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PHARMACOLOGICAL EVALUATION OF ANTI-PYRETIC ACTIVITY OF *POLEMONIUM REPTANS* FLOWER EXTRACT IN EXPERIMENTAL RATS

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ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. According to World Health Organization, approximately 85% population of the developing countries are facing difficulties to afford synthetic drugs and are relying on traditional medicines mainly of plant origin in order to maintain their primary health care needs. Plants are being used in various disorders. The aim of the study was to evaluate the antipyretic activity of methanolicflower extract of *Polemonium reptans* using Brewer's yeast-induced pyrexia model in Wister strain albino rats. The methanolicflower extract at a dose of 100mg/kg &200 mg/kg were evaluated forantipyretic activity. The extract of *Polemonium reptans* plant showed a significant ($P<0.05$) dose dependentantipyretic effect in yeast induced elevation of rectal temperature in experimental rats when compared with the standardparacetamol. So it can be recommended for further studies. Phytochemical analysis revealed the presence of glycosides, carbohydrates, saponins, alkaloids. The total phenolic content of methanolicflower extract of *Polemonium reptans* was found to be 56mg/100mg followed by flavonoids content was found to be 16.66mg/100mg respectively. The activities of methanolic flowers extract against DPPH assay method were concentration dependent with IC_{50} values of ascorbic acid and extracts 21.77 and 48.85µg/ml respectively. These data therefore suggest that extract of *Polemonium reptans* possesses significant antipyretic activity and it mechanism could be by inhibition of release inflammatory mediators and prostaglandins.

KEYWORDS: *Polemonium reptans.* Phytochemicals screened, Anti-pyretic activity, Yeast induced pyrexia, Antioxidant activity.

INTRODUCTION

India is an ironic source of therapeutic florae and a number of plants derived oils and extracts are used against various ailments related to human health by traditional healers by different systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Secondary metabolites derived from plants as natural products such as flavonoids, terpenes, phenols and alkaloids[1,2] have increased significant consideration by the researcher in recent years due to their diverse multi pharmacological properties these plants still represent an enormous cradle of natural antioxidants that might serve as leads for the development of novel drugs. Numerous anti-inflammatories, neuroprotective, antipyretic, analgesic activities digestive, hepatoprotective, anti-cancer, antidiabetic and antinecrotic medicines have lately been exposed to have an antioxidant and/or radical scavenging mechanism as part of their activity.[3-5] Fever or pyrexia is an elevated body temperature above the normal level characterized by an increase in thermoregulatory set-point, the average oral temperature is 37°C (98.6°F).[6] Which results from the interaction of the central nervous and immune system? Fever is body's natural defense mechanism against infectious agents which can damage the tissue. This intern triggers the enhanced formation of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin 1β, α and β, these pro-inflammatory mediators increase the synthesis of prostaglandin E_2 (PGE_2) near hypothalamus area and thereby trigger the hypothalamus to elevate the body temperature. The thermoregulatory system governed by nervous feedback mechanism alters the fever by vasodilation and vasoconstriction of blood vessels. Although fever is body's defensive mechanism, some studies have suggested that raising temperature may be harmful. Therefore, in clinical practices in which fever-associated risks offset benefits, antipyretic treatment is necessary.[7] Most of the marketed anti-inflammatory drugs possess antipyretic activity like paracetamol, aspirin, nimesulide, etc. These non-steroidal anti-inflammatory drugs inhibit the synthesis of PG to reduce

the inflammation, as well as fever. Greater of these drugs have toxic effect to the various organs of the body.[8] Therefore, the development of novel compounds having antipyretic and anti-inflammatory activities with improved safety profiles remains a clinical need.[9] *Polemonium reptans* (Polemoniaceae) herbaceous perennial plant is ½–1½' tall, branching occasionally. The leafy stems have a tendency to lean to one side or sprawl across the ground. The stems are light green to reddish green, glabrous to hairy, and often somewhat angular. The alternate compound leaves are simple odd-pinnate and up to 8" long, consisting of about 5-15 leaflets. The petioles and rachises of these compound leaves are light green to reddish green and glabrous to hairy; they are grooved above and convex below. The leaflets are ¾-1¼" long and about one-third as much across; they are elliptic to broadly elliptic-oblong, smooth (entire) along their margins, medium green, glabrous (or nearly so), and sessile (or nearly so). The upper stems terminate in rather loose panicles of floppy or nodding flowers spanning 1½-3" across. Some panicles are also produced from the axils of upper leaves on long peduncles up to 6" long. The pedicels are up to 1" long. Both the peduncles and pedicels of these panicles are light green to reddish green and glabrous to hairy. The campanulate (bell-shaped) flowers are up to 16 mm. (2/3") across. Each flower has 5 rounded petals that are light blue-violet, a short-tubular calyx with 5 triangular teeth, 5 stamens with white anthers, and a pistil with a slender white style that become tripartite toward its tip. The calyx is light green to reddish purple and glabrous to hairy. There are fine veins that run along the length of the petals. The stamens are the same length as, or shorter than, the petals of the flowers. The blooming period usually occurs during the late spring, lasting about 2-3 weeks. Afterwards, the flowers are replaced by 3-celled seed capsules; these capsules are about 6 mm. (¼") in length and ovoid in shape; they are few-seeded. The root system consists of a short vertical crown with abundant fibrous roots. This plant spreads by reseeding itself.[10] The dried roots have a slightly bitter and acrid taste. *P. reptans* has been traditionally used as anherbal medicine for febrile and inflammatory diseases, to ease coughs, colds and bronchial complaints, and to encourage perspiration.[11] It is furthermore said to bring relief in cases of inflammations and infections. The root is rarely used in modern herbalism. It is harvested in the autumn and dried for later use. Therefore, the present study aimed to evaluate the antipyretic effect of methanol flower extract of *Polemonium reptans* using Brewer's yeast-induced pyrexia model in Wister strain albino rats.

MATERIALS AND METHODS

Plant material

The medicinal plant *Polemonium reptans* (300 gm) was collected. After cleaning, plant part(flower) were dried under shade at room temperature for 3 days and then in oven dried at 45°Ctill complete dryness. Dried plant part (flower) was stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Authentication of selected traditional plant-Medicinal plant *Polemonium reptans* was authenticated by a plant taxonomist in order to confirm its identityand purity.

Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Polemonium reptans* 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 observe 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. Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour)and was packed in air tight container and labelled till further use.[12, 13]

Figure1: Continuous Hot Extraction.

Phytochemical screening

Methanolic extract of *Polemonium reptans* flowers was subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures.[14,15]

QuantitativePhytochemicalEstimation

TPC

The total phenolic content of *Polemonium reptans* extract was determined using the Folin- Ciocalteu Assay. The *Polemonium reptans* 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. Tangco ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically.[16]

TFC

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of Polemonium reptans 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.[16]

DPPH

The antioxidant activity of *Polemonium reptans* extract was determined by using the DPPH free radical scavenging assay. 1 mg/ml methanol solution of extracts/standard was prepared. Different concentration of *Polemonium reptans* extracts /standard (20-100µg/ml) were 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.[18] Percentage antioxidant activity of sample/standard was calculated by using formula:

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

Acute toxicity study

The acute toxic class method set out in guideline is a stepwise procedure with the use of 3animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4steps may be necessary to allow judgment on theacute toxicity of thetest substance. The substance is administered orally to a group of experimental animals at one ofthe defined doses. The substance is tested using a stepwise procedure, each step using threeanimals of a single sex. Absence or presence of compound-related mortality of the animals dosedat one stepwill determine the next step, i.e.; no further testing is needed, dosing ofthreeadditional animals, with the same dose and, dosing of three additional animals at the next higheror the next lower dose level. Three animals are used for each step. The dose level to be used asthe starting doseis selected from oneoffourfixed levels, 5, 50, 300and 2000 mg/kg bodyweight.[19]

Experimentalwork

AnimalsProtocol

IAEC Approval All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).

Animal used

Weight 200±50gm
Strain Wistar rat
Sex Either

Housing Condition- Animals were housed in a group of six in separate cages under controlledconditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, NewDelhi) and water regularly.

Brewer's Yeast Induced Pyrexiain Rats

The animals were divided into five groups (n = 6). Fever was induced by administration of 15 % w/v Brewer's yeast suspension subcutaneously below the nape of the neck.[20,21] The rectal temperature was recorded using tele thermometer immediately before and 18 h after Brewer's yeast injection.[22] After 18 h of yeast injection different groups received vehicle (1% v/v Tween 80 in distilled water), *Polemonium reptans* extract (100 and 200 mg/kg body weight) and reference drug (Paracetamol, 150 mg/kg body weight) through oral route. The rectal temperature was then periodically recorded for an observation period of 1st, 2nd, 3rd and 4th hour after drug administration.[23]

Experimental design

Rats (n=30) were randomized into following group.
Group1-Normal control
Group2-Yeastinduced pyrexiagroup
Group 3- Treated with *Polemonium reptans* extract 100 mg/kg bw
Group 4- Treated with *Polemonium reptans* extract 200 mg/kg bw
Group5-Treatedwith standard drug(Paracetamol) 150mg/kgbw

RESULTS

Inphytochemicalextractionthepercentageyieldisverycrucialinordertodeterminethestandard efficiency of extraction for a specific plant, various sections of the same plant ordifferent solvents used. The yield of extracts received from the *Polemonium reptans* is shown in Table 1.

Table 1: Percentage yield of crude extracts of Polemonium reptans extract.

S.No	Plantname	Solvent	Theoretical weight	Yield(gm)	%yield
1	*Polemonium reptans*	Petether	287	1.61	0.56%
2		Methanol	299.15	6.10	2.03%

Table 2: Phytochemical test of plant extract.

S.No.	Experiment	Presenceorabsenceofphytochemicaltest	
		Pet.Etherextract	Methanolicextract
1.	**Alkaloids**		
1.1	Dragendroff'stest	Present	Present
1.2	Mayer'sreagenttest	Present	Present
1.3	Wagner'sreagenttest	Present	Present
1.3	Hager'sreagenttest	Present	Present
2.	**Glycoside**		
2.1	Borntragertest	Absent	Present
2.2	Legal'stest	Absent	Present
2.3	Killer-Killianitest	Absent	Present
3.	**Carbohydrates**		
3.1	Molish'stest	Absent	Present
3.2	Fehling'stest	Absent	Present
3.3	Benedict'stest	Absent	Present
3.4	Barfoed'stest	Absent	Present
4.	**ProteinsandAminoAcids**		
4.1	Biurettest	Absent	Absent
5.	**Flavonoids**		
5.1	Alkalinereagenttest	Present	Absent
5.2	LeadAcetatetest	Present	Absent
6.	**Tanninand PhenolicCompounds**		
6.1	FerricChloridetest	Absent	Absent
7.	**Saponin**		
7.1	Foamtest	Present	Present
8.	**TestforTriterpenoidsandSteroids**		
8.1	Salkowski'stest	Absent	Absent
8.2	Libbermann-Burchard'stest	Absent	Absent

Table 3: Total Phenolic and flavanoids Content of extract *Polemonium reptans.*

Extracts	TotalPhenoliccontent(mg/g mequivalentof Gallicacid)	TotalFlavonoidcontent(mg/ gmequivalentofrutin)
Methanol	56	16.66

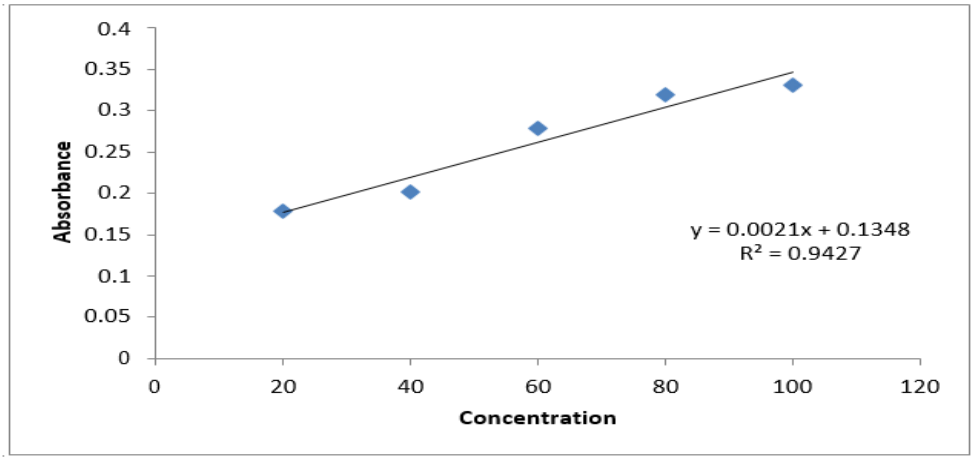

Figure 2: Represent standard curve of Gallic acid.

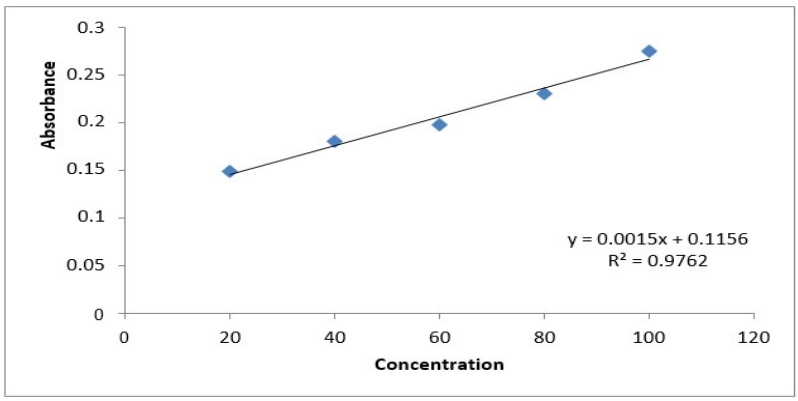

Figure 3: Represent standard curve of Rutin.

In the present investigation, the *in vitro* anti-oxidant activity of extracts of *Polemonium reptans* was evaluated by DPPH radical scavenging activity. The results are summarized in Tables 4.

Table 4: DPPH radical scavenging activity of Std. Ascorbic acid and methanol extract of *Polemonium reptans.*

Concentration (µg/ml)	Absorbance	% Inhibition of Ascorbicacid	Absorbance	% Inhibition of methanolextract
20	0.483	51.261	0.519	44.012
40	0.436	56.004	0.465	49.838
60	0.343	65.388	0.454	51.024
80	0.284	71.342	0.413	55.447
100	0.144	85.469	0.367	60.409
Control	0.991			0.927
IC5	21.77			48.85

Table 5: Brewer's Yeast Induced Pyrexia in Rats.

Rectal temperature(°C)					
Groups	18h after Yeast administration	Temperature after treatment			
		1h	2h	3h	4h
Normal control	37.44±0.1	37.66±0.2	37.85±0.1	37.75±0.2	37.70±0.1
Yeast induced Pyrexia group	40.21±0.1	40.17±0.3	40.13±0.2	39.99±0.1	39.92±0.3
Polemonium reptans extract(100mg/kg)	40.20±0.1	40.12±0.3	39.99±0.1	39.92±0.2	39.75±0.3
Polemoniumr eptans extract(200mg/kg)	40.12±0.1	39.01±0.1	39.84±0.2	38.99±0.1	38.21±0.2
Paracetamol (150mg/kgbw)	39.92±0.2	39.49±0.2	38.99±0.2	38.43.±0.1	37.76±0.2

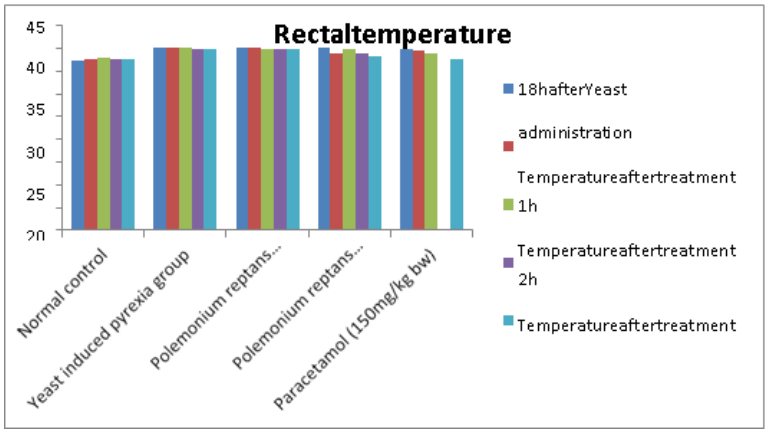

Figure 1: Brewer's Yeast Induced Pyrexia in Rats.

DISCUSSION

Phytochemical analysis of methanolic extract of *Polemonium reptans* showed the presence of alkaloids, carbohydrate, saponins, glycoside and saponin. Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. Results shown in Table 8 & table11. DPPH radical scavenging activity of *Polemonium reptans* extract exhibited percent inhibition 60.40% and its IC 50value was found to be 48.85µg/ml. Ascorbic was used as a reference compound which exhibited percent inhibition 85.46% and showed IC 50 value of 21.77µg/ml. In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study. Brewer's yeast was used to induce fever in albino rats. Fever was recorded 18 hrs after yeast injection since yeast takes a total of about 18 hrs to cause the elevation of body temperature. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins. The inhibition of prostaglandin synthesiscould is the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect.

The oral administration of *Polemonium reptans* significantly attenuated rectal temperature of yeast induced albino rats. Thus it can be postulated that *Polemonium reptans, contained* pharmacologically active principles that interfere with the release of prostaglandins. After four hours of the test period, the methanolic extract of *Polemonium reptans* produced appreciable antipyretic activity against brewer's yeast induced pyrexia in albino rat. It was revealed that the extract showed dose dependent antipyretic activity.

CONCLUSION

The study showed the extract of *Polemonium reptans* flower possessed antipyretic effect in yeast induced elevation of body temperature in experimental rats. It was revealed that the extract showed dose dependent antipyretic activity since 100mg/kg dose of test solution is considered as a slow acting compared to the higher dose preparation. This effect may be related to the extract's analgesic and anti-inflammatory activity. There is evidence to believe that this plant contains compounds acting as NSAI drugs. These findings provided strong arguments and validation of the popular use of this plant as antipyretic herb.

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