



PRELIMINARY INVESTIGATION AND IN VIVO ANTIPYRETIC ACTIVITY OF  
ETHANOLIC EXTRACT OF *RHEUM EMODI*

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ABSTRACT

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*Rheum emodi* Wall. ex Meissn. is a perennial herbaceous plant traditionally used in various indigenous systems of medicine for its therapeutic properties. This study aimed to investigate the phytochemical composition and antipyretic activity of the ethanolic extract of *Rheum emodi*. The ethanolic extract was prepared from the roots of *Rheum emodi* and subjected to phytochemical screening for alkaloids, flavonoids, phenolics, proteins, carbohydrates, saponins, and diterpenes. The extract's total flavonoid and phenolic content were quantified. The antipyretic activity was evaluated using a yeast-induced pyrexia rat model. The ethanolic extract showed a % yield of 8.4% (w/w) from the roots. Phytochemical analysis revealed the presence of flavonoids, phenolics, proteins, and carbohydrates, while alkaloids and diterpenes were absent. The extract exhibited a total flavonoid content of 0.745 mg/100 mg and a total phenolic content of 0.822 mg/100 mg. In the antipyretic activity study, the extract demonstrated a dose-dependent reduction in rectal temperature in rats compared to the control group. The ethanolic extract of *Rheum emodi* possesses significant antipyretic activity, likely attributed to its rich flavonoid and phenolic content. These findings support its traditional use and suggest its potential as a natural antipyretic agent.

**Keywords:** *Rheum emodi*, ethanolic extract, antipyretic activity, flavonoids, phenolics, phytochemical screening.

INTRODUCTION

*Rheum emodi* Wall. ex Meissn., commonly known as Indian rhubarb or Himalayan rhubarb, belongs to the family Polygonaceae. It is a perennial herbaceous plant native to the Himalayan region and is widely distributed across the subalpine and alpine regions of India, Pakistan, Nepal, and Bhutan. The roots of *Rheum emodi* have been traditionally used in various indigenous systems of medicine for their therapeutic properties, including anti-inflammatory, antipyretic, antioxidant, antimicrobial, and hepatoprotective activities.

Phytochemical studies have revealed that *Rheum emodi* contains several biologically active compounds, including anthraquinones (e.g., emodin, rhein), stilbenes, flavonoids, tannins, and phenolic acids. These phytoconstituents are known to contribute to the plant's pharmacological activities (Ali *et al.*, 2011, Sharma *et al.*, 2013, Subedi *et al.*, 2013, Kapoor *et al.*, 1990, Ayurvedic Pharmacopoeia; 2004).

Antipyretic activity is an important pharmacological property of medicinal plants and natural products, especially in the treatment of febrile conditions.

Fever is a common symptom of various infectious and inflammatory diseases and is associated with an elevation in body temperature due to the action of endogenous pyrogens on the hypothalamus. The search for effective and safe antipyretic agents from natural sources remains a significant area of research. This study aims to investigate the preliminary phytochemical profile and in vivo antipyretic activity of the ethanolic extract of *Rheum emodi* using standard experimental models.

## MATERIALS AND METHODS

### Material

#### Collection of plant material

Roots of *Rheum emodi* were collected from rural area of Bhopal (M.P), India in the months of January, 2024.

#### Extraction by maceration process

Extraction is the first step to separating the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing, and sublimation according to the extraction principle. 63 gm dried powdered roots of *Rheum emodi* has been extracted with ethanolic solvent using maceration process for 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

Phytochemical tests were done as per the standard methods (Kokate, 1994; Audu et al., 2007).

#### Total flavonoids content estimation

The calibration curve is constructed by plotting the analytical response, typically measured by spectrophotometric techniques, against a range of known concentrations of quercetin. The resulting curve allows for the estimation of flavonoids concentrations in the sample based on its corresponding response. In this study, a calibration curve using quercetin as a standard was developed to quantify the total flavonoids content in the extract. Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% AlCl<sub>3</sub> 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.

#### Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Parkhe and Bharti, 2019). 50 mg Gallic acid was dissolved in 50 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 extract or each 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.

### **In vivo anti-pyretic activity**

#### **Animals**

Wistar rats (150–200 g, 10-12 weeks old) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum*. Rats 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 rats 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.

#### **Chemicals**

All the chemicals used were of analytical grades, obtained from commercial suppliers.

#### **Acute oral toxicity study**

Toxicity studies were carried out by OECD guidelines, acute oral toxicity study of ethanolic extract of *Rheum emodi*. Acute toxicity study was performed based on OECD guideline no. 423. The rats were assessed for signs of toxicity throughout the next 14 days. Ethanolic extract of *Rheum emodi* was given orally by the safe dose. Clinical symptoms like behavioural alterations, changes in the eyes, body weight, skin and fur were noted (Gilani et al., 2022, Kazmi et al., 2023).

#### **Experimental design**

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 *ethanolic extract of Rheum emodi* (100mg/kg b.w.), orally

**Group V** animals were administered with yeast (10ml/kg,) and with *ethanolic extract of Rheum emodi* (200mg/kg b.w.), orally.

#### **Yeast induced pyrexia**

Pyrexia was induced by the subcutaneous injection of 20% w/v of brewer's yeast (10ml/kg) in distilled water. The basal rectal temperature was measured before the injection of yeast, by inserting a digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 18 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 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> hour after drug administration (Al-Saedi, 2021).

#### **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 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

Table 1 presents the % yield of the ethanolic extract of *Rheum emodi* obtained from its roots. The yield of the extract was found to be 8.4% (w/w), indicating a moderate extraction efficiency.

Table 2 outlines the results of phytochemical screening of the ethanolic extract of *Rheum emodi*. The extract tested negative for alkaloids (Dragendroff's and Hager's tests) and diterpenes (Copper acetate test). However, it tested positive for flavonoids (Lead acetate alkaline test), phenolics (FeCl<sub>3</sub> test), proteins (Xanthoproteic test), and carbohydrates (Fehling's test). Saponins were absent as indicated by the foam test.

Table 3 provides the total flavonoid and phenolic content of the ethanolic extract of

*Rheum emodi*. The extract was found to contain 0.745 mg of flavonoids per 100 mg of extract and 0.822 mg of phenolics per 100 mg of extract.

Table 4 presents the antipyretic activity of the ethanolic extract of *Rheum emodi* against yeast-induced pyrexia in rats. The rectal temperatures of rats were measured at different time points after yeast injection. Group II (control) showed a significant increase in temperature compared to Group I (normal control). Treatment with the ethanolic extract (Groups III, IV, and V) resulted in a dose-dependent reduction in rectal temperature compared to the control group (Group II), indicating antipyretic activity.

**Table 1: % Yield of ethanolic extract of *Rheum emodi***

S. No.	Part	% Yield (W/W)
1.	Roots	8.4

**Table 2: Phytochemical screening of ethanolic extract of *Rheum emodi***

S. No.	Constituents	Ethanolic extract
1.	<b>Alkaloids</b> Dragendroff's test Hager's test	-ve -ve
3.	<b>Flavonoids</b> Lead acetate Alkaline test	-ve +ve
4.	<b>Phenolics</b> FeCl <sub>3</sub>	+ve
5.	<b>Proteins</b> Xanthoproteic test	+ve
6.	<b>Carbohydrates</b> Fehling's test	+ve
7.	<b>Saponins</b> Foam test	-ve
8.	<b>Diterpenes</b> Copper acetate test	-ve

**Table 3: Total flavonoid content of extract of *Rheum emodi***

S. No.	Extract	Total flavonoid content (mg/100mg)	Total phenolic content (mg/100mg)
1.	Ethanolic extract	0.745	0.822

**Table 4: Antipyretic activity of ethanolic extract of *Rheum emodi* 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	37.70 ± 0.9	37.30 ± 0.8	37.50 ± 0.5	37.00 ± 0.8
Group II	41.50 ± 0.15	40.40 ± 0.05	39.80 ± 0.10	39.40 ± 0.10
Group III	39.80 ± 0.13	38.60 ± 0.13	38.00 ± 0.11*	37.20 ± 0.10*
Group IV	40.00 ± 0.13	39.20 ± 0.13	38.60 ± 0.10*	38.10 ± 0.10 *
Group V	40.40 ± 0.13	39.20 ± 0.13	38.10 ± 0.11*	37.50 ± 0.10*

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

## CONCLUSION

The ethanolic extract of *Rheum emodi* demonstrated significant antipyretic activity in a yeast-induced pyrexia rat model, supported by its phytochemical composition rich in flavonoids and phenolics. These findings validate its traditional use and suggest its potential as a natural antipyretic agent for further development and clinical evaluation.

## DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

## REFERENCES

- Ali, S. et al. (2011) Phytochemical, ethnomedicinal uses and pharmacological profile of genus *Rheum* (Polygonaceae): A review. *Journal of Ethnopharmacology*, 137, 647–663.
- Sharma, N. et al. (2013) Pharmacological properties and therapeutic potential of *Rheum emodi* Wall. ex Meissn. *African Journal of Pharmacy and Pharmacology*, 7, 70–80.
- Subedi, L. et al. (2013) Chemical constituents and biological activities of Nepalese medicinal plants. *Journal of Medicinal Plants Research*, 7, 538–599.
- Kapoor, L.D. (1990). *Handbook of Ayurvedic Medicinal Plants: Herbal Reference Library*, Vol. 2. CRC Press: Boca Raton, FL, USA.
- *The Ayurvedic Pharmacopoeia of India, Part 1* (2004), Vol. 4. Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homoeopathy: New Delhi.

- Kokate, C.K., editor Practical Pharmacognosy (1994), 4th edn, Vallabh Prakashan., 112, 120.
- Audu, S.A., Mohammed, I. & Kaita, H.A. (2007) Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Science Journal*, 4, 75–79.
- Parkhe, G. & Bharti, D. (2019) Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitex trifolia* Linn. JDDT [Internet], Vol. 9(4-A), pp. 705–707.
- Gilani, S.J., Bin-Jumah, M.N., Al-Abbasi, F.A., Albohairy, F.M., Nadeem, M.S., Ahmed, M.M., Alzarea, S.I. & Kazmi, I. (2022) The ameliorative role of Hibiscetin against high-fat diets and streptozotocin-induced diabetes in rodents via inhibiting tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and malondialdehyde level. *Processes*, 10, 1396.
- Kazmi, I., Al-Abbasi, F.A., Afzal, M., Shahid Nadeem, M. & Altayb, H.N. (2023) Sterubin protects against chemically induced Alzheimer's disease by reducing biomarkers of inflammation- IL-6/ IL- $\beta$ / TNF- $\alpha$  and oxidative stress- SOD/MDA in rats. *Saudi Journal of Biological Sciences*, 30, 103560.
- Al-Saaedi, A.M. (2021) Antipyretic Activity of the Aqueous Extract of Cumin (*Cuminum cyminum* L.) with Yeast Induced Pyrexia in Female Rats. *Journal of Science*. University of Thi-Qar, 8, 33–35.