

ANTIOXIDANT AND ANTIPYRETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF HERBAL PLANT *ONOSMA BRACTEATUM*

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ABSTRACT

This study was carried out to evaluate the antipyretic effects of hydroalcoholic extract of leaves of *Onosma bracteatum* in rats. The antipyretic potential was done using yeast as pyrexia inducing agent. The findings from the present study were in agreement with other studies on antipyretic potential medicinal plants in animal models. The antipyretic effects of hydroalcoholic extract of leaves of *Onosma bracteatum* at different dose levels exhibited a dose-independent response on yeast-induced fever in rats. Lower dosages of 100 mg/kg and higher 200 mg/kg body weight were as effective as dose dependent manner. The results of present study indicate that the hydroalcoholic extract of *Onosma bracteatum* leaves possesses significant antipyretic effect on yeast induced hyperthermia in rats. This may be attributed to the presence of chemical constituents in the extracts which may be involved in inhibition of prostaglandin synthesis. Also, there are several mediators or multiprocessors underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis. The hydroalcoholic extract of *Onosma bracteatum* leaves at the 200 mg/kg body weight was marginally effective than 100 mg/kg body weight dose. These findings suggest a better or a similar prostaglandin synthesis inhibition by the active components in the plant's extracts. There is therefore, a possibility of the plant extracts working effectively by blocking alternative mechanisms during fever inhibition.

Keywords: *Onosma bracteatum*, leaves extract, Antipyretic effects, hydroalcoholic extract.

INTRODUCTION

Herbal medicines continue to be a major market in US pharmaceuticals and constitute a multi-billion dollar business. Approximately 1500 botanicals are sold as dietary supplements; formulations are not subject to Food and Drug Administration (FDA) clinical toxicity testing to assure their safety and efficacy. The Indian herbal drug market size is about \$1 billion and the export of plant based crude drug

is around \$100 million. The current market potential of herbal medicine is estimated about \$ 80-250 billion in Europe and USA ¹.

In India, around 15000 medicinal plants have been recorded ² however traditional communities are using only 7,000 - 7,500 plants for curing different diseases ³⁻⁵. The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700), Amchi (600), Unani (700) and Allopathy (30) plant species for different ailments ⁶. According to another estimate 17,000 species of medicinal plants have been recorded out of which, nearly 3,000 species are used in medicinal field ⁷. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants ^{8,9}. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters ¹⁰.

Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As mentioned in the introduction only a certain percentage of plants are used in traditional medicines. The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments¹¹.

Many medicinal plants contain large amount of antioxidants such as polyphenols, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Phenolic compounds in plants provide an array of natural source of antioxidants for use in foods and nutraceutical. Polyphenols are antioxidants with redox properties which allow them to act as reducing agents, hydrogen donor and singlet oxygen quenchers. The interest in polyphenol antioxidant has increased remarkably over the last decade because of their protective effects against different diseases including cardiovascular, inflammatory disease as well as cancers. Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. The source of natural antioxidants may be all or any part of plants such as fruits, vegetables, nuts, seeds, leaves, roots, barks, peels, plant, *etc.*

Pyrexia is not a disease but is a sign of numerous diseases usually caused by various bacteria and viruses. Data regarding ethno-botanical or ethno-pharmacologically characteristics of this region plants are almost non-existent except very few reports from our group. The folklore importance of medicinal plants from *Onosma bracteatum* prompted us to systematically investigate their potential for different ailments.

Onosma bracteatum wall. (*O. bracteatum*), Family: Boraginaceae. It is known as Gaozaban in the Unani system of medicine and as Sedge in the Middle East. Present research paper deals with antioxidant and antipyretic activity of selected medicinal plant.

MATERIALS AND METHODS

Plant material

The roots of plant *Onosma bracteatum* were collected from rural area of Bhopal (M.P) in the month of February, 2020.

Chemical reagents

Ascorbic acid (AA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid and quercetin (QT) were acquired from Sigma-Aldrich, USA and All the other chemicals and reagents were of analytical grade and were purchased from S.D. fine Chemicals Pvt. Ltd., Mumbai, India and SRL Pvt. Ltd. (Mumbai, India).

Methods

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs¹²:

Defatting of plant material

Flower of *Onosma bracteatum* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

50.47 gm of dried powdered flower of *Onosma bracteatum* has been extracted with hydroalcoholic solvent (ethanol : water, 80:20 v/v) using soxhlet extraction process for 24-48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, viz. carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and Phytochemical procedures¹².

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method¹³. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm¹⁴.

Antioxidant activity of hydroalcoholic extract of *Onosma bracteatum* using DPPH method

DPPH scavenging activity was measured by the spectrophotometer with slightly modification¹³. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Antipyretic activity of hydroalcoholic extract of *Onosma bracteatum*

Albino Wistar rats of either sex (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Animals were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rat was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD, 2002). Hydroalcoholic extract of Leaves of *Onosma bracteatum* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-pyretic activity.

Anti-arthritis activity

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

Group I served as normal

Group II served as control- animals were treated with yeast via subcutaneous injection (10ml/kg).

Group III animals were administered with yeast (10 ml/kg) and the standard drug paracetamol (150mg/kg b.w.), orally

Group IV animals were administered with yeast (10ml/kg,) and with hydroalcoholic extract of leaves of *Onosma bracteatum* (100mg/kg b.w.), orally

Group V animals were administered with yeast (10ml/kg,) and with hydroalcoholic extract of leaves of *Onosma bracteatum* (200mg/kg b.w.), orally.

Yeast induced pyrexia

Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10ml/kg) in distilled water. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 19 h after yeast injection. Paracetamol 150mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments. The temperature was measured at 1st, 2nd, and 3rd hour after drug administration ¹⁵.

Statistical analysis

The values were expressed as mean $\pm$ SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered to be statistically significant.

Results and Discussion

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.039x + 0.001$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.042x + 0.008$, $R^2 = 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance.

This study was carried out to evaluate the antipyretic effects of hydroalcoholic extract of leaves of *Onosma bracteatum* in rats. The antipyretic potential was done using yeast as pyrexia inducing agent. The findings from the present study were in agreement with other studies on antipyretic potential medicinal plants in animal models. Similar work by demonstrated antipyretic effects of aqueous and methanolic root extracts of *Asparagus racemosus* in yeast-induced pyrexia. Besides, similar findings were reported by on the antipyretic effect of *Urtica dioica* (L.) aqueous leaf extract against brewer's yeast-induced fever in animal models. A study by demonstrated the antipyretic effects of selected medicinal plants in albino rats. Further, *Mangifera indica* and *Ocimum gratissimum* extracts reduced brewer's yeast-induced pyrexia in animals.

The antipyretic effects of hydroalcoholic extract of leaves of *Onosma bracteatum* at different dose levels exhibited a dose-independent response on yeast-induced fever in rats. Lower dosages of 100 mg/kg and higher 200 mg/kg body weight were as effective as dose dependent manner. The results of present study indicate that the hydroalcoholic extract of *Onosma bracteatum* leaves possesses significant antipyretic effect on yeast induced hyperthermia in rats. This may be attributed to the presence of chemical constituents in the extracts which may be involved in inhibition of prostaglandin synthesis. Also, there are several mediators or multiprocessors underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis. The hydroalcoholic extract of *Onosma bracteatum* leaves at the 200 mg/kg body weight was marginally effective than 100 mg/kg body weight dose. These findings suggest a better or a similar prostaglandin synthesis inhibition by the active components in the plant's extracts. There is therefore, a possibility of the plant extracts working effectively by blocking alternative mechanisms during fever inhibition

Table 1: % Yield of flower of *Onosma bracteatum* extract

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	10.85%

Table 2: Phytochemical screening of extract of flower of *Onosma bracteatum*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Mayer's Test: Wagner's Test: Dragendroff's Test: Hager's Test:	-ve -ve -ve +ve
2.	Glycosides Legal's test	+ve
3.	Flavonoids Lead acetate Alkaline Reagent Test:	-ve +ve
4.	Phenolics Ferric Chloride Test	+ve
5.	Proteins Xanthoproteic test	-ve
6.	Carbohydrates Molisch's Test: Benedict's Test: Fehling's Test:	-ve -ve +ve
7.	Saponins Froth Test:	+ve
8.	Diterpins Copper acetate test	-ve
9.	Tannins Gelatin Test:	+ve

Table 3: Total phenolic and total flavonoid content of flower of *Onosma bracteatum*

S. No.	Total Phenol content (mg/100mg)	Total flavonoid content (mg/100mg)
1.	0.825 mg/100mg	0.961 mg/100mg

Table 4: % Inhibition of ascorbic acid and Hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	43.65	18.65
2	20	50.02	26.85
3	40	62.96	30.55
4	60	70.75	48.96
5	80	80.87	50.52
6	100	89.75	53.84
IC 50		19.44	80.81

Table 5: Antipyretic activity of hydroalcoholic extract of leaves of *Onosma bracteatum* against yeast induced pyrexia in rats

Rectal Temperature in °C after 18hrs of Yeast Injection				
Group	0 hr	1 hr	2 hr	3 hr
Group I	38.50±0.9	38.00±0.9	37.60±0.9	37.20±0.9
Group II	41.70±0.10	41.00±0.12	40.50±0.12	40.00±0.12
Group III	40.50±0.12	38.20±0.12	38.10±0.11*	37.10±0.11*
Group IV	41.00±0.12	40.10±0.12	39.50±0.12	38.50±0.12*
Group V	41.00±0.12	40.05±0.11	38.30±0.11*	37.60±0.12*

Values expressed as mean ± SEM (n=6) *P<0.05 as compared to pyretic control

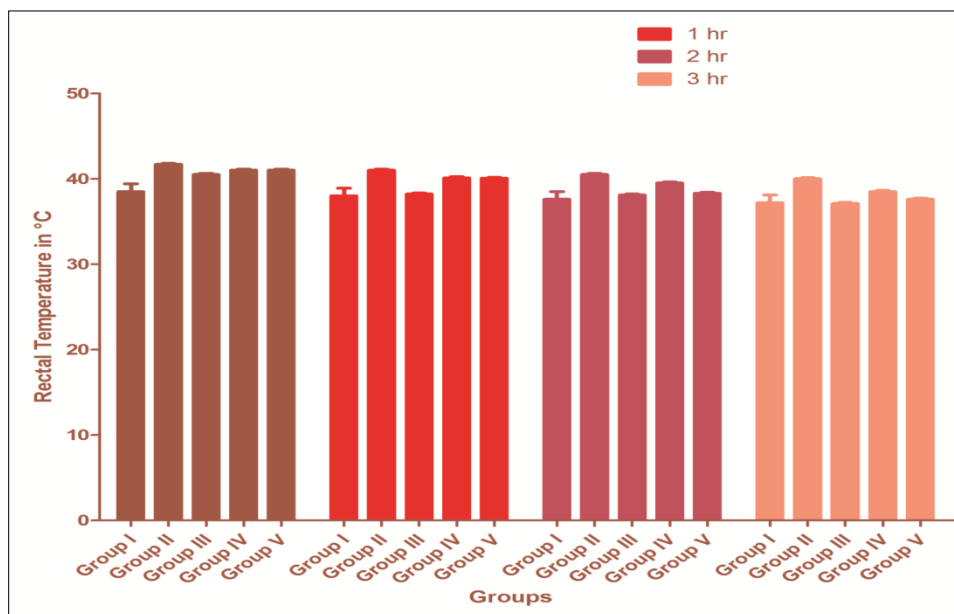


Figure 1: Antipyretic activity of hydroalcoholic extract of leaves of *Onosma bracteatum* against yeast induced pyrexia in rats

Conclusion

In conclusion, this study has revealed that these plants are endowed the many bioactive compounds which possess antipyretic activity in rats. The effect of hydroalcoholic extract of leaves of *Onosma bracteatum* were determined after administration at two dose levels (100 and 200 mg/kg b.w.) in yeast induced pyrexia rats. It can be said that *O. bracteatum* leaves contain significant antipyretic properties, which may be attributed to its antioxidants and constituents. This study brought out a number of biological activities that can be regarded as promising results. As a result of these contributions, plants can be authenticated, as well as new drugs can be developed based on their activity. Our findings suggest a medicinal plant with multiple therapeutic properties that could serve as an antipyretic drug. To elucidate the exact mechanism of action of these active constituents, further investigation is required.

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