International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 1267-1273 www.ijprajournal.com ISSN: 2456-4494

Antipyretic and anti-oxidant potential of hydroalcoholic extract of Gendarussa Vulgeris

Mateshwari M. Banwale*, Mrs. Disha Chaouhan, Dr. Manjeet Singh, Dr. Rajesh mujariya, Miss. Priya Bisen

Institute of pharmaceutical science & Research (IPSR), Sardar Patel University, Balaghat, Madhya pradesh, India.

Submitted: 05-04-2022 Accepted: 17-04-2022

ABSTRACT: G. vulgaris Nees of the family Apocynaceae is a medium sized tree grown in semishade or no shade and is common in the Ernad and Nilambur taluks of Kerala. Various parts of this plant have been used in the treatment of ulcers, sores, inflammation, dyspepsia, healing of wounds, etc. The present study aimed at the evaluation of anti-pyretic activity of the hydroalcoholic extracts of the leaves by in vivo methods. Phytochemical screening reveals the presences of Alkaloids, Saponins, Carbohydrates, Flavonoids and Phenols. The total phenolic content was found 0.691mg/100mg of dry weight of extract, expressed as gallic acid equivalents and the total flavonoid content was found 0.847mg/100mg, expressed as Quercetin equivalents. Antioxidant activity was performed using DPPH method. The IC50 value of Hydroalcoholic extract of Gendarussa vulgaris was found to be 55.79µg/ml. Yeast induced pyrxia and anti-pyretic property of hydroalcoholic extract of leaves of Gendarussa vulgaris. The effect of hydroalcoholic extract of leaves of Gendarussa vulgaris were determined after administration at two dose levels (100 and 200 mg/kg b.w.) in yeast induced pyrxia rats. From the results, it may be concluded that hydroalcoholic extract of leaves of Gendarussa vulgaris possess significant anti-pyretic effect may be due to the effect of antioxidants and constituent present in the leaves.

Key words: G. vulgaris Nees, Phytochemical screening, Anti-pyretic, Hydroalcoholic extract, TPC, TFC

I. INTRODUCTION

Ayurvedic medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Safe effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas especially in India and China. Information from ethnic groups or indigenous traditional medicines has played vital role in the discovery of novel products from plants as chemotherapeutic agents. Herbal medicines have been main source of primary healthcare in all over the world. From ancient times, plants have been catering as rich source of effective and safe medicines. About 80 % of world populations are still dependent on traditional medicines. Herbal medicines are finished, labeled medicinal products that contain as active ingredients, aerial or underground part of plants or other plant materials, or combination thereof, whether in the crude state or as plant preparations. Medicines containing plant materials combined with chemically defined active substances, including chemically defined isolated constituents of plants are not considered to be herbal medicines[1].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature[2]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increasing sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV[3]. Drugs having anti-inflammatory activity generally possess antipyretic activity (e.g) non-steroidal anti-inflammatory drugs (NSAIDs). It has been suggested that prostaglandin

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 1267-1273 www.ijprajournal.com ISSN: 2456-4494

(PGE) mediates pyrogen fever; the ability of NSAIDs, to inhibit prostaglandin synthesis could help to explain their antipyretic activity.

According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine[4]. Due to poor hygiene practices and malnutrition, children in developing countries frequently suffer from various forms of infections which present as fevers. These fevers are often accompanied by aches and pains which all lead to morbidity and mortality[5].

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature[6].

Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α[7] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain[8].

Various injuries and diseases are most often presented with pain and fever. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed drugs for their management but significant gastrointestinal complications like perforation, bleeding, peptic ulcers, and obstructions have limited their uses in clinical settings. Selective COX-2 inhibitors have some benefits on lowering such side effects while risk of cardiovascular adverse events demands important consideration. The social abuse and other side effects like psychological dependency, addiction, tolerance, sedation, constipation, and respiratory depression associated with narcotic analgesics are playing negative role in management of chronic pain and sometimes being inadequate.

G. vulgaris Nees of the family Apocynaceae is a medium sized tree grown in semishade or no shade and is common in the Ernad and Nilambur taluks of Kerala. Various parts of this plant have been used in the treatment of ulcers, sores, inflammation, dyspepsia, healing of wounds, etc. The present study aimed at the evaluation of anti-pyretic activity of the hydroalcoholic extracts of the leaves by in vivo methods.

II. MATERIAL AND METHODS

MaterialS

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Methods

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [9-10]:

Defatting of plant material

Leaves of Gendarussa vulgaris 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

46.85 gm of dried powdered leaves of Gendarussa vulgaris 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:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug taken}} \text{x100}$$

Phytochemical Screening

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

International Journal of Pharmaceutical Research and Applications
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Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [11].

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method[12]. 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% $AlCl_3$ 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 nm[13].

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Antioxidant activity of hydroalcoholic extract of Gendarussa vulgaris using DPPH method

DPPH scavenging activity was measured by the spectrophotometer with slightly modification[12]. 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 Gendarussa vulgaris

and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Animas 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. Hydroalcoholic extract of leaves of Gendarussa vulgaris (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. 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 Gendarussa vulgaris (100mg/kg b.w.), orally
Group V animals were administered with yeast (10ml/kg,) and with hydroalcoholic extract of leaves of Gendarussa vulgaris (200mg/kg b.w.), orally.

Yeast induced pyrxia

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[13].

Statistical analysis

The values were expressed as mean ± 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.

III. RESULTS AND DISCUSSION

Herbal medications produced from plant extracts are rapidly being used to treat a wide range of clinical illnesses, despite the fact that little is known about their mechanism of action[14]. For the field of modern medical science, the herbal drugs are to be subjected for several processes such as identifications, isolation, purification, characterization, structural elucidation and therapeutic evaluation. The strategy of bioassay guided fractionation and isolation using chromatographic separation techniques revolutionized what could be achieved in medicinal plant research[15].

Medicinal plants have bioactive compounds which are used to curing of various diseases. In this present investigation involves Gendarussa vulgaris medicinal plant was studied.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 1267-1273 www.ijprajournal.com ISSN: 2456-4494

The Hydroalcoholic extract was subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presences of Alkaloids, Saponins, Carbohydrates, Flavonoids and Phenols. The total phenolic content was found 0.691mg/100mg of dry weight of extract, expressed as gallic acid equivalents and the total flavonoid content was found 0.847mg/100mg, expressed as Quercetin equivalents. Antioxidant activity was performed using DPPH method. The IC50 value of Hydroalcoholic extract of Gendarussa vulgaris was found to be 55.79µg/ml.

A natural antipyretic agent with reduced or no toxicity is therefore, essential. Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds, which have an inhibitory activity on prostaglandins biosynthesis, the yeast induced hyperpyrexia in rat model was employed to investigate the antipyretic activity of the extract. Yeast induced pyrexia is called pathogenic fever which is due to the production of prostaglandins (PGE2) which set the thermoregulatory center at a higher temperature. The hydroalcoholic extract of Gendarussa vulgaris leaves had a more dramatic impact in reducing hyperthermia than the aqueous extract, but after the 3 hr of treatment, it was shown to have a similar effect as the conventional medication Paracetamol. The extracts are likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine.

Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α (Adesokan et al., 2008) and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain. This study also correlates with the study of Zakaria et al., (2007) that the compounds like flavonoids and saponins are suggested to act synergistically to exert the observed pharmacological activity. The results of present study indicate that the hydroalcoholic extract of Gendarussa vulgaris 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.

Table 1: Total phenolic and total flavonoid content of Gendarussa vulgaris

S. No.	Total Phenol content (mg/100mg)	Total flavonoid content (mg/100mg)
1.	0.691	0.847

Table 2: % Inhibition of ascorbic acid and Hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	26.65
2	20	48.62	34.56
3	40	65.34	42.74
4	60	69.65	51.43
5	80	77.41	62.87
6	100	84.13	70.24
IC 50		**17.68**	**55.79**

Table 3: Antipyretic activity of hydroalcoholic extract of leaves of Gendarussa vulgaris against yeast induced pyrxia in rats

Rectal Temperature in °C after 18hrs of Yeast Injection				
Group	**0 hr**	**1 hr**	**2 hr**	**3 hr**

International Journal of Pharmaceutical Research and Applications
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Group I	38.00±0.8	37.00±0.7	37.50±0.6	37.40±0.6
Group II	41.50±0.11	40.15±0.15	39.50±0.13	39.10±0.12
Group III	40.00±0.15	38.50±0.13	38.00±0.13*	37.50±0.12*
Group IV	40.50±00.15	39.50±0.15	39.00±0.15	38.00±0.12*
Group V	40.30±0.15	39.00±0.14	38.10±0.14*	37.50±0.14*

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

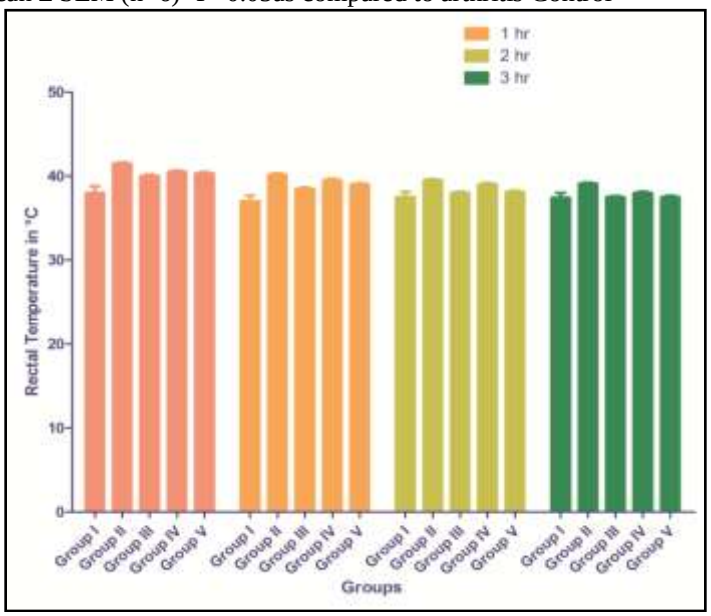

Figure 1: Antipyretic activity of hydroalcoholic extract of leaves of Gendarussa vulgaris against yeast induced pyrxia in rats

IV. CONCLUSION

Yeast induced pyrxia and anti-pyretic property of hydroalcoholic extract of leaves of Gendarussa vulgaris. The effect of hydroalcoholic extract of leaves of Gendarussa vulgaris were determined after administration at two dose levels (100 and 200 mg/kg b.w.) in yeast induced pyrxia rats. From the results, it may be concluded that hydroalcoholic extract of leaves of Gendarussa vulgaris possess significant anti-pyretic effect may be due to the effect of antioxidants and constituent present in the leaves. All these biological activities may be said to be a promising findings brought out by the present study. These contributions can be used as parameters for the authentication of plant as well as for developing newer drugs based on their activity. It can be optimistic that the present work suggests an herbal drug of multiple therapeutic advantages and likely to be an anti-pyretic drug. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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