

Pharmacological Monitoring of Antiarthritic Activity of a Polyherbal Formulation in Rat

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ABSTRACT

The current study investigates the phytochemical and pharmacologic screening of selected Indian herbal herbs. Early analyses of the phytochemistry of Poly herbal extracts revealed the presence of tannins and flavonoid alkaloid, as shown in Table. It has been established that flavonoids, which are frequently obtained from plants, have analgesic, antipyretic, and anti-inflammatory activities. Rats were administered oral doses of the plant Polyherbal extract. After 24 hours, there was no sign of mortality was observed. The antipyretic, analgesic, and anti-inflammatory and anti-arthritic activities of poly herbal extracts may be attributed to the presence of phytochemicals such flavonoids, tannins, and terpenes in the leaf extracts.

Keywords: Poly herbal extract, Anti-pyretic, Anti-inflammatory, Flavonoid, Arthritis.

I. INTRODUCTION

Arthritis is defined as joint inflammation and its consequences. An all-encompassing term for arthritis, arthro-itis is derived from the Greek words for "joint" and "inflammation." A significant contributor to impairment is arthritis. For instance, data gathered between 2007 and 2009 showed that 21 million persons in the United States had arthritis and were limited in their activities as a result of their illness. In general, arthritis was becoming more common in that nation, with 67 million persons predicted to receive a diagnosis by 2030. Similarly, more than 10 million persons in the United Kingdom visit their doctors each year for treatment for arthritis and related illnesses [1-3]. In India, the prevalence of arthritis is higher than that of several other well-known diseases including diabetes, AIDS, and cancer, affecting more than 180 million people. Although osteoarthritis and rheumatoid arthritis are the most prevalent varieties, there are many other types of arthritis, such as psoriatic arthritis, gout, and lupus, as well as others that are brought on by infections and metabolic abnormalities [4-8]. Resting the joint and alternately administering ice and heat are possible treatments. [6] Exercise and weight loss may both be

beneficial [9] The type of arthritis may affect the drugs that are advised. [10] These might include acetaminophen-based pain relievers like ibuprofen and paracetamol. [11] In certain situations, a joint replacement may be beneficial. [6] More than 3.8% of people have osteoarthritis, and 0.24% of people have rheumatoid arthritis. [12] About 1% to 2% of people in the West will experience gout at some point in their life. Around 15% of Australians and more than 20% of Americans have some kind of arthritis, respectively. Overall, as people age, the disease becomes more prevalent. People frequently miss work due to arthritis, which can also lower their quality of life. [8] The word is a combination of the words arthr-, which means "joint," and -itis, which means "inflammation." [13]

Many herbal medicines were discovered for treating human illnesses thousands of years ago, either by experience, observation, or error –and – test methods [14]. The earliest reference to the plants use for medicinal purposes can be seen in the 'Rig Veda,' which had been written around 2400 and 1800 B.C. We see a more diverse use of drugs in the "Atharva Veda." The definite characteristics of medications and its applications have been provided in considerable detail in the "Ayurveda," which is called a "Upa Veda." Another early treatise on "Ayurveda" (600 BC) is the "Charka samhita," which lists 341 plant products for community well-being [15]. The "Sushruta Samhita" also interacted with medicinal plants. Dhanvantari and Nagarjuna were very well for their detailed understanding of pharmaceutical drug features. Rauwolfia, that has gained worldwide acclaim, is mentioned in both ancient Hindu scriptures and Charaka's historic work. The plant has been suggested as a snake's venom and bug sting remedy [25-26]. Herbal medicines have become common due to their efficacy, ease of use, low cost, and relative lack of significant toxic effects (time tested) [16]. The existence of phytochemical compounds, generally known as secondary metabolites in different plant tissues gives pharmaceutical products their importance. Polyphenols, alkaloids, essential and edible oils,

polymers, mucilage, gums, astringency, and other widely used materials are among them. Such effective concepts can be found in the plant's tissues, such as stems, roots, plants, foliage, and wood [17-20].

II. MATERIALS & METHODOLOGY

Plant collection and authentication

A Polyherbal extract plant's leaves were gathered and degraded around the beginning of March 2022. The plant was recognised by the Head of Office, Botanical Survey of India, Campus, and Lucknow. They also provided the reference number.

Drying & Pulverization of plant material

After being collected and authenticated, the leaflets were cleaned to remove any impurities and allowed to fully dry by air drying in the sun. The leaf extract was then thoroughly dry-ground in a mixer [21].

Plant extracts preparation:

The fine powder was safely packed in a Soxhlet device and recovered with the solvent alcohol for 72 hours while being shaken occasionally at 60°C. The extraction was reduced in volume by evaporation to a very small amount. The resulting methanol extract of Polyherbal was used for phytochemical studies [22].

DPPH Assay:

Blois first designed the DPPH radical scavenging test, which was used to check the antioxidant activity of the compounds (1958). In a brief, 0.1mM DPPH was produced in 95 percent ethanol. At a concentration of 100g/mL, this solution (1mL) was added to 3 mL of sample (methanolic leaf extract and copper nanoparticles) in ethanol. The absorbance was then measured using a UV-Vis spectrophotometer at 517 nm. Ascorbic acid was utilised as a reference standard chemical, and the experiment was repeated three times [23].

HPTLC and TLC analysis:

The ethyl acetate precursor, methanol, formic acid, and water [20:2.5:0.5:2 (v/v)] made up the mobile phase. and HPTLC was carried out using 20 x 10 cm silicon gel 60 f 254 HPTLC plates. The conventional solutions were then applied to the plate as 10 mm bands, as well as the CAMAG TLC Scanner 3 and WIN CATS software

were used to densitometrically measure the bands [24].

Screening of phytochemicals

The methanolic extract of plant extract was tested qualitatively for alkaloids, polysaccharides, glycosides, phenolic compounds, peptides, free amino acids, and triterpenes, among other elements [25].

Animals

150-200 g Wistar rats were used in this study. The creatures were held at college name and came from there. When the animals showed up, they were assigned at random to those who were being cared for and put in polyethylene enclosures with rice husks as a bedding. The animals were housed at 24 °C and between 30 and 70 % relative humidity. We followed the A12:12 light:day cycles. Every animal received unlimited access to water and commercial pelleted food. All experimental approaches used in this study were approved by the college's Animal Ethics Committee [26].

Acute Toxicity Studies

OECD guideline 423 governs the acceptance of toxicological tests. On the selected albino rats, toxicity tests were conducted. Five groups of the creatures, each containing three creatures, were created. The creatures were fed night before the acute experimental technique. Rats received oral dosages of 6, 120, 600, 1200, and 2600 mg.kg⁻¹ per kilogramme of extract. For the first four hours following treatment, the animals were closely monitored for behavioural abnormalities such as ataxia, agitation, seizures, sweating, tremor, diarrhoea, fatigue, and sleep. Additionally, they were observed for a period of fifteen days after getting the medication to check for any mortality [27].

Analgesic activity: Hot-plate method in mice

The hot-plate test method was utilised to investigate the possible anxiolytic effects of methanol as the preparations of Poly botanical extracts when given systemically. Pentazocine, an important analgesic, was used on the +ve, comparison group. In this investigation, four sets (n = 6) of wister mice were exposed for 15 minutes on a heated plate at room temperature. Food had been put away the previous evening before the testing. CMC (0.5%) was administered to Group I, pentazocine (3 mg.kg⁻¹i.p.) was administered to Group II, and methanolic extracts of Polyherbal

extract (were administered to Groups III and IV [27]).

Anti-pyretic activity

The brewer's yeast provoked pyrexia technique was in use to determine the anti-pyretic activity of methanolic extract. Fever was generated by administering 10.0 ml.kg^{-1} of a 20.00 percent w/v solution of brewer's yeast in NaCl solution under the skin. Only animals had a rectal temperature increase of approximately 1.0°C after getting a subcutaneous vaccination of 10.0 ml.kg^{-1} of a 20.00 percent w/v brewer's yeast solution in NaCl solution. The research only involved rats with rectal temp raised by minimum 1°C after 18 hours of yeast vaccination. A flexible tail thermometer probe covered with lubricants were used to detect the usual rectal temp of each animal, and the temp was documented utilizing an electronic telethermometer. The experimental group were separated into four subgroups, each of which had six animals. The control group (I) received 0.5 mL saline, the control group (II) received 150 mg.kg^{-1} paracetamol, and group III & IV received doses I & II of methanolic extracts of test medicines, accordingly [27].

Anti-inflammatory activity: Carrageen an-induced paw edema in rats

The rat (120g - 150g) were put in 4 subgroups (n = 6) for this study. Group I was given 0.50 percent CMC (10 ml.kg^{-1}) whereas Group II was given Indomethacin (10 mg.kg^{-1}). The methanolic extracts of Polyherbal extract was given vocally to Groups III and IV at doses of 300 mg/kg and 600 mg.kg^{-1} , respectively.

Completed Freund's adjuvant-induced arthritis

The animals were divided into six groups of animals each as follows:

Non-arthritic animals

Grp I: Normal animals were given a 5 percent DMSO aqueous solution (10 ml/kg , p.o.)

Arthritic animals

Group II: Control animals: Normal animals were given a 5 percent DMSO aqueous solution (10 ml/kg , p.o.)

Group III: VPME (75 mg/kg , p.o.) was given to the drug-treated rats.

Group IV: VPME (150 mg/kg , p.o.) was given to the drug-treated rats.

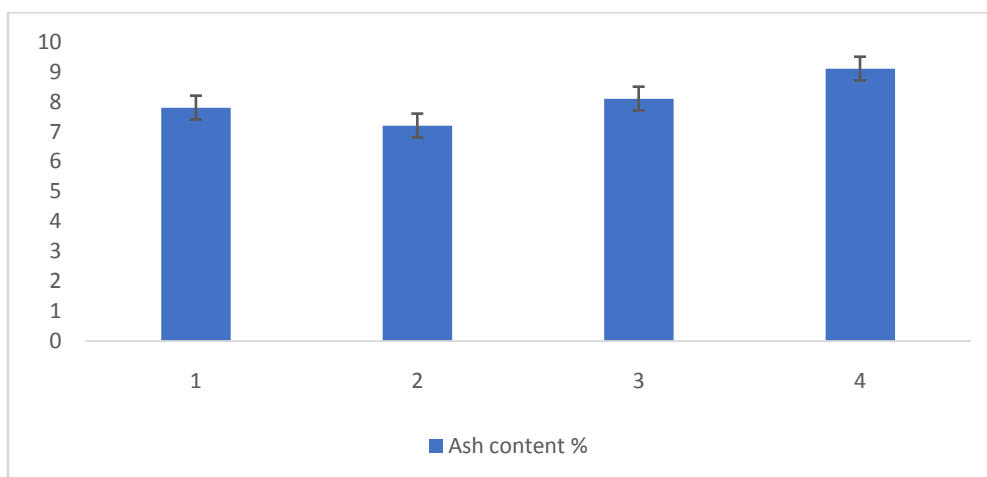
Group V: VPME (300 mg/kg , p.o.) was given to the drug-treated rats.

Group VI: Diclofenac (4 mg/kg , p.o.) was given to the drug-treated rats.

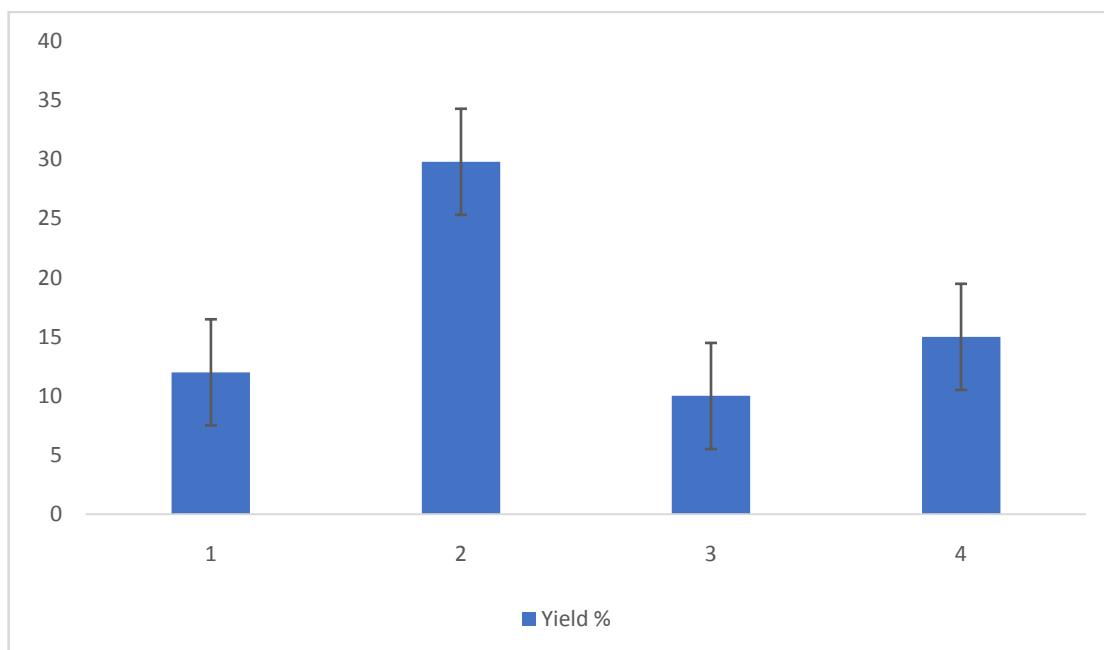
The subplantar area of the left hind paw of the rat was injected with $100 \mu\text{l}$ of CFA (having 1 mg/mL of inactive Mycobacteria in paraffin oil and mannide monooleate). Day 1 was defined as the day of CFA injection. The oral delivery of PE and diclofenac vehicle to all groups began on day 14 and continued until day 28. On days 28, 24, 21, 19, 14, 12, 10, 7, 4, 0, the anti-arthritic activity of PE was assessed using joint diameter, paw volume, pain release latency, and arthritis score [27].

III. RESULTS & DISCUSSIONS

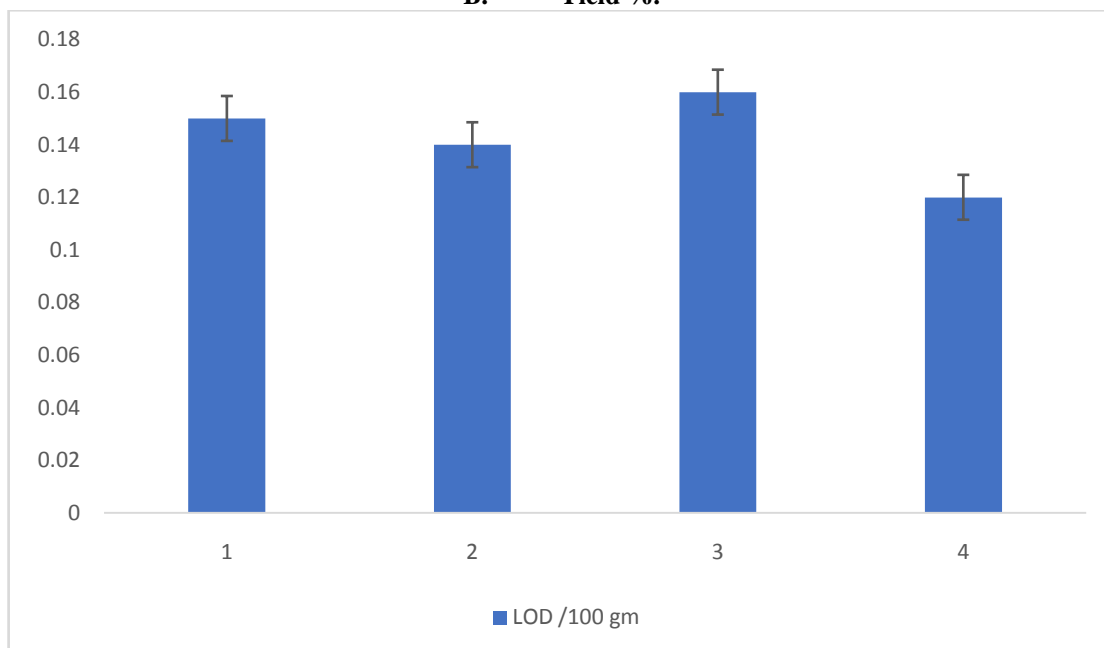
Physicochemical analysis of the extracted compounds: The extraction of plant secondary metabolites was successfully carried out by using Soxhlet apparatus and then analysed for the pharmacological, physicochemical and phytochemical analysis as shown in figure and tables.



A. Ash content.



B. Yield %.



C. Loss of drying.

Figure 9: Physicochemical analysis of the extract

Table 1: Antioxidant analysis of Extracts

S no.	DPPH
1	191±2.43
2	173±2.87
3	281±1.25
4	119.6 ±0.25

Table 2: Phytochemical screening of Polyherbal extract.

Test	Hexane	Ethanol	Water
Phenols	+	+	-
Glycosides	+	+	+
Terpenoids	+	+	+
Flavonoids	+	-	+
Alkaloids	+	-	+
Saponin	+	-	+
Tannins	+	+	+
Carbohydrate	+	+	-
Steroids	-	+	-

Note: ++:high content,+:moderate,-:Negative,

Polyherbal extract. methanolic extract: preliminary phytochemical evaluation Alkaloids, tannins, phenolic compounds, sterols, saponins,

protein and amino acids, and a large amount of flavonoids are found in this plant.



Figure 2: Phytochemical test for the identification of compounds.

HPTLC and TLC analysis of extracts.

Using silica gel HPTLC plates, four different mobile phases previously described for the separation of flavonoids were tested: ethyl acetate: formic acid: water (6:1:1, v/v), ethyl acetate: formic acid: acetic acid: water (100:11:11:26, v/v), ethyl acetate: methyl ethyl ketone: formic acid: water (50:30

The newly created mobile phase ethyl acetate: methanol: formic acid: water (20:2.7:0.5:0.2 v/v) was the only one that permitted us to see variations between the extracts analysed.

With regard to Fro, the use of these solvent solutions allows for excellent separation of

flavonoids. This mobile phase is an improvement over the older approach described by Brasseur and Angenot, and it may eventually replace the approach specified in the European Pharmacopoeia's most recent edition. All of the species investigated contained Quercetin, Rutin, Luteolin, and Vitexin ($R_f = 0.97, 0.53, 0.59,$ and $0.78,$ respectively). Rutin was found in the majority of the plant species as a typical compound.

The flavonoids contained in these plants are arranged in the following order: Rutin > Luteolin > Vitexin > Quercetin. The fluorescence bands of the majority of flavonoids are not visible at 254 nm, however they are visible at 366 nm.

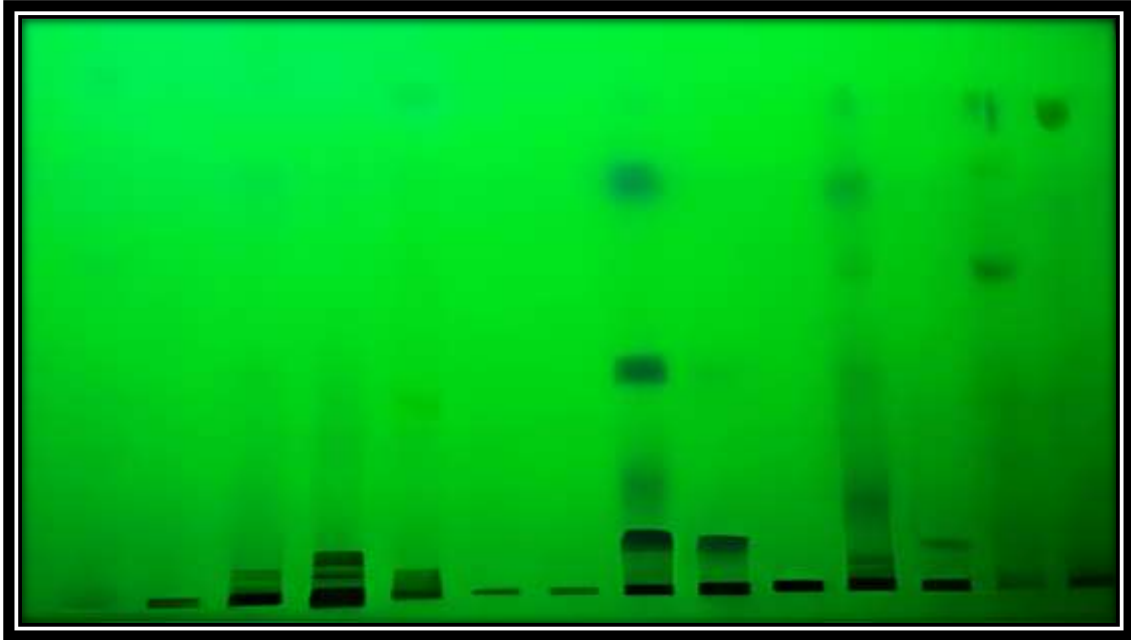


Figure 3: HPTLC fingerprint

Analgesic activity: Hot plate Method in Mice

The analgesic effectiveness of Poly botanical extracts in methanolic extracts of leaves in rats was examined using the hot plate method.

Leaf extract made of methanol from *Quisqualis indica*. It had significant analgesic effectiveness at both 200 and 400 mg/kg.



Figure 4: The analgesic effectiveness was tested using the hot plate method.

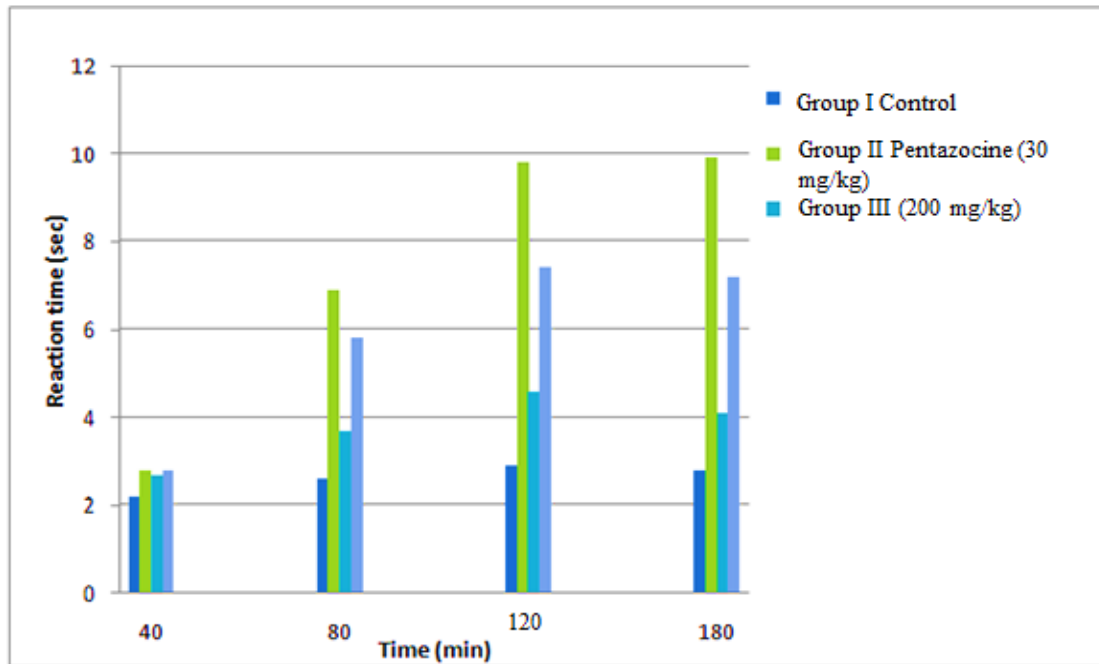


Figure 5: Analgesic effect of methanolic leaves extract of Poly herbal extract on hotplate method in mice.

Anti-pyretic activity: Brewer's Yeast Induced Pyrexia in Rats

Shows the anti-pyretic effectiveness of ethanol leaf extract of Poly herbal extract towards yeast-induced pyrexia. At concentrations of 200 and 400 mg/kg, the methanolic leaf extract of Poly

herbal extracts demonstrated a substantial impact towards the Brewer's yeast generated pyrexia technique. The temp of rats treated with the extracts was reduced in a daily dosage manner. When compare to control, the extract generated a considerable reduction.

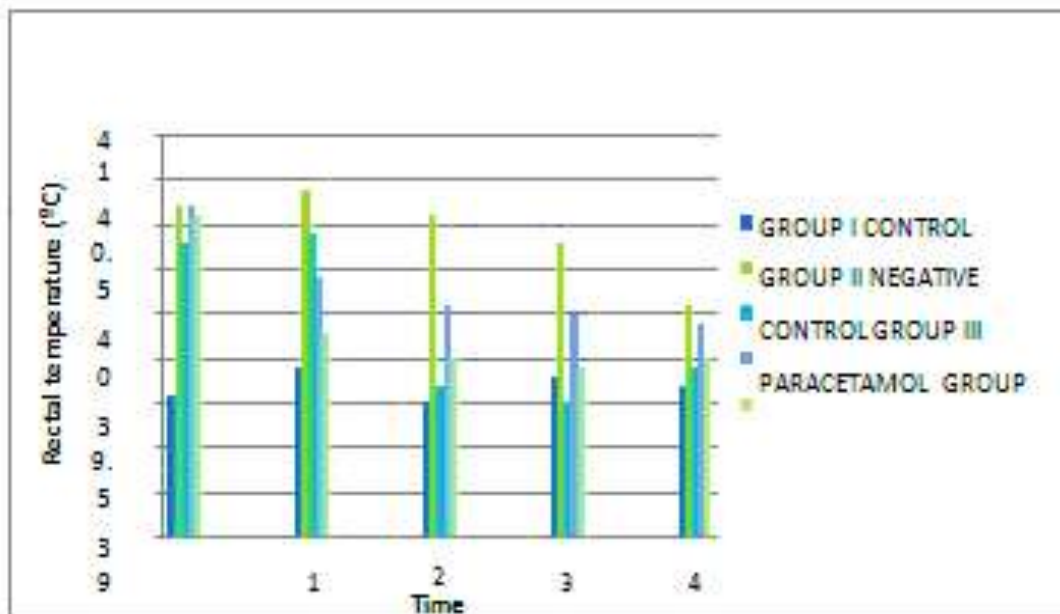


Figure 6: On Brewer's Yeast Generated Pyrexia in Rats, methanolic Leaf Extraction of Poly herbal extract has Anti-Pyretic Action.

Anti-Inflammatory Activity: Carrageenan induced Paw Edema in Rats

The anti-inflammatory activity of Poly herbal extractsmethanolic leaf extracts on carrageenan-triggered hind paw edoema. At dosages of 200 and 400 mg/kg, the methanolic leaf extract of Poly herbal extractshad a substantial anti-inflammatory activity against carrageenan-

triggered inflammation. After 3 hours, the dose of 400 mg/kg showed a significant reduction of 48 percent, with the impact increasing after 3 hours (52 percent). The anti-inflammatory efficacy of Poly herbal extractsmethanolic extract was substantial and comparable to that of indomethacine (10mg/kg).

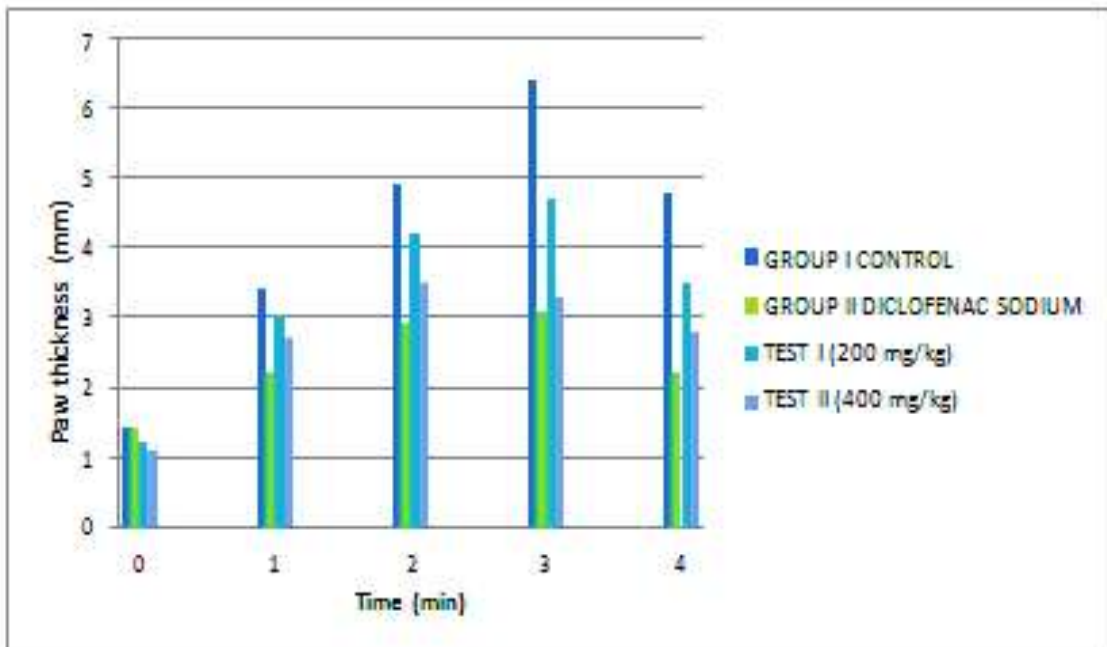


Figure 7: Poly herbal extractsmethanolic leaf extract has anti-inflammatory action in Wistar rats using the carrageenan generated paw edoema technique.

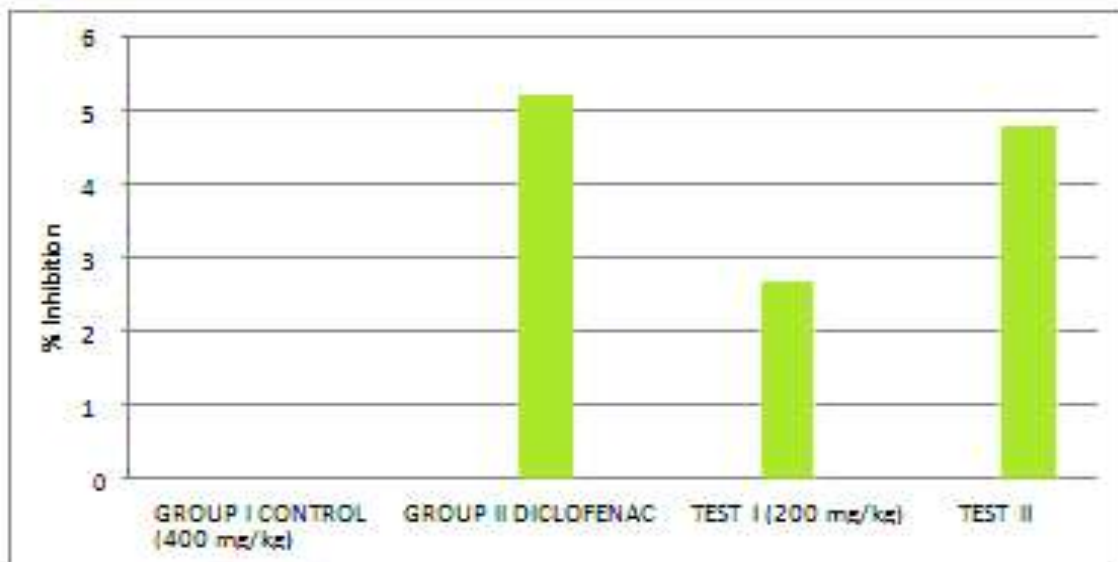
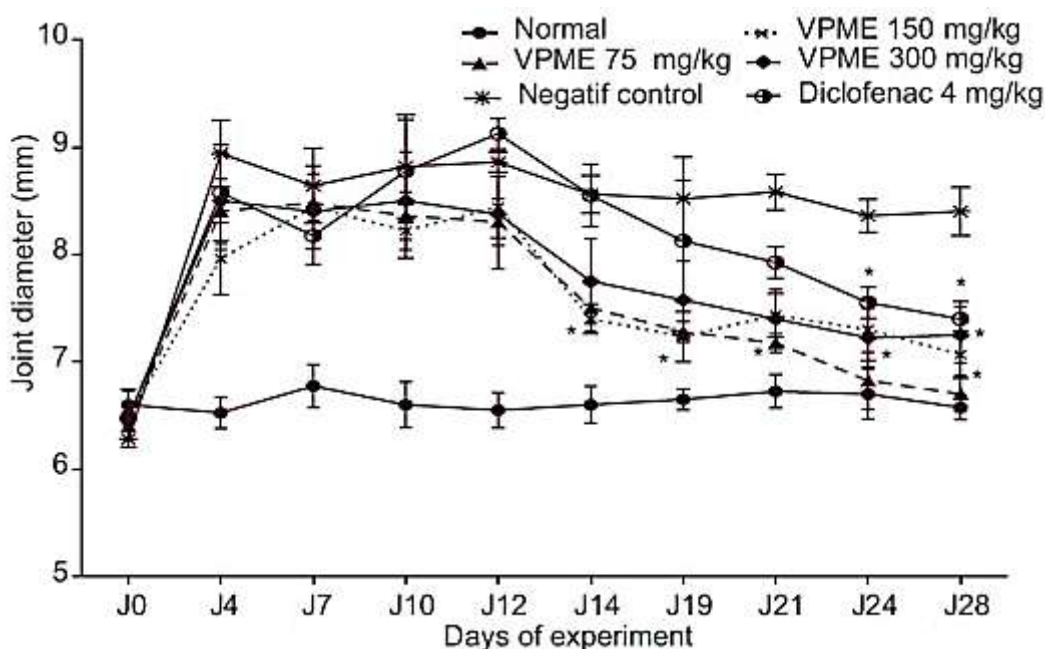


Figure 7 : Poly herbal extracts methanolic leaf extract has anti-inflammatory action in Wistar rats using the carrageenan triggered paw edoema technique. The results are given as a percentage of inhibition.



Oral administration of a methanol extract of Poly herbal extracts has an influence on joints width. The figures indicate the average of five animals. *P <0.05, **P <0.01 when compared to animals in the control group.

When contrasted to the reference arthritic group 2 days after the start of medication, VPME (75 & 150 mg/kg) animals treated showed a dramatic and significant (P <0.05) decrease in joint width, whereas diclofenac-treated mice exhibited a meaningful (P < 0.05) outcome a few days later (24 days).

IV. CONCLUSION

The current research examines the pharmacologic and phytochemical screening of herbal plants, a chosen Indian medicinal. Early phytochemical assessment of Poly herbal extracts reported the existence of flavonoid alkaloid and tannins, as shown in Table. Flavonoids, which are commonly derived from plants, have been shown to have analgesic, antipyretic, and anti-inflammatory properties.

The leaves was dried in the shade and pulverised. Utilizing a soxhlet apparatus, it was crushed and recovered with ethanol. The extract that resulted was highly concentrated The plant Polyherbal extract was studied utilizing rats that were given oral doses of 6, 60, 100, 1000, and 2000 mg/ kg body weight of extract, with no mortality detected after 24 hours. As a result, the dose was determined using the OECD 423 guidelines.

Utilizing a hot plate, tail immersion, and an acetic acid-triggered writhing mouse model the leaves extract appears to work via central and peripheral pathways of analgesia. The antipyretic effect of the leaves extraction was determined utilizing the yeast triggered pyrexia method, and the anti-inflammatory impact of the leaf extracts was determined using the carrageen anti-triggered paw edoema in mice and cotton pellet granuloma methods.

Poly herbal extracts possesses anti anti-pyretic, & analgesic properties these benefits could be due to existence of phytochemicals in the leaf extracts such as flavonoid, tannin, and terpenes.

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