

SCREENING OF ANTIPYRETIC EFFECTS OF ETHANOLIC EXTRACT OF *HIBISCUS ROSA SINENSIS* LINN (ROOTS) IN RABBITS

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ABSTRACT: Pyrexia is an increase in the normal body temperature as a result of some adverse reactions. This occurs due to many factors like malignancy, secondary impact of infection or other disease state. The aim of the present study was to evaluate the antipyretic activity of the ethanolic extract of roots of *Hibiscus rosa sinensis* (HRSR). The extract was tested on rabbits at doses of 250 mg/kg and 500 mg/kg, Paracetamol was used as standard drug (100 mg/kg), by using yeast induced pyrexia method. The results of the present research work were compared with saline control group, it was found that 250 mg/kg and 500 mg/kg doses respectively produced significant effects ($P < 0.05$). Ethanolic extract of *Hibiscus rosa* at dose level of 500 mg/kg have significant reduction in yeast induced elevated rectal temperature when compared with the dose of 250 mg/kg. The results of the present study showed that the roots of China rose have significant dose dependent antipyretic activity.

Keywords: *Hibiscus rosa sinensis* roots (HRSR), Ethanolic extract, Rabbits, Yeast induced pyrexia.

INTRODUCTION

Human beings and medical management systems are utilizing the essential resources like animals, minerals, plants and marine since time immemorial [1]. In terms of herbal treatment, the World Health Organization (WHO) has been reporting about 21,000 plants that are used in folk medicines producing beneficial effects in the medical care system in the universe. There are various folk medicine systems in the universe; every system has different types of cultural origins and philosophies, such as traditional Tibetan medicine, traditional Hindu medicine and traditional Chinese medicine. These are mostly used in various areas of the universe [2]. Since last century, herbal medications has been getting more attention, both in the establishing and established countries, because of human compatibility, natural origins, fewer side effects and economical [3].

The genus of the *Hibiscus* contains almost 275 species in the tropical zone and subtropical zone [4]. *Hibiscus rosa sinensis* (HRS) belongs to the family Malvaceae, commonly known as China rose [5]. *Hibiscus rosa sinensis* is used as vitaliser in palpitation, urinary diseases, fever, antiestrogenic, natural health for contraception, antispasmodic, cough, laxative, anti-inflammatory, menorrhagia, refrigerant, antifertility, hair loss, controlling uterine bleeding, leucorrhoea and venereal diseases [6-10]. Researchers are searching the natural antipyretic drugs in all over the universe as an alternative of antipyretic drugs because these drugs are having side effects like gastric lesion, dependence, tolerance and toxicity.

Pyrexia is defined as increases the normal body temperature in term of centigrade ($^{\circ}\text{C}$) or Fahrenheit ($^{\circ}\text{F}$). The moderate oral body temperature is 37°C (98.6°F). Pyrexia occurs due to malignancy, secondary impact of infection or other disease condition. The human body has natural mechanisms to produce an environment where malignancy, infective agents, graft rejection, damaged tissues and inflammation cannot survive [11]. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases around the hypothalamic region and through hypothalamus to increase the body temperature. Under these conditions, the agents like Tumor Necrosis Factor α (TNF- α), interferon, interleukin and cytokines are produced in large quantities and increases the normal body temperature [12].

Symptoms of pyrexia are included depression, inability to concentrate, sickness behavior, lethargy and anorexia. Shivering and increased muscle tone occur when the body temperature evaluated [13].

Mostly, antipyretic medicines are inhibiting the enzymatic activity of cyclooxygenase (COX-2) pathway and decreasing the levels of prostaglandin E2 (PGE2) within the hypothalamic region [14].

There are varieties of antipyretic medicines that are either chemical in nature or chemically substituted in order to prolong the action of medicines. Several studies are ongoing Worldwide to search the natural antipyretic agents with better efficacy and fewer effects because of;

- Higher dosing caused severe toxicity.
- Patient with hepatic ulceration or impaired hepatic functions do not administer these medications.
- Duration of action is lesser [15-17].

MATERIAL AND METHOD

Plant material

Roots of *Hibiscus rosa sinensis* were collected from Karachi and identified by Madam Afsheen Ather in Herbarium of the Department of Botany, Karachi University, Pakistan, allotted the voucher specimen G.H. No: 92098

Extraction of roots of *Hibiscus rosa sinensis*

The root parts of plant were taken and properly washed with distilled water, dried under shade and then ground to collect coarse powder. About 450g of coarse powdered was subjected to the soxhlet apparatus (Model: HMFT) for extraction, using ethanol as a solvent. The extract was concentrated in a rotary evaporator (Buchi, Switzerland) at $30-40^{\circ}\text{C}$ to obtain semi-solid material [18]. Its percentage was calculated 3.111% (14g). The extract was stored at 4°C in a properly labeled airtight container. A fresh dilution of dried extract in 2% Tween 80 was prepared for the experiments and administered by oral route at two different doses of 250 mg/kg and 500 mg/kg.

Study Animals

For the assessment of antipyretic effects, rabbits of both genders were used. Standard iron cages (01 per cage) were used to kept the experimental animals [19] at Dr. HMI (Institute of Pharmacology) Hamdard University, Karachi,

Pakistan to maintain the new environment of the animals under conditions of standard lighting (12 h of darkness and light) and temperature (27±2°C), for at least one week before the starting of the assessment. Animals were fed with standard diet and water. The departmental animal ethical committee approved standard procedures for the rabbit's handling and uses of rabbits in antipyretic activity.

Chemicals

Ethanol (analytical grade), Brewer's yeast and Paracetamol.

Evaluation of antipyretic activity

Fever induced by brewer's yeast in rabbits

Rabbits were divided into four groups (I, II, III and IV) of six animals (n=6) weights between 1.08-1.48 kg. The rectal temperatures of the animals were recorded by inserting the well lubricated thermometer into the rectum. It is carefully inserted with equally deepness at each animal (3-6 cm) [20]. After taking the rectal temperature at 0 hours, the animals were injected subcutaneously with 20% w/v aqueous suspension of brewer's yeast [21] and the animals with elevated temperatures up to 0.5°C preferred for the study. Normal saline was orally administered in group I and group II treated with standard drug (Paracetamol, 100mg/kg). The extract of *Hibiscus rosa sinensis* was orally administered at a dose level of 250 mg/kg and 500 mg/kg in group III and IV. The rectal temperatures of the groups were then recorded at 01 hour interval up to 05 hours after yeast injection.

STATISTICAL ANALYSIS

The results were represented as mean ± S.E.M. The calculations of the experimental collected data were done by using Two-Way analysis of variance (ANOVA) followed LSD post hoc test when compared to control (Saline group). All the data were processed with SPSS software version no. 20 and *p* < 0.05 was considered as significant.

RESULT

The present study has been carried out to evaluate and compare the in vivo antipyretic activity of the ethanolic extract of *Hibiscus rosa* roots by the yeast induced pyrexia method. The effect of ethanolic extract of *Hibiscus rosa* roots on brewer's yeast induced pyrexia has been shown in table 1. The rectal temperatures of animals were increased when subcutaneously injected 20% w/v aqueous suspension of brewer's yeast in experimental rabbits. Ethanolic extract of *Hibiscus rosa sinensis* roots at a dose level of 500 mg/kg results a highly significant reduction in yeast induced elevated rectal temperature when compared with dose of 250 mg/kg. The extract of *Hibiscus rosa* roots (250 mg/kg and 500 mg/kg) respectively caused significant (*p* < 0.05) reduction in temperature when compared with the saline control group.

Table 1: Effect of Hibiscus rosa sinensis roots extract on yeast brewer's induced pyrexia in rabbits

Groups	Dose (mg/kg)	Initial rectal temperature (°C)	Rectal temperature (°C)					
			0Hr	1Hr	2Hr	3Hr	4Hr	5Hr
Control negative	-	37.86 ± 0.20	38.83 ± 0.30	39.02 ± 0.26	39.15 ± 0.29	39.30 ± 0.27	39.53 ± 0.27	39.70 ± 0.26
Paracetamol	100	36.86 ± 0.22	38.76 ± 0.48	38.00 ± 0.30	37.61 ± 0.18	37.33 ± 0.20	37.06 ± 0.22	36.9 ± 0.08
Ethanol extract	250	37.20 ± 0.24	39.00 ± 0.27	38.80 ± 0.24	38.40 ± 0.16	38.00 ± 0.16	37.80 ± 0.16	37.70 ± 0.12
Ethanol extract	500	36.85 ± 0.30	38.70 ± 0.31	38.02 ± 0.20	37.66 ± 0.22	37.3 ± 0.18	37.16 ± 0.20	37.00 ± 0.14

Rabbits were pretreated with 20% (w/v) of brewer's yeast in 0.9% saline solution. After subcutaneous injection measuring the rectal temperature of animals were recorded. Animals showing raise temperature were oral administered with extract of *Hibiscus rosa sinensis* roots (250 mg/kg and 500 mg/kg), Paracetamol (100 mg/kg) and normal saline. The results are represented as mean ± S.E.M (n=6). The statistical analysis was performed by analysis of variance (A.N.O.V.A) followed by LSD post hoc test when compared to the saline control group *p* < 0.05.

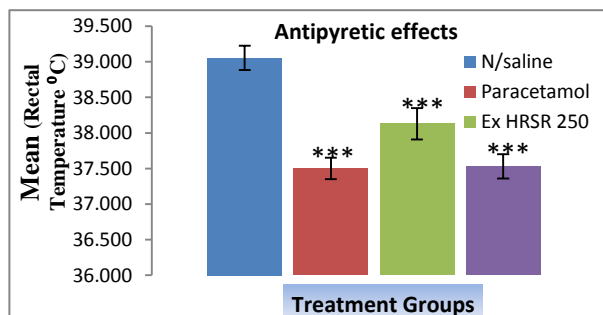


Fig. 1 Antipyretic effect of Hibiscus rosa sinensis roots extract by yeast brewer's response

The decreased rectal temperature of rabbits by Paracetamol, ethanolic extract of *Hibiscus rosa sinensis* roots at dose level of 250 mg/kg and 500 mg/kg are statistically significant (*P* < 0.05) when compared to the control group, where as extract dose of 500 mg/kg highly significant and have almost similar effects as standard drug (Paracetamol). Results are expressed as mean ± S.E.M. The asterisks (*) denote the significance levels compared with control group and significantly different from control group (*P* < 0.05).

DISCUSSION

The present study has been carried out to evaluate and compared the in vivo antipyretic activity of the ethanolic extract of *Hibiscus rosa sinensis* roots (HRSR) in rabbits at a dose of 250 mg/kg and 500 mg/kg by using brewer's yeast induced pyrexia method and using Paracetamol as standard drug. Brewer's yeast induced pyrexia called pathogenic fever, which is due to activation of the production of prostaglandins (PGE₂) in the central nervous system (CNS) [22].

The results of *Hibiscus rosa sinensis* roots showed the significant antipyretic activity. Previous phytochemical studies are showed that the plant *Hibiscus rosa sinensis* have saponins, steroids, anthocyanin and flavonoids [23-24]. Flavonoids are known to target TNF- α , which are involved in the antipyretic effects [25]. The constituents responsible for the antipyretic activity are not exactly known, but may be the flavonoids responsible for this activity.

Although the *Hibiscus rosa sinensis* roots were not much investigated in earlier studies, but it also has great curing potential against different diseases. The results of the present study are closely related to a previous study where aqueous extract of roots of *Hibiscus rosa sinensis* has a powerful antipyretic activity in albino rats [26]. Ethanolic extract of HRSR in our research work has been used for further evaluations.

Antipyretic medicines have been shown to reduced pyrexia by inhibiting the COX-2 pathway to suppress the increased body temperature by inhibiting the prostaglandin biosynthesis or inhibiting the evaluated formation of cytokines and interleukin 1 β , α , β [25]. This may be attributed to the presence of flavonoids in the extract that may be involved in inhibition of prostaglandin synthesis.

In this present study, two different doses of ethanolic extract (250 mg/kg and 500 mg/kg) of *Hibiscus rosa sinensis* were used to evaluate the antipyretic effect of this plant. The results obtained from *Hibiscus rosa sinensis* extract and Paracetamol treated rabbits were compared with control group and produced highly significant ($P < 0.05$) reduction in yeast induced elevated rectal temperature was observed.

Hibiscus rosa sinensis extract at a dose level of 500 mg/kg have a highly significant reduction in yeast induced elevated rectal temperature when compared with dose of 250 mg/kg.

Extract dose of 500 mg/kg almost produced similar results when compared with standard drug administration. It is thus evident that the plant has a significant antipyretic activity. It might be possible that the increased dose of extract above 500 mg/kg may show better results as compare to Paracetamol. In future, the plant of *Hibiscus rosa sinensis* may prove as an alternative medicine of pyrexia.

CONCLUSION

In conclusion, the ethanolic extract of *Hibiscus rosa sinensis* roots has shown significant antipyretic activity in rabbits. However, further in depth studies to isolate bioactive compounds and their structure assessments are required to acknowledge the accurate antipyretic mechanism of action of *Hibiscus rosa sinensis* roots (HRSR).

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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