International Journal of Phytopharmacology
Research Article
www.onlineijp.com
e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

EXPLORING THE ANTI-ARTHRITIC EFFICACY OF *GLOBULARIA Alypum* ON FORMALDEHYDE-INDUCED ARTHRITIS

Dinesh Aarun R, Poorna Chandra T.M*, Jeevana K, Jothieswari D

Assistant Professor, Department of Pharmacology, Sri Venkateswara College of Pharmacy, RVS Nagar, Tirupati Road, Chittoor - 517 127, Andhra Pradesh, India.

ABSTRACT

Aim: To evaluate the anti-arthritic activity of Methanol extract of leaves of Globularia alypum in Formaldehyde induced paw edema of rats. Methods: The leaves of Globularia alypum were subjected to methanol extraction, producing a yield of 24.55% w/w and containing 225.76 ± 75.44 mg/g of phenolic compounds, measured as quercetin equivalents. An acute toxicity study (OECD 423) at 2000 mg/kg confirmed the extract's safety, with no observed adverse effects. Five groups of six Wistar albino rats were assigned as follows: Group I (normal, saline-treated), Group II (control, formaldehyde 0.05 ml/kg, 10% v/v), Group III (standard, formaldehyde + Ibuprofen 15 mg/kg), Group IV (formaldehyde + MGA 200 mg/kg), and Group V (formaldehyde + MGA 400 mg/kg). Treatments were given orally, and paw edema was assessed at 0, 60, 120, 180, and 240 minutes post-formaldehyde injection. Liver enzymes (ALP, ALT), inflammatory markers (CRP, RF), and liver tissue histopathology were also examined. Results: The methanol extract of Globularia alypum (MGA) significantly lowered paw edema at both doses, with the 400 mg/kg dose achieving a 61.72 ± 0.19% reduction at 240 minutes, comparable to Ibuprofen's 64.84 ± 0.13% inhibition. Biochemical tests revealed decreased levels of liver and inflammatory markers in treated groups, while histopathological analysis showed a dose-dependent protective effect on the liver, with the higher dose notably reducing cellular damage and inflammation. Conclusion: The Methanol extract of Globularia alypum exhibits substantial anti-arthritic and hepatoprotective effects, likely due to its phenolic constituents and capacity to suppress inflammatory pathways, positioning it as a promising natural option for arthritis treatment.

Key words: Cnidoscolus aconitifolius, pharmacognostical studies, phytochemical screening, anti-diabetic activity

Corresponding Author: - **Poorna Chandra T.M Email: - poornadailybiologyedu@gmail.com**

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Received:20.02.2025	**Revised**:02.03.2025	**Accepted**:18.04.2025

INTRODUCTION

The therapeutic use of natural products dates back to the earliest civilizations, where minerals, plants, and animal-derived substances were the primary sources of medicinal agents. For centuries, traditional medicine relied on natural products to treat a wide range of ailments. However, with the advent of the Industrial Revolution and the rapid advancements in organic chemistry, the pharmaceutical industry began favoring synthetic compounds for pharmacological treatments. This shift was largely driven by the ease of obtaining pure synthetic compounds, the ability to structurally modify molecules to enhance efficacy and safety, and the increasing economic dominance of pharmaceutical corporations. Additionally, historical and cultural perspectives played a role in diminishing the credibility of natural products, as industrialized societies often dismissed them as remedies for the uneducated or low-income populations or relegated their use to religious or superstitious beliefs without pharmacological merit.

Despite this transition, the significance of natural products in medicine remains undeniable. One of the most impactful discoveries in medical history—the development

of penicillin from microorganisms—revolutionized anti-infective therapy, underscoring the vital role of naturally derived compounds in modern medicine. It is estimated that approximately 25% of prescription drugs worldwide originate from plants, with 121 bioactive plant-derived compounds currently in medical use. Among the 252 essential drugs listed by the World Health Organization (WHO), 11% are exclusively plant-derived, while many others are semi-synthetic derivatives of natural precursors. Notable examples of plant-based drugs include digoxin from Digitalis spp., quinine and quinidine from Cinchona spp., vincristine and vinblastine from Catharanthus roseus, atropine from Atropa belladonna, and morphine and codeine from Papaver somniferum. Furthermore, an estimated 60% of anti-tumor and anti-infective drugs currently available or undergoing clinical trials are of natural origin.

Many of these naturally occurring compounds cannot yet be synthesized economically and continue to be sourced from wild or cultivated plants. Beyond direct medicinal applications, natural compounds serve as lead structures, inspiring the rational design of novel drugs, fostering biomimetic synthesis, and enabling the exploration of new therapeutic properties. Plant-derived compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine, and phorbol esters have been instrumental in pharmacological, physiological, and biochemical research. However, one of the major challenges associated with natural product-based drug development is the structural complexity of these molecules, making their synthesis and modification through conventional synthetic chemistry difficult and cost-intensive.

In recent years, there has been a renewed global interest in alternative medicine and plant-based therapeutics. This shift can be attributed to several factors, including inefficiencies in conventional medicine (e.g., side effects, treatment resistance, and ineffective therapies), concerns over the misuse or overuse of synthetic drugs, and limited access to conventional pharmacological treatments in various parts of the world. Additionally, folk medicine, traditional knowledge, and growing ecological awareness have contributed to the widespread perception that natural products are safer and more sustainable. However, the lack of regulatory oversight and quality control issues surrounding the production, commercialization, and prescription of phytomedicines remain significant concerns. Many studies highlight the need for stringent quality assurance protocols to ensure the efficacy, safety, and standardization of herbal medicines in modern healthcare systems.

Despite these challenges, natural products continue to be a cornerstone of drug discovery and therapeutic innovation. Their role in providing novel bioactive compounds, guiding drug synthesis, and offering new treatment approaches highlights their enduring importance in medicine. Moving forward, integrating traditional knowledge with scientific advancements and establishing robust regulatory frameworks will be essential in unlocking the full potential of natural product-based therapeutics.

Herbal Medicine

Plants have been the basis of different traditional medicinal systems throughout the world and continue to provide mankind with new remedies. World Health Organization (WHO) defines traditional medicine (including herbal drugs) as therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Herbal medicine is the synthesis of therapeutic experience of generation of practicing physicians of indigenous systems of medicine. The traditional preparations include medicinal plants, minerals, organic matter etc. Herbal drugs have been in use in Indian, Chinese, Syrian, Roman, Egyptian, Greek texts for thousands of years as per recorded evidences. The Indian texts include Rig-Veda, Atharvaveda, Charak Samhita and Sushruta Samhita. Folk medicines also play a vital role in healthcare system of ethnic people. Thus, herbal medicines have been derived from rich traditions of ancient civilizations and scientific heritage (Joy et al., 1998; Kamboj, 2000; Mukherjee, 2008). Herbal medicines serve as major remedy in traditional system of medicine, even in 21st century these are the primary source of health care system in rural areas and poor countries. According to WHO, about 80% of the world populations still depend on herbal medicines for primary health care. Herbal medicine practices continue still today because of their biomedical superiority over modern medicine (Sen et al., 2012; Verma and Singh, 2008).

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is strong and can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products. Opportunities for multidisciplinary research that joins the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry and pharmacology to exploit the vast diversity of chemical structures and biological activities of natural products are high.

The one reason for the popularity and acceptability is belief that all natural products are safe. The demand for plant-based medicines, health products, pharmaceuticals, food supplement, cosmetics etc. are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less

side effects and easily available at affordable prices. Now a days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine and the herbal drug industries is now very fast-growing sector in the international market.

Importance of herbal medicine

Herbal medicines are used for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in plants are a part of the physiological functions of living system and hence they are believed to have better compatibility with the human body. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity.

Medicinal plant, Traditional medicinal system and India

India is one of the world's 12th biodiversity zones with the presence of over 45 thousands diverse plant species, though only half of the plants are used for their medicinal values. Ayurveda, Siddha, Unani and Folk medicines are the indigenous medicinal systems in India. About 8,000 herbal medicines have been codified in Ayurveda. The Rig-Veda has documented 67 medicinal plants, Yajurveda 81 species, Atharvaveda 290 species, Charak Samhita and Sushruta Samhita had discussed properties and uses of 1100 and 1270 species respectively are still used in classical formulations (Thomas, 1997).

Traditional medicine is a medical system based on cultural beliefs and practices handed down from generation to generation. Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern Western medicine. In view of the progress of Western medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. The development procedure of herbal drugs for world-wide use has to be different from that of synthetic drugs.

Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian Medical System-Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. Also, Siddha, Unani and Tibb are traditional health care systems have been flourishing for many centuries. Apart from these systems there has been a rich heritage of ethno botanical usage of herbs by various colorful tribal communities in the country.

In many rural communities of developing countries, the use of remedies based on traditional medicine form the basic core of health care. The concept includes mystical and magical rituals, herbal therapy, and other treatments, which may not be explained by modern medicine. Traditional systems in general have had to meet the needs of local communities for many centuries. Over the years, the World Health Assembly has adopted a number of resolutions drawing attention to the fact that most of the populations in various developing countries around the world depend on traditional medicine for primary health care, that the work force represented by practitioners of traditional medicine is a potentially important resource of the delivery of health care and the medicinal plants are of great importance to the health of individuals and communities. The reasons for inclusion of traditional healers in primary health care are manifold: the healers know the sociocultural background of the people; they are highly respected and experienced in their work; economic considerations; the distances to be covered in some countries; the strength of traditional beliefs, the shortage of health professionals, particularly in rural areas.

Vast ethno botanical knowledge exists in India from ancient time. Our work over four decades, both in the field and literary studies, has resulted in a dictionary of Indian folk-medicine and ethno botany that includes 2532 plants. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs or their derivatives and excels in home consumption too. India has about 45,000 plant species; medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500. Despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, chemistry, pharmacology and biotechnology of herbal drugs. The value of ethno medicine has been realized; work is being done on psychoactive plants, household remedies and plants sold by street drug vendors. Statistical methods are being used to assess the credibility of claims. Some recent work in drug development relates to species of *Commiphora* (used as a hypolipidaemic agent), *Picrorhiza* (which is hepatoprotective), Bacopa (used as a brain tonic), *Curcuma* (anti-inflammatory) and *Asclepias* (cardio tonic). Medicinal plants play a key role in the development of potent therapeutic agents. Plant based drugs provide excellent contribution to modern therapeutics. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. The use of plant material used as indigenous cure in folklore or traditional system of medicine (Verma and Singh, 2008)

MATERIALS AND METHODS

The leaves of ***Globularia alypum*** was collected washed and dried at room temperature. After complete

drying, it was powdered and passed through a 60-mesh sieve and stored in air tight container. Dried powdered drug was used to prepare extract. A weighed quantity of air-dried powdered drug was taken and treated with petroleum ether for dewaxing as well as to remove chlorophyll. Then same powder after drying, extracted with ethanol in a soxhlet apparatus by continuous hot extraction for 72 h. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60^0C. The extract was concentrated in water bath and stored in desiccator.

Determination of Total Phenolic Content

Total phenolic content

Following the literature and the results of preliminary phyto chemical screening for plant to contain poly phenols, it was tested to estimate the total phenolic content. It was measured colorimetrically using quercetin and FC (Folin Ciocault's) reagent (Mariela González et al., 2003).

Standard curve of Quercetin

1mg of quercetin was weighed and dissolved in 100ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. A volume from above aliquots was taken and mixed with 1.25ml of FC reagent. It was left for 5 mins. Then 2.5ml of 20% sodium carbonate was added and it was let to react for 30 min then the volume was made up to 10ml. Then the absorbance was measured at 765 nm. The calibration curve was drawn plotting the absorbance and concentrations.

Sample preparation

0.5g of Methanolic extracts was weighed and dissolved in 100ml of water. From this 0.1ml was taken into 10ml standard flask and 1.25ml of FC reagent was added and let to react for 5 min. then 2.5ml of 20% sodium carbonate was added and the column was made up to 10ml. it was kept for 30 min for complete reaction. Now the absorbance was measured at 765 nm. Total phenolic content was calculated from the calibration curve of quercetin and the value was expressed in quercetin equivalents

RESULTS

Table 1: Total phenol content of the methanol extract of Globularia

Extract	% yield w/w	Total Phenol Content (mg/g of extract)
Methanol	24.55	225.76±75.44

Acute toxicity

Acute toxicity is the toxicity produced by a pharmaceutical when it is administered in one or more doses during the period of not exceeding 24 hours. Single escalating dose was given and mortality, body weight and behavioral changes were observed. Mortality was not observed in the animals treated orally with 2000 mg/kg of methanol extract of Globularia. There was no characteristic, statistically significant change in the behavioral patterns, body weight, and food and water intake.

Formalin induced paw edema

The study evaluated the anti-arthritic activity of methanolic extract of *Globularia alypum* (MGA) at two different doses (200 mg/kg and 400 mg/kg) using a formalin-induced paw edema model in rats. The treatments were compared to a control group (formalin only) and a standard group treated with Ibuprofen (15 mg/kg). The results are summarized in three tables, focusing on paw diameter, paw edema, and the percentage inhibition of paw edema over time.

The table 1 presents the effect of MGA (Methanolic extract of Globularia Alypum) on paw edema in rats at different time intervals (0 min, 60 min, 120 min, 180 min, and 240 min). The study aims to assess the anti-inflammatory potential of MGA compared to a control group (which received an inflammatory agent without treatment) and a standard treatment group (Ibuprofen 15 mg/kg). The paw diameter measurements indicate the extent of paw edema (inflammation) over time.

The normal group (administered normal saline) maintained a constant paw diameter of 2.00 ± 0.00 mm throughout the experiment, indicating the absence of any induced inflammation. In contrast, the control group (injected with the inflammatory agent but not treated) showed a significant increase in paw diameter, peaking at 4.50 ± 0.030 mm at 120 min before gradually reducing to 3.75 ± 0.042 mm at 240 min. This confirms the development of inflammation following the administration of the inflammatory agent. The standard treatment group (Ibuprofen 15 mg/kg) exhibited a significant reduction in paw swelling compared to the control group. Paw edema peaked at 2.70 ± 0.045 mm at 60 min, followed by a progressive decrease to 2.15 ± 0.025 mm at 240 min. This demonstrates that Ibuprofen effectively reduced inflammation, supporting its established anti-inflammatory action.

The Test-1 group (MGA 200 mg/kg) showed moderate anti-inflammatory effects. The paw diameter increased to 3.35 ± 0.072 mm at 60 min, reaching 3.28 ± 0.070 mm at 120 min, and then gradually decreased to 3.05 ± 0.045 mm at 240 min. This suggests that MGA at 200 mg/kg possesses noticeable anti-inflammatory activity, though less potent than Ibuprofen. The Test-2 group (MGA 400 mg/kg) demonstrated a stronger anti-inflammatory response, with paw swelling peaking at 2.78 ± 0.083 mm at 60 min and progressively decreasing to 2.18 ± 0.030 mm at

240 min. The reduction in edema was comparable to the Ibuprofen-treated group, indicating that MGA at 400 mg/kg has significant anti-inflammatory potential.

Table 2: Effect of MGA on paw diameter of rats

Groups	0min	60min	120min	180min	240min
Normal	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Control	2.18 ± 0.030	4.10 ± 0.065	4.50 ± 0.030	4.30 ± 0.045	3.75 ± 0.042
Standard (Ibuprofen 15 mg/kg)	2.22 ± 0.038	2.70 ± 0.045***	2.45 ± 0.038***	2.30 ± 0.030***	2.15 ± 0.025***
Test-1 (MGA 200 mg/kg)	2.25 ± 0.026	3.35 ± 0.072***	3.28 ± 0.070***	3.20 ± 0.065***	3.05 ± 0.045***
Test-2 (MGA 400 mg/kg)	2.20 ± 0.040	2.78 ± 0.083***	2.58 ± 0.060***	2.30 ± 0.028***	2.18 ± 0.030***

All values are shown as mean ±SEM and n=6. ***p<0.001 when compared with control (II) group.

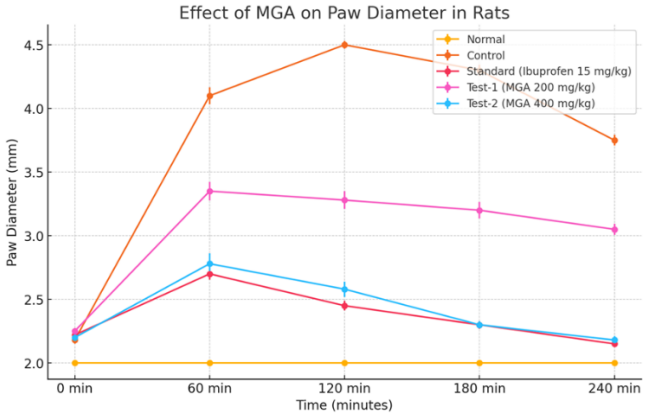


Figure 1: Effect of MGA on paw diameter of rats

Table 2 presents the effect of MGA on paw edema, measured as the difference in paw volume. The control group showed a substantial increase in paw edema, peaking at 120 minutes (0.875±0.025 ml). Ibuprofen significantly reduced paw edema at all-time points, with a final value of 0.275±0.025 ml at 240 minutes. The low dose of MGA resulted in a moderate reduction in paw edema, with significant differences noted at 180 and 240 minutes compared to the control (0.575±0.047 ml and 0.475±0.047 ml, respectively). The high dose of MGA demonstrated a stronger anti-inflammatory effect, with significant reductions at all-time points, reaching 0.30±0.040 ml at 240 minutes, close to the effect seen with Ibuprofen.

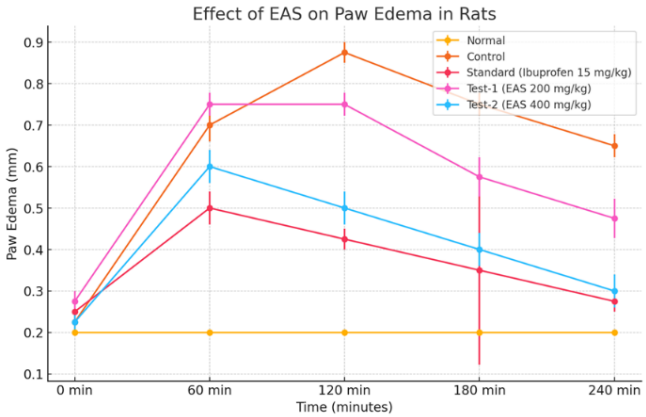


Figure 2: Effect of EAS on paw edema of rats

Table 3: Effect of EAS on paw edema of rats

Groups	0min	60min	120min	180min	240min
Normal	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00	0.2±0.00
Control	0.225±0.025	0.70±0.040	0.875±0.025	0.75±0.028	0.65±0.028

Standard (Ibuprofen 15 mg/kg)	0.25±0.028	0.50±0.040**	0.425±0.025***	0.35±0.228***	0.275±0.025***
Test-1 (MGA 200 mg/kg)	0.275±0.025	0.75±0.028	0.75±0.028	0.575±0.047*	0.475±0.047*
Test-2 (MGA 400 mg/kg)	0.225±0.025	0.60±0.040	0.50±0.040***	0.40±0.040***	0.30±0.040***

All values are shown as mean ±SEM and n=6. ***p<0.0001 when compared with control (II) group.

Table 3 summarizes the percentage inhibition of paw edema. Ibuprofen achieved a high percentage inhibition throughout the study, peaking at 64.84±0.13% at 240 minutes. The low dose of MGA showed significant inhibition, though consistently lower than Ibuprofen, with values ranging from 41.10±0.27% at 0 minutes to 47.18±0.26% at 240 minutes. The high dose of MGA exhibited a strong inhibitory effect on paw edema, closely mirroring the performance of Ibuprofen. At 0 minutes, the inhibition was 44.49±0.42%, which increased to 61.72±0.19% at 240 minutes. Notably, the inhibition rates for the high dose of MGA at 120 and 180 minutes were not significantly different from Ibuprofen, indicating a comparable efficacy.

Table 4: Effect of MGA on the % inhibition of paw edema

Groups	% inhibition of edema				
	0min	60min	120min	180min	240min
Ibuprofen	46.18±0.25	54.83±0.25	63.46±0.36	64.49±0.13	64.84±0.13
Test-1 (MGA 200 mg/kg)	41.10±0.27***	44.80±0.22***	46.97±0.27***	43.63±0.36***	47.18±0.26***
Test-2 (MGA 400 mg/kg)	44.49±0.42***	45.87±0.36***	63.18±0.39^{ns}	64.22±0.18^{ns}	61.72±0.19***

The values were expressed as mean±SEM (n=6) where ***P<0.001 indicates significance compared to the Standard, Ibuprofen group, ^{ns}indicates non-significant

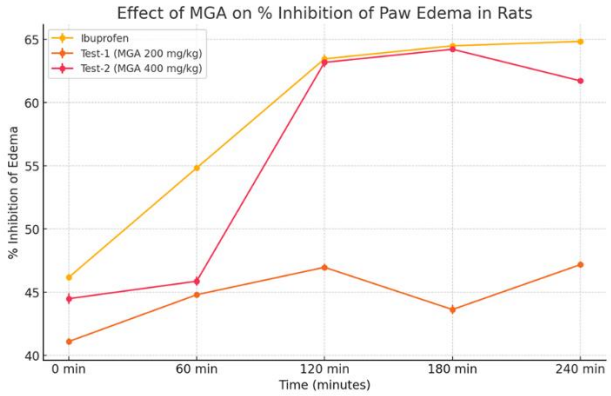


Figure 3: Effect of MGA on the % inhibition of paw edema

Both doses of MGA reduced paw diameter and paw edema compared to the control group, with the high dose (400 mg/kg) showing effects similar to the standard anti-inflammatory drug, Ibuprofen.

Table 5: Effect of MGA on the Hepatic function parameters

Groups	Treatment	ALP (IU/L)	ALT (IU/L)	Serum Urea (mg/dL)	CRP (mg/L)	RF (IU/mL)
Normal	Normal Saline (0.9% p.o)	176.5 ± 18.90	31.10 ± 2.75	2.74 ± 0.40	3.48 ± 0.42	17.40 ± 2.35
Control	Formalin (2% v/v, 0.02 ml s.c)	210.8 ± 22.15**a	68.20 ± 2.18**a	3.05 ± 0.45**a	1.10 ± 0.40**a	32.00 ± 2.30**a
Standard (Ibuprofen 15 mg/kg)	Formalin (2% v/v, s.c) + Ibuprofen (15 mg/kg, p.o)	105.2 ± 2.00***	46.50 ± 1.40***	1.30 ± 0.22***	5.30 ± 0.30***	22.55 ± 1.30***
Test-1 (MGA 200 mg/kg)	Formalin (2% v/v, s.c) + MGA (200 mg/kg, p.o)	138.5 ± 10.20***	45.00 ± 1.35***	0.55 ± 0.10***	4.10 ± 0.38***	23.10 ± 1.25***
Test-2 (MGA 400 mg/kg)	Formalin (2% v/v, s.c) + MGA (400 mg/kg, p.o)	117.0 ± 16.50***	48.00 ± 1.15***	1.15 ± 0.30***	4.30 ± 0.75***	25.00 ± 2.00***

The values were expressed as mean±SEM (n=6) where ***P<0.001 indicates significance compared to the Standard, Ibuprofen group, ^{ns}indicates non-significant

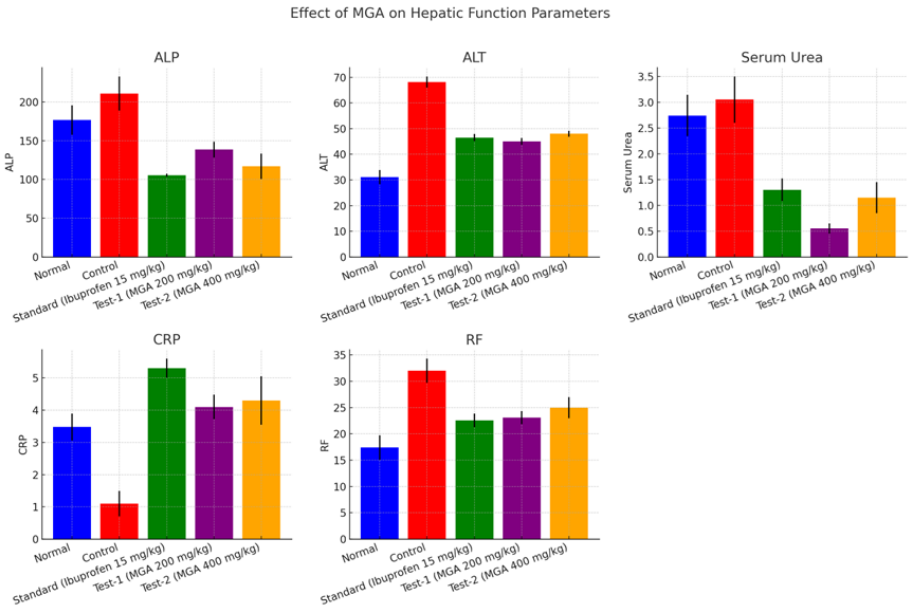
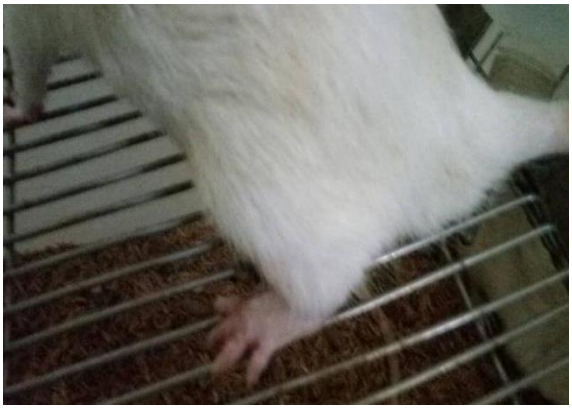


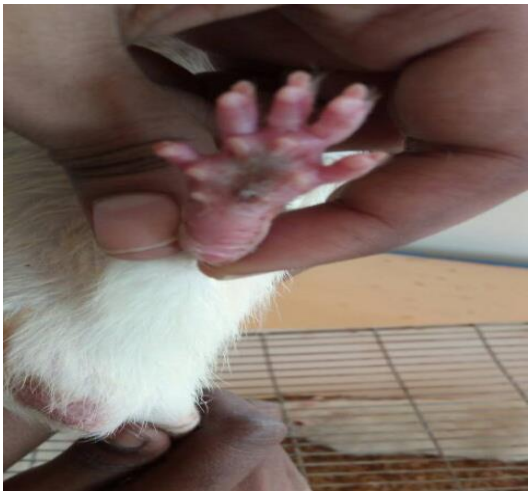
Figure 4: Effect of MGA on the Hepatic function parameters

The table evaluates the effect of MGA (Methanolic extract of Plant) on liver function markers (ALP, ALT), kidney function (Serum Urea), and inflammatory markers (CRP, RF) in formalin-induced inflammation. The control group (formalin only) exhibited significantly elevated ALP, ALT, Serum Urea, and RF levels, along with reduced CRP levels, indicative of hepatic and renal dysfunction associated with inflammation. The standard treatment (Ibuprofen 15 mg/kg) significantly reduced ALP, ALT, and Serum Urea levels, while restoring CRP and RF levels towards normal. The Test-1 group (MGA 200 mg/kg) and Test-2 group (MGA 400 mg/kg) demonstrated dose-dependent improvements in all biochemical parameters, with Test-2 (higher dose) showing a greater reduction in liver and kidney dysfunction markers.

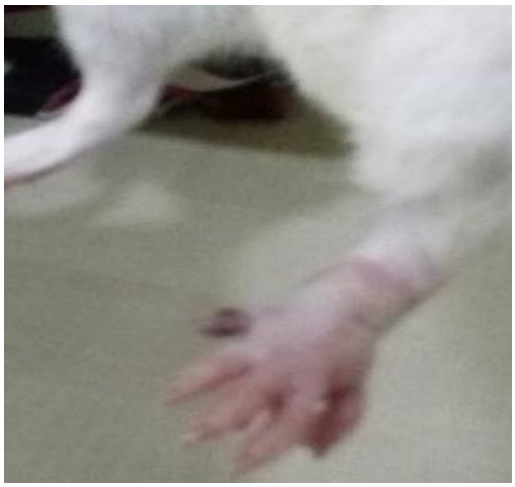
Induced group



Standard group



Test 1



Test 2

Figure 5: Pictures showing effect of MGA in inhibiting paw edema

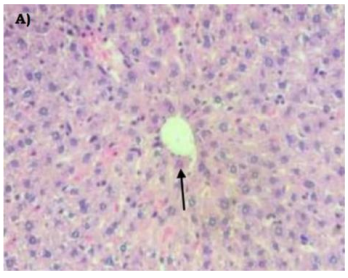
Histopathological Assessment of Liver Tissue in Various Groups

The histopathological evaluation of liver sections from different experimental groups provides critical insights into the extent of liver damage, inflammation, and hepatoprotective effects of the tested compounds. The liver tissue was stained with haematoxylin and eosin (H&E), which allows for a detailed examination of cellular architecture, necrotic changes, and inflammatory infiltrates.

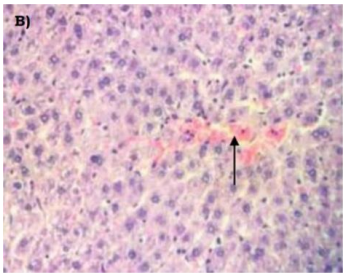
The liver sections from the disease control group (formalin-induced inflammation) exhibited severe histopathological alterations, including hepatocellular degeneration, necrosis, and inflammatory cell infiltration. Vacuolar degeneration and cytoplasmic swelling were evident, indicating liver injury and oxidative stress. The presence of dilated sinusoids, congested blood vessels, and inflammatory cell infiltration suggested hepatic inflammation and damage due to formalin exposure. These findings confirm the hepatic toxicity and inflammatory response induced by the disease condition. Liver sections from the Ibuprofen-treated group exhibited considerable protection against hepatic damage compared to the disease control group. The hepatic architecture was relatively preserved, with fewer necrotic hepatocytes and less inflammatory cell infiltration. Minimal congestion of blood vessels and mild vacuolation of hepatocytes were observed, suggesting that Ibuprofen provided partial hepatoprotective effects by reducing inflammation and oxidative stress.

The liver sections from mice treated with MGA at 200 mg/kg showed moderate hepatoprotective effects. While some hepatocellular degeneration and vacuolation were present, the extent of inflammation and necrosis was reduced compared to the disease control group. The sinusoids and central vein appeared less congested, and inflammatory infiltration was mild, suggesting that MGA at 200 mg/kg exhibited partial liver protection but was not as effective as Ibuprofen or the higher MGA dose.

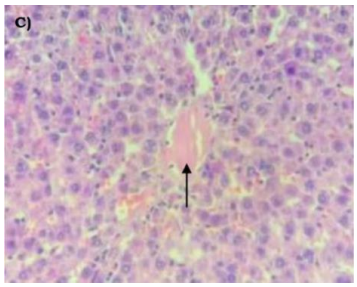
Liver sections from mice treated with MGA at 400 mg/kg exhibited significant hepatoprotection, comparable to the standard treatment group. The hepatic cords were well-organized, with minimal inflammatory infiltration and reduced necrotic hepatocytes. The sinusoids were less congested, and hepatocytes maintained a normal structural appearance with only mild vacuolation. These findings suggest that MGA at a higher dose (400 mg/kg) effectively protected the liver against formalin-induced damage, likely due to its anti-inflammatory and antioxidant properties.



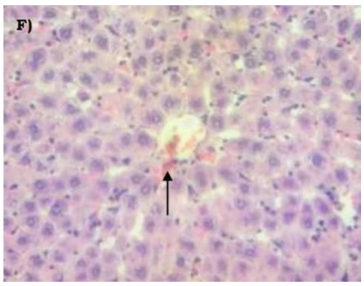
Induced Group



Standard Group



Test 1



Test 2

Figure 6: Pictures showing effect of MGA in inhibiting Hepatic damage

DISCUSSION

The extract of Globularia was evaluated for its anti-inflammatory activity. The effect of MGA at the dose of 200 and 400 mg/kg showed significant anti-inflammatory activity. Significant anti-inflammatory activity was observed in Formalin, mediators induced edema.

The Formalin-induced inflammation was a standard model of screening for anti-inflammatory activity in various experimental compounds (M. Di Rosa, *et al., 1971)* Formalin-induced edema is characterized by the presence of PGs and other compounds of slow reaction. COX-2 is an inducible isoform found in activated inflammatory cells that generates prostanoid mediators of inflammation (D. A. Sawatzky *et al*., 2005,). Inhibition of COX-2 protein expression has also become the most popular target for screening anti-inflammatory agents and the study of pathogenesis and pathology of the inflammatory and nociceptive processes in animal models (F. Nantel, et al., 1999). TNF-α is a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages and stimulating the secretion of other inflammatory cytokines (Beutler and A. Cerami, 1989).

As cytokines are critical to the pathogenesis of inflammatory disorders, inhibition of their production and action can provide therapeutic benefits. Previous studies have shown significant correlations among cytokine production, COX-2 protein expression and PG synthesis in the paw tissues of rats in which edema was invoked by intraplantar injection of formalin. The results suggest that MGA played a role in the anti-inflammatory activities in the model of Formalin-induced paw edema of rats through the inhibition of TNF-α and COX-2 level.

The Formalin-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radicals, as well as to the release of other neutrophil-derived mediators. Some studies demonstrate that the inflammatory effect induced by Formalin is associated with free radicals. Free radicals and PG are released when Formalin is administered for 1–6 h. Also, the paw edema was raised to maximum at the third hour. Research has demonstrated that MDA production is caused by free radicals attacking plasma membranes. Thus, Formalin-induced inflammatory effect results in the accumulation of MDA. Glutathione is a known oxyradical scavenger. The enhancement of glutathione levels reduces MDA production. Cuzzocrea suggests that endogenous glutathione plays an important role against Formalin-induced local inflammation (S. Cuzzocrea, et al., 1999).

This study demonstrated that MGA exhibited anti-arthritic activity against Formalin-induced paw edema. There are two possible mechanisms associated with the anti-inflammatory effect of EAS. One is reducing the amount of AA transformed to PGs by suppressing TNF-α and COX-2 level. The other is cleaning away free radicals by increasing the activity of anti-oxidant enzymes, such as SOD, GRx and GPx.

In the present study the result indicated that oral administration of MGA can inhibit the exudation on the process of acute inflammation, and the early phase inflammatory responses related to the release of pro-inflammatory mediators, such as histamine, serotonin, kinins. This anti-inflammatory activity was due to the presence of polysaccharides and fixed oil. The study reveals that the MGA has shown significant anti-inflammatory activity than that of the individual extracts.

CONCLUSION

Inflammation is a complex process characterized by swelling, redness, warmth (fever), and pain. It is the body's natural response to injury and plays an important role in healing and the fighting of infection. While essential for host defense and repair, the inflammatory response may has also been implicated in a myriad of disease including arthritis. The extract of Globularia leaves has shown anti-inflammatory activity with a significant decrese in Formalin induced paw edema. The study also reveals that the extract of Globularia has shown significant anti-inflammatory activity than that of individual extracts. Hence, the results obtained in this study proved the efficacy of extract of the plant as anti-inflammatory and the effect was observed to be dose dependent.

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