

Antipyretic effects of hydro-methanol extract of *Melia azedarach* Linn. seeds and *Cucumis melo* Linn. seeds in experimental rabbits

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Abstract: To investigate the antipyretic activity of hydro-methanol extract of *Melia azedarach* Linn. (HMEMA) seeds and *Cucumis melo* Linn. (HMECM) seeds in experimental animals. Baker's yeast was used to induce fever in rabbits which were divided into six groups. The animal groups were thereafter administered distilled water (control), paracetamol (reference standard, 150mg/kg), HMEMA (250mg/kg), HMEMA (500mg/kg), HMECM (250mg/kg) and HMECM (500mg/kg) respectively. HMEMA and HMECM were also phytochemically screened for tannins, alkaloids, phenols, flavonoids, saponins and cardiac glycosides. Results indicate that hydro-methanol extract of *M. azedarach* Linn. Seeds (250mg/kg and 500mg/kg) significantly ($p < 0.001$, $p < 0.05$ respectively) reduced the elevated body temperature in dose dependant manner. Insignificant to no antipyretic effect was produced by hydro-methanol extract of *Cucumis melo* L. seeds. Phytochemical analysis of the HMEMA showed the presence of flavonoids, saponins, tannins, phenols, alkaloids and cardiac glycosides While HMECM was positive for flavonoids, phenols and saponins. The result shows that there exists a potential benefit in utilizing *Melia azedarach* L. seeds in treating fever. This property can be attributed to the presence of phytochemical constituents present in the hydro-methanol extract of *Melia azedarach* L. seeds and the exact mechanism need to be evaluated.

Keywords: Medicinal plants, antipyretic activity, Baker's yeast, hydro-methanol extract, phytochemical screening.

INTRODUCTION

Fever, also called pyrexia is a complex physiologic process characterized by elevated body temperature above normal range, accompanied with increased pulse, increased tissue destructions, chills, aches, restlessness and other symptoms (Sandhar *et al.*, 2003). Fever can be caused by any factor that effect the temperature-regulating centre, any abnormality in the brain itself, bacterial diseases, brain tumors and environmental conditions like heatstroke. Hypothalamus keeps the body temperature at a set point and it regulates the temperature time to time either by increase in body temperature (through increase muscles tone and shivering) or prevention of heat loss by vasoconstriction (Guyton, 1998). There is controversy regarding the beneficial effects of fever; however high temperature is considered a medical emergency due to its serious side effects such as intracranial haemorrhage, sepsis, thyroid storm, serotonin syndrome and Kawasaki syndrome. The search for an ideal antipyretic herbal drug is never ending challenge. Therefore it is worth searching for herbal materials that have potential antipyretic activity with less toxicity and are free from side effects and acting as substitutes of synthetic drugs such as paracetamol (Balunas *et al.*, 2005).

The *Melia azedarach* Linn. commonly known as Bakayain

belongs to family Maliaceae is a large ever green tree native to tropical Asia. It is widely distributed in Pakistan, India, Indonesia, Southeast Asia and Australia. *M. azedarach* L. used extensively against intestinal worms, in skin diseases, stomach ache, intestinal disorders, uterine illnesses, cystitis, diuretic and febrifuge (Sultana *et al.* 2013). It seeds are considered anthelmintic, aphrodisiac and are useful in typhoid fever, also prescribed in rheumatism. Seed oil is used in skin disease (Sultana *et al.*, 2014). The plant is known to possess terpenoids, Flavonoids such as Apigenin- 5-O- β -D-glactopyranoside, Rutin Steroids such as β -sitosterol, stigma sterol, Anthraquinone like 1, 3, 5, 8-Tetrahydroxy-2-methyl anthraquinone (Sultana *et al.*, 2014).

Cucumis melo L. commonly known as Kharboza in Hindi and musk melon or cantaloupe in English belongs to Cucurbitaceae family. Musk melon is growing in tropical and subtropical areas of the world (Asif *et al.*, 2014). *Cucumis melo* L. known to possess analgesic, anti-inflammatory, antiplatelet, anthelmintic, antifertility, diuretic and febrifuge activities. Musk melon composed of many volatile compounds, biosynthetically derived from fatty acid, carotenoids, amino acid and terpenes. The volatile constituent's presents are Methyl acetate, Ethanol, Ethyl butanol, Eugenol. It contain terpenoids such as β pinene; 1,8-Cineol; Limonene; p-Cymene etc. Non volatile constituents such as β Carotenes, Flavonoids, Carbohydrates, Amino acids, Phenolic glycosides (Asif *et al.*, 2014).

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To the best of our knowledge, there is no report regarding the antipyretic activity of the hydro-methanol extract of the seeds of *Melia azedarach* L. and seeds of *Cucumis melo* L. The aim of the present work, therefore, was to evaluate the hydro-methanol extract of *M. azedarach* L. and *C. melo* L. seeds for their antipyretic activity.

MATERIALS AND METHODS

Animals

In this study adult healthy rabbits 1000-1200gm of either sex were obtained from laboratory animal centre, The Islamia University Bahawalpur. All the animals were maintained in air conditioned animal house located in the faculty of Pharmacy and Alternative Medicine, The Islamia University Bahawalpur. They were fed with standard rodent diet. The animals were acclimatized in an environment of controlled temperature 22-25°C and light/dark 12h/ 12h cycle for seven days prior to study. All the animals were fasted one hour before drug treatment. Food and water was continued after the administration of drug.

Plant materials

The seeds of *Melia azedarach* L. were collected from Bahawalpur City during the month of March and samples of *Cucumis melo* L. were purchased from the local market of Bahawalpur. Both the specimens were identified by Dr. Shazia Anjum Director of Cholistan Institute of Desert studies, The Islamia University of Bahawalpur. The voucher specimen of *M. azedarach* seeds (3414/CIDS/IUB) and *Cucumis melo* L. seeds 3418/CIDS/IUB were deposited in the herbarium of CIDS, The Islamia University of Bahawalpur.

Preparation of extract

The seeds of *Melia azedarach* L. were separated, cleaned and washed with distilled water and dried under shade at temperature between 21-30°C for 30 days. The *C. melo* L. seeds were washed to removed all the external dirt and unwanted material, shade dried for 72h. 500gm of powdered material of *Melia azedarach* L. and *Cucumis melo* L. seeds were soaked in 2 liter of 70% methanol in a beaker for 72 hour followed with occasional shaking and stirring. The soaked material of each plant was filtered through several layers of musclien cloth one by one for coarse filtration. The filtrate was filtered through a whattman # 1 filter paper. The residues were extracted thrice with the same fresh solvent and extract combined. The filtered extracts were concentrated under reduced pressure at 40°C, rotary evaporator. The crude extract so obtained were weighed to calculate the yield of *M. azedarach* L. seeds (18.6%w/w) and *C. melo* L. seeds (3.9%w/w) and were stored in a refrigerator (-8°C), until used for analysis.

Drugs and chemicals

Paracetamol (Islamia University Bahawalpur; GlaxoSmithKline, Pakistan, Limited), Methanol (Merk KGaA Darmstadt, Germany), Normal Saline (Siza International Lahore, Pakistan), Distilled water (Department of pharmacy, IUB, Pakistan), Baker yeast (Rossmoor food products, Karachi, Pakistan).

Phytochemical screening

The hydro-methanol extract of *M. azedarach* L. seeds and *C. melo* L. seeds were subjected to standard phytochemical screening for flavonoids (sodium hydroxide, ferric chloride and lead acetate test), alkaloids (Dragondroff,s and Mayer's test), Tannins (Ferric chloride test), Saponins (Foam test) as described by (Harborne 1973). Phenols (Ferric chloride test), Cardiac glycosides (Chloroform and sulphuric acid test) described by (Rasool *et al.*, 2010; Sofowora 1993) respectively.

Antipyretic activity

Yeast induced pyrexia method

Antipyretic activity was performed by slightly modifying the method described by (Bose *et al.*, 2007). Rabbits of either sex weighing 1000-1200gm were divided in to six groups of six rabbits each. The rectal temperature of all the rabbits was measured with digital thermometer before the administration of yeast suspension. Pyrexia was induced by subcutaneously injecting 3ml/kg/bodyweight of 10% w/v yeast suspension in the back of the rabbits. After the 18 hours, of the administration of yeast suspension temperature was measured. The rabbits showed 0.5°C to 1.5°C increase in temperature were selected for the study. Group I is negative control and received distilled water. Paracetamol at a dose of 150mg/kg body weight was given orally to positive control group II. Hydro-methanol extract of *Melia azedarach* L. seeds at a dose of 250 and 500mg/kg body weight were administered to group III and IV respectively, while *Cucumis melo* L. seeds extract at a dose of 250mg/kg and 500mg/kg was administered to group V and VI respectively. The rectal temperature was recorded at the interval of one hour for 6 hours.

STATISTICAL ANALYSIS

Values are expressed as mean $\pm$ SEM. Statistical significance was determined by ANOVA one way analysis of variance followed by LSD post hoc test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of hydro-methanol extract of *Melia azedarach* L. seeds gave positive test for alkaloids, flavonoids, saponins, tannins and phenols (table I). While *C. melo* extract gave positive test for saponins, phenols and flavonoids (table 1).

Table 1: Results of phytochemical analysis of hydro-methanol extract of *Melia azedarach* L. seeds and *Cucumis melo* L. seeds

Test Sample	Alkaloids	Tannins	Flavonoids	Saponins	Phenols	Cardiac glycosides
<i>Melia azedarach</i> seed	+++	++	+++	+++	++	-
<i>Cucumis melo</i> seeds	-	-	++	++	++	-

(++) Present; (-) Absent; (+++) Highly present

Table 2: Antipyretic effect of hydro-methanol extract of *Melia azedarach* L. seeds and *Cucumis melo* L. seeds against baker yeast induced pyrexia in rabbits

Nos.	Treatment	Rectal Temperature °C		Rectal temperature °C after drug administration					
		-18hr	0hr	1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr	6 th hr
Groups	Dose mg/kg								
Control	-	38.37 ±0.06	40.13 ±0.02	40.03 ±0.02	40.00 ±0.00	39.86 ±0.03	39.70 ±0.07	39.65 ±0.06	39.7 ±0.06
Paracetamol	150mg/kg	38.38 ±0.07	40.05 ±0.04	39.42 ±0.04**	38.93 ±0.04**	38.57 ±0.06**	38.53 ±0.06**	38.33 ±0.04**	38.15 ±0.02**
MASE	250mg/kg	38.77 ±0.17	40.07 ±0.03	39.58 ±0.06**	39.17 ±0.06**	38.92 ±0.08**	38.73 ±0.05**	38.53 ±0.05**	38.38 ±0.06**
MASE	500mg/kg	38.90 ±0.19	40.08 ±0.04	39.80 ±0.06*	39.70 ±0.06	39.50 ±0.12*	39.26 ±0.14	38.88 ±0.07**	38.76 ±0.07**
CMSE	250mg/kg	38.57 ±0.17	40.12 ±0.04	40.15 ±0.05	39.97 ±0.03	39.63 ±0.08	39.37 ±0.08	39.00 ±0.04**	38.87 ±0.10**
CMSE	500mg/kg	38.77 ±0.10	40.05 ±0.09	40.0 ±0.06	39.88 ±0.30	39.72 ±0.06	39.75 ±0.06	39.73 ±0.04	39.53 ±0.02

Values are expressed as mean± SEM N= 6, Multiple comparison between treatment groups were performed by LSD Test. *P<0.001, **P<0.0001, when compared with the control value of corresponding hours. -18hr; the initial temperature of rabbits before the administration of yeast suspension, 0hr; temperature at the time of drug administration. MASE (*Melia azedarach* seeds extract); CMSE (*Cucumis melo* seed extract)

Antipyretic activity

The initial rectal temperature of untreated rabbits was in the range of (38.17°C-38.57°C). 18h after the administration of yeast suspension, the rectal temperature was elevated by 1.00°C-1.5°C in all the groups, indicating the onset of fever. Treatment with paracetamol significantly (p<0.0001) reduced the rectal temperature during all periods of observation compared to control group. The treatment group of *M. azedarach* seeds extract 250mg/kg reduce the rectal temperature highly significantly (p<0.0001) during the all period of observation compared to control and significantly (p<0.01) reduce temperature compared to positive control (Paracetamol) as shown in fig. I. The treatment group of *M. azedarach* seeds extract 500mg/kg reduced the rectal temperature significantly during all period of observation as shown by the (table II). However, the effect of the extract 500mg/kg was slow compare to that of extract 250mg/kg. The treatment groups of *C. melo* seeds extract 250mg/kg did not reduce the rectal temperature during the first three hours of observation. However, significant (p<0.05) reduction in rectal temperature was observed during the third and fourth hour of observation period compare to control.

The treatment group of *C. melo* seeds extract 500mg/kg did not reduced the rectal temperature significantly during

all period of observation as shown by the (table 2) and (fig. 1).

DISCUSSION

Fever or pyrexia is a complex physiologic processes triggered by infectious or aseptic stimuli. Increase in body temperature occurs when concentration of Prostaglandin E2 (PGE2) increases in certain area of brain. These prostaglandins alter the firing rate of neurons that control the thermoregulatory mechanism in the hypothalamus (Shah *et al.*, 2010). The ability of HMEMA to reduce the experimentally elevated temperature shows that it possesses significant antipyretic effect in yeast induced pyrexia. The reduction in the temperature might be due to its influence on the biosynthesis of prostaglandin, as it is involved in the regulation of body temperature (Dascombe *et al.*, 1985). Generally it is believed that several mediators play a vital role in the pathogenesis of fever. Antipyretic inhibits these mediators and is said to bring about anti-pyresis effect and how they interfere in the biosynthesis of PG is not clearly established (Akio *et al.*, 1985).

The available antipyretic drugs such as Paracetamol, Nimusulide and aspirin present a wide range of side effect as they inhibit none selectively cyclooxygenase COX1

and cyclooxygenase COXII (Vane *et al.*, 1995). By the activation of cyclooxygenase, the level of prostaglandin (PGE₂) markedly increase and its production provoke fever (Dannhardt *et al.*, 2001). Fever induction studies in experimental animals have shown that expression of COX II enzyme in the vasculature of the brain is the cause for the onset of fever (Matsumura *et al.*, 1998). Therefore we assume that some active metabolites present in the HMEMA could have played a role in the inhibition of cyclooxygenase activity. Phytochemicals such as flavonoids, alkaloids, saponins, tannins and anthraquinones have been reported to possess antipyretic activity in experimental animals (Tariq *et al.*, 1989) and the present study also reports the presence of these constituents in HMEMA. Further study is required to identify the active principle responsible for the antipyretic activity of *M. azedarach* seeds and its mechanism of action.

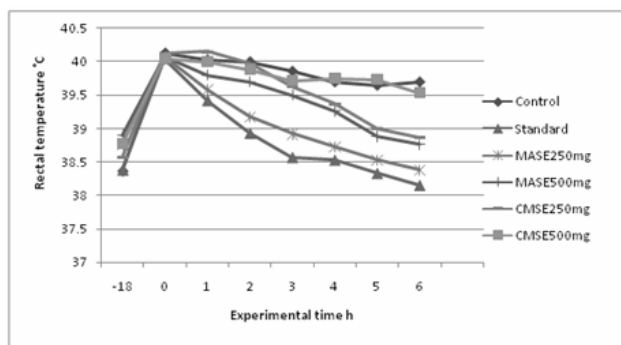


Fig. 1: Effect of antipyretic activity challenged against baker yeast induced pyrexia was observed in control (Distilled water), Standard drug (Paracetamol 150mg/kg b.wt.) and doses (250mg/kg b.wt. and 500mg/kg b.wt.) of extract *M. azedarach* L. and *C. melo* L. MASE (*Melia azedarach* seed extract); CMSE (*Cucumis melo* seed extract) -18hr; the initial temperature of rabbits before the administration of yeast suspension, 0hr; temperature at the time of drug administration.

Hydro-methanol extract of *Cucumis melo* L. seeds did not give appreciable antipyretic activity, although the extract was positive for saponins and flavonoids. It might be assumed that those flavonoids responsible for the antipyretic activity may not be present in the HMECM. However it must be evaluated by using other fractions.

CONCLUSION

Over all the results of the present study lead to conclude that the hydro-methanol extract of *Melia azedarach* seeds, at a dose of 250mg/kg possesses significant antipyretic activity against yeast induced pyrexia in animals. The effect was similar to standard drug paracetamol 150mg/kg.

However detailed phytochemical study is required to fractionate, purify and identify the structure of the active principle present in these extracts responsible for antipyretic activity of these plants and also the mechanism of action of the active principle.

ACKNOWLEDGEMENTS

The authors would like to express sincere thanks to Dean and Chairman of University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine providing support and necessary facilities for research.

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