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SCIENTIFIC EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF HERBAL PLANT THROUGH IN VITRO AND IN VIVO STUDIES

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ABSTRACT

Background: *Martynia annua* is a medicinal plant traditionally used for the treatment of inflammatory disorders owing to its rich phytochemical composition and therapeutic potential. **Objective:** The present study aimed to evaluate the phytochemical constituents, antioxidant activity, and in vitro and in vivo anti-inflammatory activities of *Martynia annua* seed extract. **Methods:** The seeds were extracted successively with petroleum ether and methanol using Soxhlet extraction. Preliminary phytochemical screening and quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) were performed. Antioxidant activity was evaluated using the DPPH assay. In vitro anti-inflammatory activity was assessed by the protein denaturation method, while in vivo activity was investigated using the carrageenan-induced paw edema model in rats. Acute oral toxicity was performed according to OECD guidelines. **Results:** The methanolic extract was rich in alkaloids, glycosides, flavonoids, tannins, phenolics, proteins, and carbohydrates, with a total phenolic content of 54 mg GAE/g and total flavonoid content of 17.66 mg RE/g. The extract exhibited significant antioxidant activity with an IC_{50} value of 50.47 µg/mL and demonstrated concentration-dependent inhibition of protein denaturation (IC_{50} = 66.74 µg/mL). In vivo,

the extract significantly reduced carrageenan-induced paw edema, with 42% inhibition at 200 mg/kg, comparable to the standard drug ibuprofen. Acute toxicity studies indicated that the extract was safe up to 2000 mg/kg. **Conclusion:** *Martynia annua* methanolic extract possesses significant antioxidant and anti-inflammatory activities, which may be attributed to its rich phenolic and flavonoid content. These findings support its traditional use and suggest its potential as a natural anti-inflammatory agent.

KEYWORD: *Martynia annua,* anti-inflammatory activity, antioxidant activity, DPPH assay, carrageenan-induced paw edema, protein denaturation assay, phytochemical screening, total phenolic content, total flavonoid content, medicinal plant.

1. INTRODUCTION

Inflammation is a complex biological response of the immune system to infection, tissue injury, toxins, or other harmful stimuli. Although it plays a crucial role in tissue repair and host defense, prolonged or uncontrolled inflammation contributes to the development of several chronic disorders, including arthritis, cardiovascular diseases, diabetes, inflammatory bowel disease, and cancer. Conventional anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are effective in controlling inflammation but are frequently associated with adverse effects, including gastrointestinal ulceration, renal toxicity, and cardiovascular complications. Consequently, there is increasing interest in identifying safer and more effective anti-inflammatory agents from medicinal plants **(Medzhitov, 2008; Libby, 2007).**

Medicinal plants have been extensively utilized in traditional systems of medicine due to their diverse pharmacological properties. Their therapeutic activities are mainly attributed to the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, and terpenoids. These phytoconstituents exhibit antioxidant, anti-inflammatory, antimicrobial, analgesic, and immunomodulatory activities by regulating inflammatory mediators and scavenging reactive oxygen species **(Kokate et al., 2006; Harborne, 1998).**

Martynia annua L., commonly known as devil's claw or tiger's claw, belongs to the family Martyniaceae and is widely distributed throughout tropical and subtropical regions. The plant has long been used in traditional medicine for the treatment of inflammation, wounds, skin disorders, epilepsy, sore throat, and rheumatic conditions. Previous phytochemical studies

have reported the presence of flavonoids, phenolic compounds, glycosides, alkaloids, steroids, and other bioactive constituents responsible for its pharmacological activities, including antioxidant, antimicrobial, analgesic, and anti-inflammatory effects **(Kirtikar and Basu, 2005; Nadkarni, 2009).**

Oxidative stress plays an important role in the initiation and progression of inflammation by generating excessive reactive oxygen species (ROS), which damage cellular components and activate pro-inflammatory signaling pathways. Therefore, plant extracts possessing both antioxidant and anti-inflammatory properties may provide better therapeutic benefits. In vitro assays such as DPPH radical scavenging and inhibition of protein denaturation are widely used for preliminary evaluation of antioxidant and anti-inflammatory activities, whereas the carrageenan-induced paw edema model in rats is a well-established in vivo model for assessing acute anti-inflammatory effects **(Winter et al., 1962).**

Considering the traditional medicinal importance of *Martynia annua* and the limited scientific evidence regarding its anti-inflammatory efficacy, the present study was undertaken to investigate the phytochemical profile, total phenolic and flavonoid contents, antioxidant potential, and in vitro and in vivo anti-inflammatory activities of the methanolic seed extract of *Martynia annua*. The findings of this study may provide scientific support for its traditional use and contribute to the development of plant-based anti-inflammatory therapeutics.

2. MATERIAL AND METHODS

2.1 Chemicals

Ammonia, sodium hydroxide, nitroprusside, and glacial acetic acid were obtained from Merck, a reputable supplier of analytical reagents. Research lab provided the petroleum ether, and Fizmerck provided the concentrated sulfuric acid. Molychem provided the ethanol, while Clorofiltind supplied the concentrated hydrochloric acid and 95% alcohol, along with chloroform. Himedia supplied the magnesium, and Rankem provided the 1% copper sulphate solution.

2.2 Plant collection

The medicinal plant *Martynia annua* (300 gm) was collected. After cleaning, plant part (Seed) were dried under shade at room temperature for 3 days and then in oven dried at 45°C

till complete dryness. Authentication of selected traditional plant - Medicinal plant *Martynia annua* was authenticated by a plant taxonomist in order to confirm its identity and purity.

2.3 Extraction

In present study, *Martynia annua* seed was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Martynia annua* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with methanol solvent. For each solvent, soxhlation was continued till no visual colour change will observed in siphon tube and completion of extraction were confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Materialused}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al*., 2002).**

2.4 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used **(Kokate *et al*., 2000).**

2.5 Quantitative Phytochemical Estimation

2.5.1 Total Phenolic Content (TPC)

The total phenolic content of *Martynia annua* extract was determined using the Folin-Ciocalteu Assay. The *Martynia annua* extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including

polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically (**Tangco *et al*., 2015)**.

2.5.2 Total flavonoid Content (TFC)

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Martynia annua* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy *et al*., 2009).**

2.6 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of *Martynia annua* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared. Different concentration of *Martynia annua* extracts/standard (20 – 100µg/ml) was prepared from 1mg/mL stock solution and 2mL of 0.1mM solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using UV spectrophotometer (Shimadzu 1700) at 517 nm. For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm **(Athavale *et al*., 2012).**

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

2.7 Acute Toxicity Study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed,

dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight (**Guideline Document on 1996**).

2.8 Evaluation of *in vitro* anti-inflammatory activity of plant extract

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of plant extract so that final concentrations become 20, 40, 60, 80 and 100 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ^{o}C in Incubator (Universal) for 15 min and then heated at 70 ^{o}C for 5 min. After cooling, their absorbance was measured at 660 nm (Shimadzu 1700) by using vehicle as blank. Diclofenac sodium at the final concentration of (20, 40, 60, 80 and 100µg/ mL) was used as reference drug and treated similarly for determination of absorbance and viscosity **(Chandra *et al.*, 2012)**.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

2.9 *In vivo* study

2.9.1 Animals

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). The animals used of either sex were of approx 200-250g body weight in range. Before the experiment for the acclimatization, the animals were kept in groups of six in individual cages with temperature controls set at 22 ± 2°C. After that every animal received a consistent supply of water and a standard meal (golden feed, New Delhi).

2.9.2 Carrageenan-Induced Edema in Rats

Four Groups of six animals were used. Paw swelling was induced by sub plantar injection of 0.1 mL 1% carrageenan in saline into the right hind paw. The *Martynia annua* extracts at dose of 100 and 200 mg/kg were administered orally 30 min before carrageenan injection. Ibuprofen (50 mg/kg) was used as reference drug. Control group received the vehicle only (10 mL/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a vernier calliper at time 1, 2, and 3 h after carrageenan injection. The difference

between the left and the right paw volumes (indicating the degree of inflammation) was determined, and the percent inhibition of edema was calculated in comparison with the control animals.

2.9.3 Experimental setup

Thirty minutes after drug or test compound (extracts) administration, 0.1 mL of 1% carrageenan in distilled water was injected into the sub plantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of vernier calliper at 1, 2, and 3 h. The difference between 1 h and subsequent hours reading was taken as actual edema volume.

The four groups are as follows:

Group I: served as control and treated with carrageenan;

Group II: standard group, ibuprofen 50 mg/kg

Group III: served with *Martynia annua* extract at dose of 100 mg/kg

Group IV: served with *Martynia annua* extract at dose of 200 mg/kg

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 1: Percentage Yield of crude extracts of *Martynia annua* extract.

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Martynia Annua*	Pet ether	290	1.64	0.56%
2		Methanol	299	6.22	2.08%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Present
2.2	Legal's test	Present	Present
2.3	Killer-Killiani test	Present	Present
3.	**Carbohydrates**		

3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

Table 3: Standard table for Gallic acid and Rutin.

S. No.	Concentration (µg/ml)	Absorbance (TPC)	Absorbance (TFC)
1.	20	0.149	0.180
2.	40	0.188	0.209
3.	60	0.197	0.279
4.	80	0.238	0.325
5.	100	0.275	0.338

Table 4: Total Phenolic Content and Total Flavonoids content of extract *Martynia Annua.*

Extracts	Methanol
Total Phenolic content (mg/gm equivalent of Gallic acid)	54 mg/gm
Total Flavonoid content (mg/gm equivalent of rutin)	17.66 mg/gm

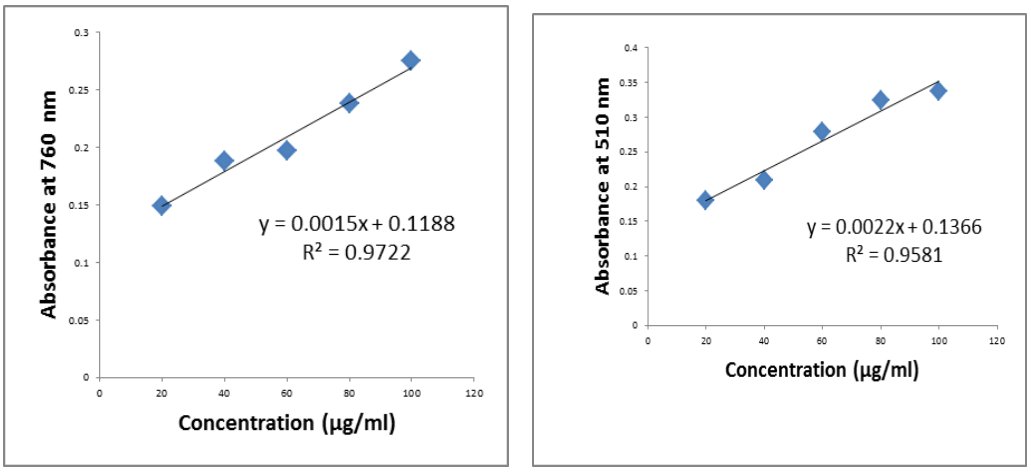


Figure 1: Represent standard curve of Gallic acid and Rutin.

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.489	50.70
40	0.440	55.64
60	0.340	65.72
80	0.287	71.06
100	0.146	85.28
Control	0.992	
IC50	22.98	

3.4 *In vitro* Antioxidant Assays

3.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid and methanol extract of *Martynia Annua.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.522	43.75
40	0.470	49.35
60	0.453	51.18
80	0.418	54.95
100	0.370	60.12
Control	0.928	
IC50	50.47	

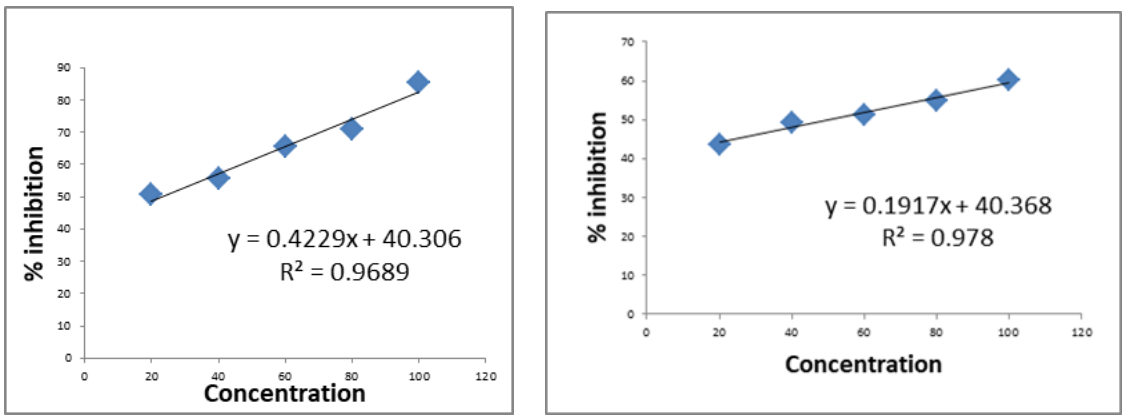


Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid and the Percentage Inhibition *Vs* Concentration of extract of *Martynia Annua.*

3.5 Evaluation of *in vitro* anti-inflammatory activity of *Martynia annua* extract

Table 6: Effect of Diclofenac sodium (Standard) and Effect of *Martynia annua* extract on Protein Denaturation.

S. No	Con. of extract (µg/mL)	Absorbance at 660nm		% Inhibition		IC_{50} Value	
		Diclofenac sodium	*Martynia annua* extract	Diclofenac sodium	*Martynia annua* extract	Diclofenac sodium	*Martynia annua* extract
1.	Control	1.104	1.056	-	-	54.65 µg/ml	66.74 µg/ml
2.	20	0.734	0.804	33.51	23.86		
3.	40	0.617	0.737	44.11	30.20		
4.	60	0.537	0.580	51.35	45.07		
5.	80	0.426	0.428	61.41	59.46		
6.	100	0.302	0.307	72.64	70.92		

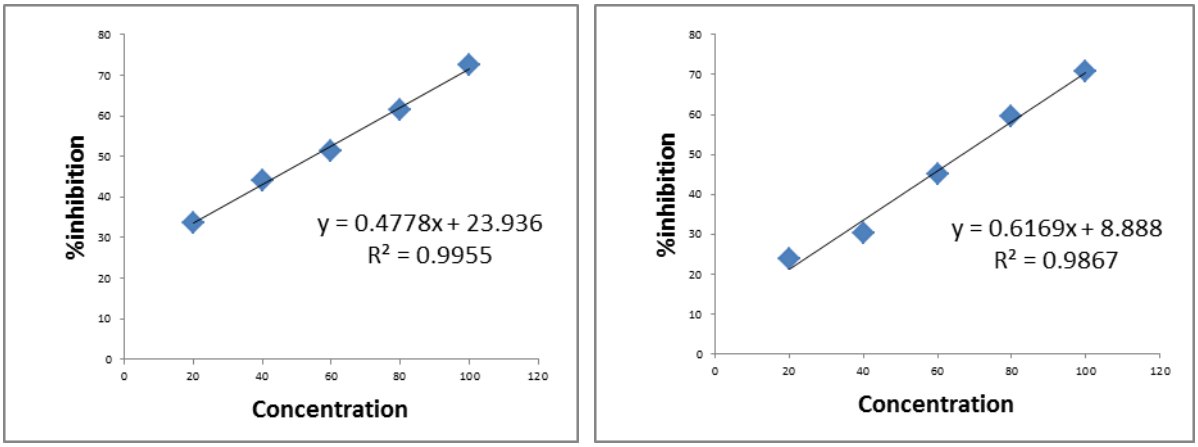


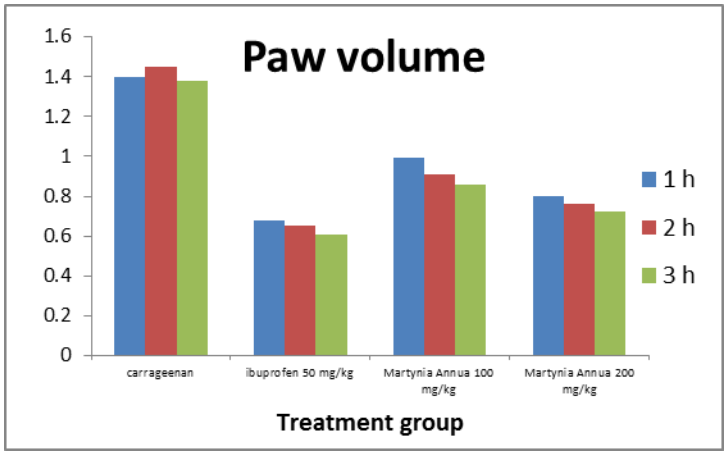
Figure 3: Graph represents the standard curve of Percentage inhibition *Vs* Concentration of Diclofenac sodium (Standard) and *Martynia annua* extract.

3.6 Carrageenan-Induced Edema in Rats

The anti-inflammatory activity of *Martynia annua* against carrageenan induced paw edema has been shown in and the results were comparable to that of ibuprofen a protype of non-steroidal anti-inflammatory agent.

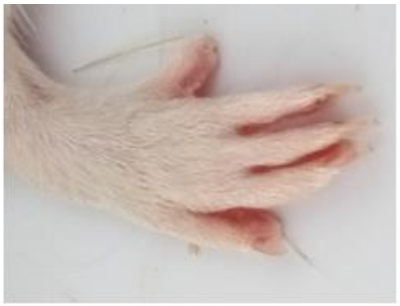
Table 7: Effect of extract *Martynia annua* against carrageenan induced paw edema in rats (n=6).

Treatment	1 h	2 h	3 h	Average reading	% of inhibition
carrageenan	1.40±0.07	1.45±0.06	1.38±0.04	1.41	0
Ibuprofen 50 mg/kg	0.68±0.05	0.65±0.07	0.61±0.03	0.64	59
Martynia annua 100 mg/kg	0.99±0.10	0.91±0.12	0.86±0.09	0.92	38
Martynia annua 200 mg/kg	0.80±0.08	0.76±0.03	0.72±0.11	0.76	42



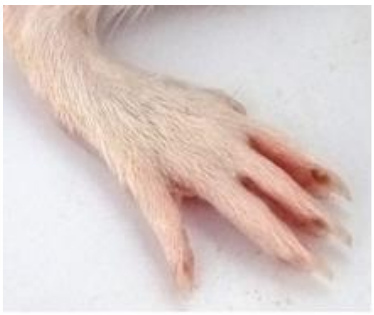
Graph 1: **Carrageenan -induced rat paw edema.**

3.7 Images

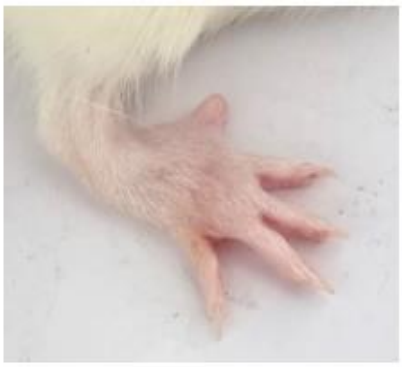


Group I: Normal control

Group II: Carrageenan induced



Group III: Ibuprofen treated



Group IV: *Martynia Annua* 100 mg/kg treated

Group V: *Martynia Annua* 200 mg/kg treated

4. CONCULSION

The present study demonstrated that the methanolic seed extract of Martynia annua possesses significant antioxidant and anti-inflammatory properties. Preliminary phytochemical screening confirmed the presence of bioactive constituents, including alkaloids, flavonoids, phenolic compounds, glycosides, and tannins, while quantitative analysis revealed appreciable total phenolic and flavonoid contents. The extract exhibited concentration-

dependent antioxidant activity in the DPPH assay and effectively inhibited protein denaturation in the in vitro anti-inflammatory model. Furthermore, in the carrageenan-induced paw edema model, the extract significantly reduced paw inflammation, with the 200 mg/kg dose showing greater anti-inflammatory activity, comparable to the standard drug ibuprofen. Acute toxicity studies also indicated that the extract was safe up to 2000 mg/kg. Overall, these findings provide scientific evidence supporting the traditional use of *Martynia annua* as an anti-inflammatory medicinal plant and suggest its potential as a natural source for the development of safer anti-inflammatory agents. Further studies are warranted to isolate the active phytoconstituents and elucidate their molecular mechanisms of action.

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