

REPORT

Antipyretic activity of hydro-alcoholic extracts of *Moringa oleifera* in rabbits

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Abstract: Pyrexia and inflammation are indicatives of various disorders. Modern medicines are available for treatment of pyrexia, but they have few side effects. Several studies are ongoing Worldwide to search natural antipyretic agents with better efficacy and fewer or no side effects. This study was aimed at evaluating the antipyretic activity of *Moringa oleifera* bark in rabbits against *E. coli* induced pyrexia. Rectal temperature was recorded with digital thermometer at 0 h and *E.coli* suspension was injected. After 1 h again rectal temperature of the animals was recorded and hydro-alcoholic extract were administered to the treatment groups and paracetamol hydro-alcoholic 50 mg/kg orally to the positive control group. Then rectal temperature was recorded at the interval of one h for 4 h. After the drug administration (at h 1), the decrease in body temperature with the dose of 25mg/kg⁻¹ during next four h ranged between 1.9-2.6°F as compared to the negative control. At the dose of 50mg/kg⁻¹ the decrease in temperature was 1.9-3.0 °F. The decrease in body temperature at the dose of 100mg/kg⁻¹ was high, which ranged from 2.3-3.1°F as compared to negative control. Paracetamol, a standard drug , also significantly lowered the temperature but *Moringa oleifera* at the concentration of 100mg/kg⁻¹ lowered the body temperature significantly as compared to the negative as well as positive control. *Moringa oleifera* bark has marked antipyretic activity in animal models and this strongly supports the ethnopharmacological uses of *Moringa oleifera* bark as an antipyretic plant.

Keywords: *Escherichia coli*, Cholistan desert, analgesic, anti-inflammatory, prostaglandin, *E. coli* induced pyrexia, traditional medicine

INTRODUCTION

Cholistan Desert, situated in the southern part of Punjab province, Pakistan occupies an area of 26000 km² with highly saline and brackish sub-soil aquifer. In this desert, a total of 128 plant species have been identified, out of which 64 are extensively used by the local people and herbal practitioners to cure different diseases (Arshad & Akbar, 2003).

Moringa oleifera is a common tree, known as "Suhanjana" belongs to family Moringaceae and is found throughout Pakistan. Although this plant is not very common in the Cholistan desert but it is commonly found in adjoining areas of this desert (Rao *et al.*, 1989). Different parts of this plant are used by the local people for the cure of various ailments. The leaves and green pods of this plant are cooked as delicious vegetable. Medicinally, the leaves of this plant are used to treat scurvy and catarrhal affections. Paste of the leaves is applied externally on wounds. Flowers are useful as tonic, diuretic and cholagogue. The seeds are used as

antipyretic drug. The oil extracted from seeds is used to treat gout and rheumatism (Arshad & Akbar, 2003). The roots of the young tree are vesicant and rubefacient (Bhattacharjee, 2004). In India decoction of the bark is used to cure the malarial fever (Singh & Kumar, 2003). Anti-inflammatory potential of crude methanol extract of the root of the *Moringa oleifera* was evaluated on rat paw edema by Ezeamuzie *et al.* (1996). The results showed that the root of *Moringa oleifera* possess anti-inflammatory activity. The ethanol and ethyl acetate extracts of seeds of *Moringa oleifera* depicted significant antipyretic activity in rats; ethyl acetate extract of dried leaves of the plant showed significant wound healing activity on excision, incision and dead space (granuloma) wound models in rats (Hukkeri *et al.*, 2006). Antipyretic effect of ethanol extract of *Moringa oleifera* leaves determined by Ahmad *et al.* (2006) in DPT induced pyrexia in rats showed significant analgesic and antipyretic effects. Most of the scientific work carried out in recent past is for the determination of biological activities of extracts of different parts of *Moringa oleifera*, which suggest important pharmacological effects of this plant. Therefore, the present study was undertaken to

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determine the antipyretic activity of different doses of the bark of *Moringa oleifera* on *E.coli* induced pyrexia in rabbits.

MATERIAL AND METHOD

Plant material

Bark of *Moringa oleifera* was collected in March, 2007 from Cholistan desert and were identified and confirmed by the Dr Arshad Mehmood plant Taxonomist at the Cholistan institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan. This study was conducted in the College of Conventional Medicine the Islamia University of Bahawalpur, Pakistan. Rabbits were used for study. Five rabbits were in each group. Two groups were used as control groups as a positive control (Paracetamol 50mg/kg administrated) and other as negative control and three groups were used as test group. Outcome measure was reduction in temperature by use of *Moringa oleifera* bark in rabbits

Preparation of hydro-alcoholic plant extract

The hydro-alcoholic plant extract were prepared by the plant material one part and solvent nine parts was used for preparation of plant extract (Banerjee, 2005). The solvent used was ethanol and distilled water (70% alcohol and 30% water). Powder of *Moringa oleifera* bark (100 g) was soaked in 900 ml of solvent (Ethanol 600 ml + Distilled water 300 ml), in a conical flask having capacity of 2 L. The material was soaked for one week and shacked vigorously for 10 min twice a day. The flask was kept in laboratory on room temperature (20°C). Finally the soaked material of each plant was filtered through several layers of muslin cloth one by one for coarse filtration. The coarse filtrate was filtered through a Whatman # 3 filter paper. The filtrates were kept in close neck plastic bottles with tight closure on (20°C) temperature.

Preparation of hydro-alcoholic of Paracetamol

Fifteen grams powder of Paracetamol was soaked in 150 ml of solvent (ethanol 105 ml + distilled water 45 ml), for ten minutes. The container was closely tightened with aluminum sheet for prevention of unwanted evaporation of solvent. The material was shacked vigorously for ten minutes twice a day. The container was kept in laboratory on room temperature (20°C).

Management of animals

The experiment was carried out on albino rabbits. They were 12-14 months old, of both sexes, weighing between 1.5 and 1.6 kg. All rabbits were inbred. All the rabbits were kept in air conditioned animal house located in the College of Conventional Medicine the Islamia University of Bahawalpur. These animals were given grass, bread, maize, wheat grains and water. The experiments were started after one week of acclimation of animals.

Preparation of *E. coli* suspension

The pure and identified cultures of *Escherichia coli* (*E. coli*) were obtained on MacCokeys agar from microbiology laboratory of Quid-e-Azam Medical College, Bahawalpur and incubated 37°C for 24 h. The colonies were counted under the colony counter. One colony picked and washed in normal saline and spread on agar plate for re-culture and incubated for 24 h. These cultures were washed with normal saline and then cultured in nutrient broth by incubating for 24 h. A tenfold dilution of the suspended broth culture was prepared with normal saline. The total number of organisms was calculated by multiplying the number of organisms in one drop to the number of drops in one ml. Total number of *E. coli* in one ml volume was 127×10^7 .

Induction of pyrexia in experimental animals

Pyrexia was induced by injecting of *Escherichia coli* (*E. coli*) suspension, in the marginal ear vein of the rabbit at the concentration of 0.01 ml per kg body weight (Riffat et al., 1982). Rectal temperature was recorded with digital thermometer before and after *E. coli* injection at a regular interval during the experiment.

Drug administration

The pyrexia was produced after 1-2 h injection of *E. coli* suspension. The rectal temperature of animals raised 2-5°F from normal body temperature of animals. The hydro-alcoholic of *Moringa oleifera* bark was administrated orally to animals at the dosage rate of 25 mg, 50 mg and 100 mg/kg body weight dissolved in 3ml of distilled water.

Study protocol

The rectal temperature was recorded with digital thermometer at 0 h and *E. coli* suspension was injected. After one h again rectal temperature of the animals was recorded and hydro-alcoholic extract were administered to the treatment groups and paracetamol hydro-alcoholic 50 mg/kg orally to the positive control group. Then rectal temperature was recorded at the interval of one h for 4 h. Five rabbits were in each group. Two groups were used as control groups as a positive control (Paracetamol 50 mg/kg administrated) and other as negative control.

Antipyretic activity

Fever in rabbits was induced by injecting of *Escherichia coli* (*E. coli*) suspension, in the marginal ear vein of the rabbits at the concentration of 0.01 ml per kg body weight (Riffat, 1982). The animals having temperature were divided into five groups having five animals in each group and treated orally as follows:

Group 1: Positive control: Given *E.coli* suspension

Group 2: Negative control: Given paracetamol (50 mg/kg)

Group 3: Treatment group 1: Treated with plant extract (25 mg/kg)

Table 1: Antipyretic activity of bark of *Moringa oleifera* hydroalcoholic extract on *E. coli* induced pyrexia in rabbits

Treatment	Dose (Mg kg ⁻¹)	Rectal temperature (°F)					
		Injecting <i>E. coli</i> suspension	Drug administration	After drug			
				0 h	1 h	2 h	3 h
Control	25mg	100.6±0.352	103.3±0.322	103.5±0.235	103.7±0.185	104.0±0.180	104.3±0.186
		101.0±0.211	102.8±0.273	100.9±0.200*	101.1±0.162*	101.6±0.163*	102.4±0.254*
Treatments	50mg	100.9±0.436	102.5±0.227	100.5±0.240*	101.2±0.182*	101.5±0.203*	102.3±0.150*
	100mg	101.0±0.305	103.5±0.395	100.4±0.228*	101.0±0.208*	101.5±0.249*	102.0±0.283*
Paracetamol	50mg	101.0±0.417	103.0±0.222	101.2±0.240*	101.4±0.254*	101.9±0.338*	102.6±0.301*

Mean ± SEM, * P<0.05

Group 4: Treatment group 2: Treated with plant extract (50 mg/kg)

Group 5: Treatment group 3: Treated with plant extract (100 mg/kg)

STATISTICAL ANALYSIS

Statistically analysis was performed by using one-way Analysis of Variance (ANOVA) test between two mean groups: control and test groups, followed by Student's t-test at p<0.05 level of significance.

RESULTS

The results with regard to antipyretic activity of hydroalcoholic extract of bark of *Moringa oleifera* bark recorded on *E. coli* induced pyrexia in rabbits are given in table 1. At 0 h, before the injection of the *E. coli* suspension the rectal temperature of rabbits was recorded. In negative and positive control group rectal temperature was 100.6±0.352°F and 101.0±0.417°F. The rectal temperature of animals in 25mg/kg⁻¹ treatment group was 101.0±0.211°F, in 50mg/kg⁻¹ was 100.9±0.436°F and in 100mg/kg⁻¹ treatment group was 101.0±0.305°F. At h1, after the injection of *E. coli* suspension to the rabbits the rectal temperature of the animals in negative control group increased up to 103.3±0.322°F and 103.0±0.222°F in positive control group, while the temperature recorded in the treatment groups was 102.8±0.273°F was in 25mg/kg⁻¹ treatment, 102.5±0.227°F in 50mg/kg⁻¹ treatment and 103.5±0.395°F in 100mg/kg⁻¹ treatment. At h 2, after the administration of plant extract dose to the treatment groups and of paracetamol to positive control group the rectal temperature noted in negative control group was almost same i.e. 103.5±0.235°F, while in positive control group it decreased upto 101.2±0.240°F. Decrease in temperature was also recorded in treatment groups as 100.9±0.200 in 25 mg/kg⁻¹ treatments, 100.5±0.240°F in 50mg/kg⁻¹ treatment and 100.4±0.228°F in 100mg/kg⁻¹ treatment. The rectal temperature of the animals recorded at h 3 remained high in negative control group and low in

positive control group (103.7±0.185°F and 101.4±0.254°F), while the temperature of the animals of treatment groups showed slight increase and reached up to 101.1±0.162°F in 25mg/kg⁻¹ treatment, 101.2±0.182°F in 50mg/kg⁻¹ treatment and 101.0±0.208°F in 100mg/kg⁻¹ treatment. At h 4 the temperature recorded in negative control was increased up to 104.0±0.180°F and in positive control group it remained almost same (101.9±0.338°F). In treatment groups the temperature recorded was 101.6±0.163°F in 25mg/kg⁻¹ treatment, 101.5±0.203°F in 50mg/kg⁻¹ treatment and 101.5±0.249°F in 100mg/kg⁻¹ treatment. The rectal temperature of the animals recorded at h 5 was 104.3±0.186°F in negative control group and 102.6±0.301°F in positive control group. In treatment groups the temperature was 102.4±0.254°F in 25mg/kg⁻¹ treatment, 102.3±0.150°F in 50mg/kg⁻¹ treatment and 102.0±0.283°F in 100mg/kg⁻¹ treatment.

DISCUSSION

The results showed that the hydroalcoholic extract of bark of *Moringa oleifera* possess a significant antipyretic effect in maintaining the normal body temperature and reducing *E. coli* induced pyrexia in rabbits. After the drug administration (at h 1), the decrease in body temperature of rabbits with the dose of 25mg/kg⁻¹ during next four h ranged between 1.9-2.6°F as compared to the negative control. At the dose of 50mg/kg⁻¹ of *Moringa oleifera* the decrease in temperature was 1.9-3.0°F. The decrease in body temperature of animals at the dose of 100mg/kg⁻¹ was high, which ranged from 2.3-3.1°F as compared to negative control. Paracetamol, a standard and analgesic and antipyretic drug, also significantly lowered the temperature of *E. coli* induced rabbits but the dose of the hydroalcoholic extract of *Moringa oleifera* at the concentration of 100mg/kg⁻¹ played a significant role in lowering the body temperature of the animals as compared to the negative as well as positive control. *Moringa oleifera* contains phytosterols, glycosides, tannins, amino acids, phenolic compounds, carbohydrates and amino acids. In general, fever is thought to be

produced by certain endogenous substances including prostaglandins (Kluger 1991; Roth & Zeisberger 1995), therefore the antipyretic action of extract of bark of *Moringa oleifera* may also be related to the inhibition of prostaglandin synthesis. The results of present study also correspond with the findings of Hukkeri *et al.* (2006); Lakshman *et al.* (2006).

CONCLUSION

In conclusion, the results achieved in the present study depicted that the hydroalcoholic extract of the bark of *Moringa oleifera* have significant antipyretic activity, particularly in the increased dose of hydroalcoholic extract (100mg/kg^{-1}). The results affirm the claim by local herbal practitioners of the area which use this plant to cure fever in humans. However, further studies are proposed to fully elucidate the mechanism of the extract of the plant bark.

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