Antipyretic potential of herbal coded formulation (Pyrexol)

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Abstract: The antipyretic effect of the aqueous extract of herbal coded formulation containing equal amount of *Salix alba, Emblica officinalis, Glycyrrhiza glabra, Adhatoda vasica, Viola odorata, Thea sinensis, Veleriana officinalis, Foeniculum vulgare, Sisymbrium irrio* and *Achillea millefolium* was investigated using the yeast induced pyrexia model in rabbits. Paracetamol was used as a control group. Rectal temperatures of all rabbits were recorded immediately before the administration of the extract or paracetamol and again at 1 hour, after this, temperature was noted at 1 hrs interval for 5 hrs using digital thermometer. At 240mg/kg dose the extract showed significant reduction in yeast-induced elevated temperature as compared with that of standard drug paracetamol (150mg/kg). It is concluded that herbal coded medicine at a dose of 240mg/kg has marked antipyretic activity in animal models and this strongly supports the ethno pharmacological uses of medicinal plants of this formulation.

Keywords: Yeast, analgesic, anti-inflammatory, prostaglandin, yeast induced pyrexia, traditional, medicine.

INTRODUCTION

Pyrexia is the elevation of body temperature above the normal range. It may be caused by abnormalities in the brain itself or by toxic substances that affect the temperature regulating centers (Varghese *et al..*, 2010). Several analgesic drugs are used to treat pyrexia but they exert side effects.

Therefore, there is need to search new drugs with fewer or no side effects. For this purpose, medicinal plants have been investigated for their antipyretic activity. The antipyretic effect of the aqueous extract of herbal coded medicine containing equal amount of *S. alba*, *E. officinalis*, *G. glabra*, *A. vasica*, *V. odorata*, *T. sinensis*, *V. officinalis*, *F. vulgare*, *S. irrio* and *A. millefolium* was investigated using the yeast induced pyrexia model in rabbits. Literature review of medicinal plants under investigation is mentioned. *T. sinensis* belongs to family Theaceae. It has been reported to be stimulant, cure flatulence (gas), regulate blood sugar and body temperature, improve mental processes and alleviate urinary problems. Several studies using modern techniques have authenticated its use as hypoglycemic, anti-inflammatory, antipyretic, anti cancer, anti-allergic, hepatoprotective, anti carcinogenic, anticataractogenic, antimicrobial and hypolipidemic (Gupta *et al.*, 2010). *G. glabra* (Liquorice) has been used in Europe since prehistoric times. It is well documented in written form starting with the ancient Greeks. Liquorice roots contain major active constituent Glycyrrhizin that is used in many herbal formulations for the management of liver disorders. The plant is prescribed as hypoglycemic, antiulcer, antidepressive, laxative, spasmolytic and anti-inflammatory agent (Dastagir *et al.,* 2016; Thiyagarajan *et al.,* 2011). *S. alba* belongs to family Salicaceae. *S. alba* contains a range of chemical constituents including salicin that is metabolically transformed in the body to the aspirin metabolite, salicylic acid. Salicylates have anti-inflammatory effects in the body. *Salix* bark extract exhibited analgesic effect in patients with osteoarthritis and proved effective in the treatment of bodyache. Ethanolic extracts of *S. alba* is also used as an anti-inflammatory, antipyretic and analgesic agent (Eisenberg *et al.,* 2000; Heide *et al.,* 2000; Joseph *et al.,* 2010). *E. ribes* Burm. f. belongs to family Myrsinaceae. *E. ribes* Burm commonly known as Baobarang, is a large woody climbing shrub and is found throughout India and Pakistan. It is used in the treatment of intestinal worm infestations. *E. ribes* is also used as anti-fertility agent. Analgesic activity of embelin and its derivatives has also been reported. It is used as an anti-inflammatory agent in the treatment of pyrexia and rheumatism. The fruit treats mental disorders, jaundice, bronchitis, ascites and tumors. It is antidiabetic, antidyslipidemic and antioxidant (Ibrahim *et al.,* 2010). Sweet viol *et al* so known as the *Viola odorata*, blooms in continental climate conditions in early spring with delicate flowers of attractive scent. It has been traditionally the part of various indigenous preparations and being used to cure respiratory and inflammatory conditions (Naveed *et al.,* 2013). *V. odorata* contains vitamin C, mucilage, methyl salicylate, saponins, glycosides, and alkaloid. Traditionally *V. odorata* is worthwhile to cure Jaundice. *V. odorata* has shown antibacterial, anti-inflammatory, antipyretic and hepatoprotective activity (Mittal *et al.,* 2015). *A.*

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millefolium belongs to family Asteraceae. *A. millefolium* has an anti-inflammatory, antipyretic and analgesic effect. *A. millefolium* had been used in popular medicine for its wound healing, analgesic and anti-hemorrhagic effects. People living in North America and Northern Europe use it as emmenagogue, abortifacient and contraceptive. The most active fraction is present in the flower heads of *A. millefolium* that posses anti-inflammatory activity (El-Sadek *et al*., 2007). *S. irio* is an annual herb of family Brassicaceae found in different areas of Pakistan. *S. irio* is prescribed to treat chest congestion, cough, rheumatism, swelling and wounds. *S. irio* has many uses in folk medicine in treatment of arthritis and inflammation. Seeds are used as febrifuge and expectorant. *S. irio* is prescribed to treat voice disorders. *S. irio* has antioxidant, antimicrobial, analgesic and antipyretic potential (Sumaira *et al.,* 2013). *A. vasica* belongs to family Acanthaceae. It is used in chronic bronchitis, cough, bodyache and inflammation. It is bronchodilator, anti-asthmatic and anti-tussive (Duraipandiyan *et al.,* 2015). *A. vasica* has been reported as anti-inflammatory agent (Singh *et al.,* 2013). *F. vulgare* (Apiaceae) commonly known as fennel is a well known and important aromatic and medicinal plant widely used as diuretic, lactagogue, digestive and carminative and in treating gastrointestinal and respiratory disorders. The major phytoconstituents of this species are fenchone, estragole, trans-anethole, phenolic glycosides and phenols. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the potential of *F. vulgare* to exhibit antifungal, antibacterial, antioxidant, antithrombotic and hepatoprotective activities, lending support to the rationale behind several of its therapeutic uses. Phenolic compounds isolated from *F. vulgare* are considered to be responsible for its antioxidant activity while the volatile aroma compounds make it an excellent flavouring agent (Manzoor *et al*., 2012). The aim of current study was to investigate the antipyretic effect of the aqueous extract of herbal coded formulation.

MATERIAL AND METHODS

Plant material

Plants materials were purchased from Judia market Karachi and were identified and confirmed by the Prof. Dr. Khan Usmanghani, Visiting Professor, Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan. This study was conducted in Herbion Pharmaceutical Pvt Limited Karachi. There were total twenty four rabbits with four groups. Each group had six rabbits. 1st group was used as negative control (Yeast suspension 1ml/kg) and 2nd group was used as test group (Plant extract 110mg/kg). 3rd group was also used as test group (plant extract 240mg/kg). 4th group was used as positive control (Paracetamol 150mg/kg administrated). Outcome measure was reduction in temperature by use of test drug in rabbits.

Preparation of aqueous plant extracts

The aqueous plants extracts were prepared by soaking powder of plants (100g) in 900ml of distilled water, 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 medicinal plants 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 aqueous extract of paracetamol

Fifteen grams powder of paracetamol was soaked in 150 ml of distilled water, 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 (800 – 1000 g; n=24) of either sex were obtained from animal house facility of Herbion Pak. Pvt. Ltd. They were housed under standard environmental conditions i.e. 25±1°C and 12h dark / light cycle. Food and water were available *ad libitum*. All rabbits were inbred. All the rabbits were kept in air conditioned animal house located in Herbion Pharmaceutical Pvt. limited, Karachi. These animals were given grass, bread, maize, wheat grains and water. The experiments were started after one week of acclimation of animals.

Yeast induced pyrexia in rabbits

Before inducing pyrexia, the initial rectal temperatures of rabbits were recorded using a digital thermometer. Care was taken to insert thermometer to the same depth each time (about 6 cm). Pyrexia was induced by intraperitoneal injection of 20% yeast suspension (1ml/kg) into the animals. One hour after the yeast injection, the rectal temperature of each rabbit was measured using a digital thermometer, animals with increase of 1°C temperature were selected for the study. After induction of pyrexia, the anti-pyretic product was administered orally (110mg/kg & 240mg/kg) to rabbits, and the temperature was measured at 1, 2, 3, 4 and 5hr after drug administration (Jude, 2010).

Lethal dose $_{50}$ (LD_{50})

Rabbits of either sex (n=10 /sex) were treated orally with doses (1 or 5g/kg) of test product. Mortality and behavioral changes were observed for 1 week.

RESULTS

The results with regard to antipyretic activity of test drug recorded on yeast induced pyrexia in rabbits are given in

Table 1: Antipyretic effect of extract and paracetamol

Groups	Dose (mg/kg)	Rectal temperature (°C)						
		Before treatment		After treatment				
		BBT	0	1	2	3	4	5
Control	-	99.2	100.4	100.8	100.9	100.9	101	101
Extract	110mg/kg	100.133	102.05	100.15	99.45	99.066	99.733	99.05
Extract	240mg/kg	100	101.33	100.1	100	99.9	99.58	99.36
Paracetamol	150mg/kg	99.4	102	101	99.5	99	99	98.7

table 1. At base line, before the injection of the yeast suspension, the rectal temperature of rabbits was recorded. In control group rectal temperature was 99.4F. The rectal temperature of animals in 110mg/kg treatment group was 101.133F, in 240mg/kg was 100F. At 0 hour, after the injection of yeast suspension to the rabbits the rectal temperature of the animals in control group increased up to 102F, while the temperature recorded in the treatment groups was 102.05F in 110mg/kg treatment, 101.33F in 240mg/kg treatment. Rectal temperature decreased 100.15, 99.45, 99.066, 99.733 and 99.05F at 1h, 2h, 3h, 4h and 5h interval respectively in 110mg/kg treatment group. Rectal temperature decreased to 100.1, 100, 99.9, 99.58 and 99.36F at 1h, 2h, 3h, 4h and 5h interval respectively in 240mg/kg treatment group. Rectal temperature decreased up to 101, 99.5, 99, 99 and 98. 7F at 1h, 2h, 3h, 4h and 5h interval respectively in paracetamol treated group. The test product did not cause mortality in rabbits at the given doses of 1 or 5g/kg. Other signs of toxicity like hair loss and weight reduction were also not observed. The behavioral changes exhibited tail erection indicating that the product is stimulant at high doses.

DISCUSSION

Acute toxicity study indicates that this formulation can be considered a nontoxic one. The results showed that the test drug extract possess a significant antipyretic effect in maintaining the normal body temperature and reducing yeast induced pyrexia in rabbits and their effects are comparable to standard drug paracetamol. Such reduction of rectal temperature of the tested animals by test drug at dose of 110 and 240mg/kg appears to be presence of bioactive compounds present in them. Effect of test product on temperature in rabbits is presented in table 1. The intraperitoneal injection of yeast suspension markedly elevated the rectal temperature after 1 hour of administration. Treatment with test product at a dose of 110, 240mg/kg decreased the rectal temperature of the rabbits in dose dependent manner. Both the standard drug paracetamol (150mg/kg) and tested extract (110, 240 mg/kg) significantly reduced the yeast-elevated rectal temperature, at 1hrs, 2hrs, 3hrs, 4hrs and 5hrs. Pyrexia and inflammation are indicatives of various disorders. Modern medicines are available for treatment of pyrexia, but they have side effects. Several studies are ongoing Worldwide to search natural antipyretic agents with better efficacy and fewer or no side effects. The present study was aimed at investigating antipyretic activity and toxicity of test drug in rabbits against yeast induced pyrexia. Rectal temperature was recorded with digital thermometer at base line and yeast suspension was injected. After 1 hour again rectal temperature of the animals was recorded and extracts were administered to the treatment groups and paracetamol 150mg/kg orally to the positive control group. Then rectal temperature was recorded at the interval of one h for 5h. After the drug administration (at 1 hour), the decrease in body temperature with the dose of 110mg/kg during next 5 h ranged between 1.9-3^{o}F. At the dose of 240 mg/kg the decrease in temperature was 1.23-1.97^{o}F. Paracetamol, a standard drug, significantly lowered the temperature ranged from 1-3.3^{o}F at the concentration of 150mg/kg. In previous studies, *T. sinensis, V. officinalis, F. vulgare, S. irrio* and *A. millefolium* has been reported as antipyretic and anti-inflammatory agents (Chattopadhyay *et al.*, 2004; Ali *et al.*, 2014; Badgujar *et al.*, 2014; Al-Jaber, 2011; Benedek *et al.*, 2007). In current study, test drug containing *S. alba, E. officinalis, G. glabra, A. vasica, V. odorata, T. sinensis, V. officinalis, F. vulgare, S. irrio* and *A. millefolium* has marked antipyretic activity in animal models and this strongly supports the ethno pharmacological uses of medicinal plants used in test drug as an antipyretic plant.

CONCLUSION

In conclusion, the results achieved in the present study depicted that test product containing equal amount of *S. alba, E. officinalis, G. glabra, A. vasica, V. odorata, T. sinensis, V. officinalis, F. vulgare, S. irrio, A. millefolium* has significant antipyretic activity, particularly in the increased dose of extract (240mg/kg). Test product is safe alternative because it exhibits significant potential in reducing fever with minimum or no side effects as compared to paracetamol. The results affirm the claim by physicians who use these plants to treat fever in humans. However, further studies are proposed to fully elucidate the mechanism of the extract of the test product.

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