all animals. The latency time for all groups was recorded at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 h after drug administration [12].

Tail immersion method

The animals were placed in upright position to set their tails in hanging position. The tail of each animal was marked up to 5 cm length and was dipped into a container of hot water. Healthy animals were selected for this experiment who responded within 15 sec to hot water stimulus. Temperature of hot water was about 55 ± 0.5 °C. Time taken to pull the tail out of hot water called reaction time was measured. The readings were taken at the time interval of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 h of administration of the test drugs. A cut-off time of 30 sec was considered i.e. time of no response [13].

Acetic acid induced writhing method

Thirty minutes after the administration of *M. pumilus* extract, each treatment group was intra-peritoneally administered acetic acid (10 mL/kg of 0.6 %). Five min after the acetic acid injection, the number of abdominal constrictions (writhes) were counted for next 10 min [14].

Determination of anti-inflammatory activity

Carrageenan-induced rat paw edema

Thirty minutes after the intra-peritoneal administration of different doses of the methanol extract and standard drug to their relevant groups, carrageenan (1%, 0.1 mL) was injected subcutaneously in the sub plantar tissue of the right hind paw of each rat. Digital vernier caliper was used to measure the inflammation right after the injection of carrageenan and then after the time interval of 0.5, 1, 2, 3 and 4 h. Percentage (%) inhibition of edema (E) was calculated using Eq 1.

$$E (\%) = \{(A – B)/A\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where A represents edema volume of control and B paw edema of tested group [15].

Evaluation of hepatoprotective activity

All the animal groups were subjected to oral administration of respective treatments in distilled water (10 mL/kg) for a period of 21 days. At the end of the study, animals were anaesthetized by

intra-peritoneal administration of 5 mL/kg of a solution of 1 % chloralose in 25 % urethane (w/v). Blood samples were drawn from the anaesthetized animals by cardiac puncture in sterile heparinized tubes and allowed to clot for 30 min. Serum was separated from the blood by centrifugation process and used for the assay of serum marker enzymes [16].

Histopathological examination

Fresh liver tissues (previously trimmed to 7 µm thick) were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. Treated tissues were fixed in paraffin, sectioned, deparaffinized and re-hydrated. The tissues were then stained with eosin and hematoxylin. The structural changes in prepared liver slides were analyzed under microscope [17].

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was carried out using SPSS 21 and GraphPad Prism 7. Statistical significance of the differences between control and treated groups was calculated using One-way ANOVA with post-hoc Tukey's HSD test. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical analysis

Phytochemical screening of methanol extract revealed presence of proteins carbohydrates, saponins, tannins, terpenoids, sterols, glycosides and alkaloids whereas lipids were absent (Table 1).

Table 1: Phytochemical profile of methanol extract of *M. pumilus*

Compound group	Test	Result
Proteins	Millon's test	+
Carbohydrates	Molisch's test	+
Saponin	Foam test	+
Lipids	Soap formation test	–
Tannins	Ferric chloride test	+
Terpenoids	Salkowaski test	+
Sterols	Salkowaski test	+
Glycosides	Keller-killani test	+
Flavonoids	Ferric Chloride test	+
Alkaloids	Dragendroff's test	+

+ = present, – = absent

Analgesic activity

The results of the hot plate test revealed that latency time significantly ($p < 0.05$) increased with 150 mg/kg as compared to the DMSO 1% treated group. The effect was significant at all test doses and the maximum effect was observed after 2 h. The most significant increase in latency time was observed with 150 mg/kg of plant extract (Table 2), 2 h after the administration of the methanol extract of *M. pumilus.* Maximum analgesic effect of diclofenac sodium was also observed at 2 h. Non-significant results were obtained with 300 and 600 mg/kg dose as presented in Table 2.

The analgesic effect of the plant extract was significant ($p < 0.05$) in tail flick test. The maximum analgesic effect was noticed 4 h after the administration of 150 mg/kg (60.88 sec ±4 .73) and 600 mg/kg (111.0 sec ± 3.62) respectively and at 2.5 h with 300 mg/kg (48.4 sec ± 3.86). The standard drug diclofenac sodium (50 mg/kg body weight) induced a significant ($p < 0.05$) decrease in the tail flick response at 2 h when compared to control untreated group as presented in Table 3.

The methanol extract of *M. pumilus* at different (150, 300, and 600 mg/kg) doses significantly ($p < 0.05$) reduced the number of abdominal constrictions (writhing) induced in mice by acetic acid 0.6 %. i.p. The results showed that the anti-nociceptive activity increased with increasing the dose of the extract (150, 300 and 600 mg/kg). This dose-dependent analgesic effect reached a maximum inhibition of 59.16 % at the dose of 600 mg/kg. The standard drug, diclofenac sodium (50 mg/kg) exerted a significant analgesic effect, with percentage inhibition of 80.89 % (Table 4). *M. pumilus* extract and diclofenac sodium showed significant ($p < 0.05$) reduction of pain in comparison with control group given DMSO and 0.6 % acetic acid (Table 4).

Anti-inflammatory activity

The methanol extract of *M. pumilus* at all doses showed significant ($p < 0.05$) reduction in paw edema at first, second, third and fourth hour as compared to reference drug diclofenac sodium. The reduction in paw size shows reduced inflammation as presented in Table 5 at different time intervals. Diclofenac Na (50 mg/kg) at 4th h showed significant and maximum reduction of paw edema in comparison with the control group at 4th hour. The reduction in size of paw in extract treated group at different time intervals is comparable to Diclofenac Na that showed decrease in paw edema starting from 1 h after the administration of drug for up to 4 h. The methanol extract at the dose of 150 mg/kg exhibited a significant anti-inflammatory activity 0.5 h after the injection of carrageenan and was

Table 2: Anti-nociceptive effect of methanol extract of *M. pumulis* herb by hot plate method in mice

Treatment	Dose mg/kg	0h	0.5h	1h	1.5h	2h	2.5h	3h	3.5h	4h
DMSO 1%	10 ml	12.79±0.90	13.16±0.90	13.35±0.89	15.65±0.78	16.59±0.75	17.34±0.58	17.60±0.67	17.19±0.66	16.77±0.65
	150	15.90*±0.59	17.38*±0.55	30.50*±1.54	46.46*±6.75	81.24*±6.99	20.15±1.00	22.85±1.02	18.70*±1.48	18.49*±1.02
	300	15.84*±0.56	31.23*±1.31	35.10*±1.61	34.03±1.46	47.68±2.30	39.53*±2.10	37.01*±2.23	29.02*±1.30	23.95*±1.13
Extract	600	16.25*±0.55	21.85*±0.85	25.17±1.11	24.45±1.76	24.30±1.82	18.32±0.94	18.49±1.02	15.85*±0.95	14.66*±0.98
Diclofenac Na	50	16.60*±0.54	17.77*±0.64	17.91±0.62	17.54±0.80	20.92±0.91	19.74±0.81	18.66±0.93	16.77±0.79	14.11±0.60

All the results are presented in seconds and values are expressed as mean ± SEM (n=5). The extract and Diclofenac Na treated groups were compared with 1 % DMSO-treated group using one-way ANOVA followed by Tukey's test; * $p < 0.05$

Table 3: Anti-nociceptive effect of methanol extract of *M. pumulis* herb by tail flick method in mice

Treatment	Dose mg/kg	0h	0.5h	1h	1.5h	2h	2.5h	3h	3.5h	4h
DMSO	10ml	1.46±0.30	1.65±0.36	2.03±0.32	2.40±0.43	2.74±0.37	2.51±0.30	2.50±0.18	1.60±0.27	1.52±0.28
	150	0.99±0.17	3.97±0.80	23.70*±2.60	25.4*±2.68	35.86*±3.06	33.72±3.22	26.38*±2.80	22.12±2.44	60.88*±4.73
	300	1.23*±0.29	21.22±2.32	22.08±2.03	15.65±1.64	15.72±1.65	31.68*±3.14	9.49±1.18	11.08*±1.28	7.28*±1.06
Extract	600	0.99±0.20	3.30*±0.82	23.66*±2.60	25.24±2.68	37.50±3.06	48.40*±3.86	27.85±2.79	21.99±2.46	111.0*±3.62
Diclofenac Na	50	1.28*±0.327	15.25*±2.078	3.21±0.704	2.58±0.442	2.73±0.386	2.69±0.501	2.70±0.300	3.05±0.540	1.76±0.343

Results are presented in seconds and values are expressed as mean ± SEM (n = 5). The data was analyzed by one way ANOVA followed by Tukey's test using SPSS. All the treatments at different time intervals were compared with their matching time interval of the control group; * $p < 0.05$

Table 4: Analgesic effect of methanolic extract of *M. pumilus* by acetic acid-induced writhing in mice

Treatment	Dose (mg/kg/ day)	Number of writhes	Protection (%)
Control (DMSO)	10 mL/kg + 0.6 % acetic acid	45.00±0.66	0
Extract	150	37.38±0.37*	16.93
	300	27.02±0.65*	39.96
	600	18.38±0.64*	59.16
Diclofenac Na	50	08.60±0.52*	80.89

The number of writhes are presented in seconds and values are expressed as mean ± SEM (n=5). The data was analyzed by one way ANOVA followed by Tukey's test using SPSS. All the treatments at different time intervals were compared with their matching time interval of the control group; * $p < 0.05$

maintained all along the experiment with a maximum effect 5.42mm ± 0.26. The extract (150 and 300 mg/kg) induced significant ($p < 0.05$) anti-inflammatory effect and the anti-inflammatory effect of diclofenac sodium (50 mg/kg) was comparable with that of the extract (Table 5).

Hepatoprotective activity

The hepatoprotective activity of *M. pumilus* treated group (group 4) showed significant $p < 0.05$ reduction in liver enzymes i.e. ALT, AST, ALP, total protein and total bilirubin levels when compared with Group 2 INH (50 mg/kg) + RMP (100 mg/kg). Significant ($p < 0.05$) results were also obtained with silymarin but more pronounced hepatoprotective effect was observed with the methanol extract (Figure 1).

Histopathological features

Histopathological examination also showed less damage to the hepatocytes and liver architecture in methanol extract- and silymarin-treated groups when compared with isoniazid (50 mg/kg and rifampicin (100 mg/kg) treated group (Figure 2).

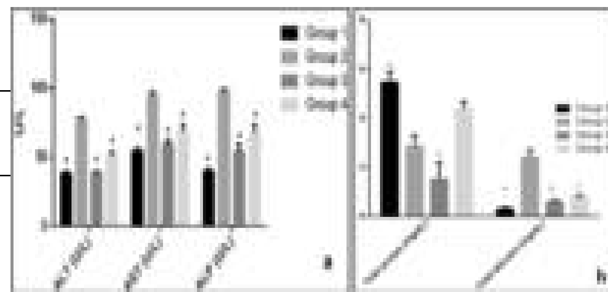

Figure 1: Comparison of the alterations in the liver markers by methanol extract of *M. pumilus* and silymarin treatments with isoniazid and rifampicin induced hepatotoxic treatment. Group 1= Control group; Group 2= INH (50 mg/kg) + RMP (100 mg/kg); Group 3 = extract of silymarin (100 mg/kg), INH (50 mg/kg) and RMP (100 mg/kg); Group 4 = extract of *M. pumilus* (400 mg/kg), INH (50 mg/kg) and RMP (100 mg/kg). All groups are compared with Group 2 using Graphpad Prizm by applying One-way ANOVA. * represents $p < 0.05$. **a:** Levels of ALT, AST, and ALP, **b:** Levels of total protein and total bilirubin

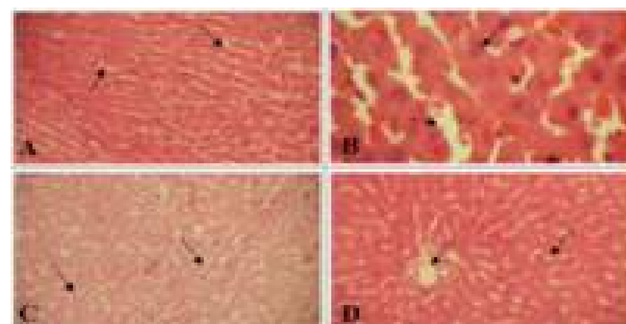

Figure 2: A: Normal group; normal hepatocytes, **B:** Toxic group; hepatocyte degeneration, **C:** Standard group; hepatocyte regeneration, **D:** Experimental group; mild hepatocyte regeneration

DISCUSSION

Pain and inflammation are connected with numerous pathological conditions. Synthetic drugs accessible for curing these disorders cause numerous undesirable effects. A number of studies are being conducted worldwide to assess natural sources for the active or lead

Table 5: Anti-inflammatory effect of MeOH extract of *M. pumilus* by carrageenan-induced rat paw edema method

Treatment	Dose (mg/kg/day)	0 h	0.5 h	1 h	2 h	3 h	4 h
DMSO	10mL	5.15±0.11	6.62±0.20	7.56±0.12	7.12±0.11	7.00±0.14	6.72±0.13
Extract	150	4.18±0.26	4.95*±0.21	5.13*±0.22	4.66*±0.24	4.71*±0.28	4.42*±0.25
	300	4.91±0.34	5.54*±0.28	6.12*±0.25	5.90*±0.19	5.84*±0.22	5.42*±0.26
	600	5.13±0.29	5.95±0.41	6.10*±0.46	6.20±0.43	5.90*±0.41	5.44*±0.41
Diclofenac Na	50	4.95±0.26	5.84±0.22	6.04*±0.23	5.96*±0.28	5.74*±0.24	5.40*±0.23

All the results are presented in mm and values are expressed as mean ± SEM (n = 5). The data was analyzed by one way ANOVA followed by Tukey's test using SPSS. All the treatments at different time intervals were compared with their matching time interval of the DMSO-treated group; * $p < 0.05$

compounds with best safety profiles [15]. Medicinal plants are accepted as a vital source of new compounds having therapeutic potential. The research on folkloric usage of plants as pain relievers, anti-inflammatory and hepatoprotective agents should therefore be viewed as a fruitful research strategy for search of a new analgesic, anti-inflammatory and hepatoprotective drugs [18].

The results of the analgesic experiments showed that *M. pumilus* exhibits both peripheral and central analgesic properties. Tail immersion and hot plate test are suitable methods for the assessment of centrally acting analgesic drugs while acetic acid-induced writhing is used for the evaluation of peripherally acting drugs. *M. pumilus* extract also showed significant (p < 0.05) analgesic effect in both the hot plate and tail immersion tests. The extract showed maximum analgesic effect after 1 h of administration and prevailed for up to 4 h with all doses (150, 300 and 600 mg/kg) in hot plate experiment. Hot plate method is the oldest method employed to study the supra spinal analgesic effect of the drugs. Hence it could be suggested that the plant extract might possess central supra spinal analgesic activity. The tail immersion test is thought to be particular to analyze compounds acting through opioid receptor [18]. The plant extract expanded pain threshold basal latency time demonstrating that it might act by means of centrally mediated analgesic mechanism.

The extract of *Mazus pumilus* exhibited significant (p < 0.05) dose dependent inhibition of acetic acid-induced writhing in mice, comparable to Diclofenac Na. Writhing is a noticeable response produced due to the intense pain produced by acetic acid. Acetic acid-induced writhing model shows pain sensation by activating localized inflammatory response. The pain stimulus results into release of free arachidonic acid from the tissue phospholipid. The acetic acid induced writhing response is an accurate method to assess peripherally acting analgesics. The response is thought to be intervened by peritoneal mast cells, acid sensing ion channels, and the prostaglandin pathways [18]. The analgesic effect of the extract might be due to the inhibition of arachidonic acid pathways involving cyclooxygenase enzyme. A dose dependent increase in the analgesic effect of the extract was observed. The number of the writhes decreased more significantly (p < 0.05), with increasing dose. The inhibitory effect might be due to the different peripheral mechanisms of pain inhibition. The flavonoids present in the extract might be responsible for the analgesic action due to interference with the prostaglandin studies and inhibition of prostaglandin synthase as have been observed in previous studies [19].

Carrageenan-induced paw edema is one of the most commonly employed animal model to evaluate the anti-inflammatory effect of natural products as well as synthetic chemical compounds. Carrageenan induces edema in paw in biphasic manner for the 1 – 4 h; the early phase (1 or 1.5 h) is predominately a non-phagocytic edema and is associated with the release of autacoids. The second phase (2 – 4 h) consist of expanded edema formation that stayed up to 4 h. The early phase has been induced due to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability. Over-production of prostaglandins might be the reason for the appearance of second phase [14,18]. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests possible inhibition of COX synthesis by the extract and this impact is similar to that produced by non-steroidal anti-inflammatory drugs whose mechanism of action is inhibition of the COX enzyme [19]. In carrageenan paw edema model of acute inflammation, extract significantly reduce the paw size at 1st, 3rd and 4th h comparable with diclofenac sodium. The herb extract showed significant dose dependent reduction in paw size. This may be because of the inhibition of the biphasic response induced by the carrageenan. The anti-inflammatory effect might be attributed to the presence of terpenes, saponins, glycosides and flavonoids [20].

It is conceivable that multiple mechanisms like inhibition of either cyclooxygenase and/or lipooxygenase enzyme or inhibition of synthesis, release and action of above inflammatory mediators may be involved in acute anti-inflammatory effect of extract. In previous studies, several plants showing anti-inflammatory effects have been proposed to act through these mechanisms. [21,22].

The phytochemical screening of *Mazus pumilus* plant extract uncovered the existence of the various phytochemicals (Table 1). The present investigation proves the analgesic and anti-inflammatory action of the plant extract in experimental animals however the exact mechanisms still requires further evaluations [15]. These activities might be because of its high flavonoid substance which are responsible for free radical scavenging activity, as these free radicals are the cause of pain stimulation, and antioxidants demonstrated decrease in such pain [23]. A number of plants extract demonstrated

analgesic and anti-inflammatory impact on animals and their impacts have been ascribed to the existence of triterpenoids, alkaloids, glycosides, flavonoids, tannins, saponins and sterols [24,25].

The hepatoprotective effect of the plant extract against INH and RMP induced liver damage might be attributed to the presence of the flavonoids and triterpenoids that have the property to scavenge the free radicals that are responsible to decrease the levels of glutathione and related thiols [26]. The significant ($p < 0.05$) decrease in the biochemical markers of the liver is presented in Figure 1 and histopathological analysis of the liver treated with extract and silymarin (Figure 2) indicate the improvement in the antioxidant status of the liver.

CONCLUSION

The anti-nociceptive, anti-inflammatory and hepatoprotective effects of the methanol extract of *M. pumilus* may be due to the presence of secondary metabolites like glycosides, saponins, flavonoids and terpenoids. The mechanisms probably involve both the central and peripheral actions that inhibit the release and synthesis of prostaglandins and cyclooxygenase or blocking of nociceptors, while the hepatoprotective effect might have been caused by the antioxidant activity of the plant extract. However, further pharmacological and phytochemical analysis is required to determine the exact mechanisms and chemical constituents responsible for these actions.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. SI, AI and NI conceived and designed the study. UH and UN helped in plant material collection. SI, SHK and MSKA collected and analyzed the data. AI and NI wrote the manuscript. All the authors read and approved the final manuscript.

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