EVALUATION OF HYDROETHANOLIC EXTRACT OF *OPUNTIA MONACANTHA HAW* CLADODES FOR ANTIPYRETIC ACTIVITY

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ABSTRACT
*Opuntia monacantha Haw* is a small tree with fibrous roots belongs to family of Cactaceae, having great potency in treating certain common diseases like fever, urinary problem, inflammations, tumors, piles, ulcers, anemia, and bronchitis traditionally. Previously studies showed that the *Opuntia monacantha Haw* has good effect against diabetes, microbes and oxidative stress, however no work was carried out for in vivo antipyretic activity. The present study was made to investigate the hydroethanolic extract of *Opuntia monacantha Haw* cladodes for antipyretic activity, compared to that of Aspirin as a reference drug.

Pyrexia was induced by s/c injection of aqueous suspension of yeast 20% w/v in normal saline at the rate of 10ml/kg body weight. The experiment was done at the doses of 200mg/kg, 400mg/kg and 800 mg/kg B/w. Rectal temperatures were recorded for 4 hrs by digital thermometer (Holden medical B.V, Netherlands). The result showed that *Opuntia monacantha Haw* has strong p< 0.01 antipyretic activity and this strongly support the ethanopharmacological uses of *Opuntia monacantha Haw* as antipyretic agent.

Keywords: Opuntia monacantha Haw, Yeast-induced pyrexia, Antipyretic, Aspirin.

INTRODUCTION
Medicinal plants are important source of new chemical substances with potential therapeutic effects (*Akuodor, G e.t al. 2012*). Approximately, 250,000-500,000 species of plants are present. Only 1% are studied phytochemically, which shows that there is great potential for discovering new compounds. Compounds such as carbohydrates, terpenoids, enzymes, fats, proteins, oils, minerals, alkaloids, quinones, vitamins, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, and saponins are derived from plants (*Olowokudejo, J., et. al.2008*). About 80% of the remote area populations rely on the traditional medicines for their
health (Kim, H.S 2005). Research on plants continues on identification and isolation of active ingredients, rather than to study medicinal properties of whole plant (Iwalewa, E., et. al. 2010). About 6000 species of flowering plants are existing in Pakistan, from which almost 400-600 are consider being medicinally important.

The genus Opuntia monacantha Haw” commonly known as dropping prickly peer, belongs to family of Cactaceae (Bari, M.N., Zubair M, Rizwan K, 2012). The plant is native to South America, but also grows in Australia, Asia, India, South Africa and Spain (Hanelt, P. 2005). A number of active chemical compounds such as tannins, phenol, terpenoids and saponins have been found in genus and exhibits antidiabetic, antimicrobial and neuroprotective properties. The plant is a shrub or small tree with erect fibrous roots growing to a height of 5m. Stems are grey green to light green. The whole plant is about 45cm long, 15cm wide and 1.5cm thick. Flowers are yellow, which are 6cm wide with red spots on the back. The cover of fruits are red, soft and contains seeds that are yellow or pale brown in color (T.K. Lim 2012). The cladodes of Opuntia monacantha Haw are known to possess remarkable medicinal properties. The literature review shown that ethanolic extract of plant has significant in vivo hypoglycemic effect in streptozocine induced diabetic rats (Zhao, M., kuin S, tony E, 2007). In Ayurvedic system of medicine various parts of plant are used, the local use includes laxative, carminative and fever. The plant is useful in the treatment of urinary problems and provides an effective treatment for inflammations, tumor, piles, ulcers, anemia and enlargement of the spleen (Ahmad, S.S 2007). The flowers has been claimed to be beneficial against bronchitis and asthma (Oils, E., et. al. 1998). Traditionally, the juice of the plant is used in ophthalmic, liver complaints, cough and expectorant (Jabeen, A., et al 2010). The cladodes of Opuntia monacantha Haw possess potential antioxidant activity (Yang, N., et al 2012). However no in vivo antipyretic activity on Opuntia monacantha Haw has been reported, hence, present study was undertaken to determine the in vivo antipyretic activity of the hydroethanolic extract of Opuntia monacantha Haw by yeast induced pyrexia in Balb C mice.

MATERIALS AND METHODS

Chemicals
Acetyl salicylic acid (made up of waku pure chemical industries, Osaka Japan), Yeast Saccharomyces cerevisiae, (Emman food products, Islamabad), Digital thermometer (Holden medical B.V, Netherland), Digital weighing balance, Absolute ethanol.
Test animals
The animals used in the study were Balb C mice (25-35gm), within age 3-4 week of either sex. The animals were kept in cages at animal house, and maintained at room temperature of 25°C±2°C with relative humidity (60 ± 10%) under 12 hrs night and light cycle. All the animals were kept at overnight fasting before to every experiment. The animals used were approved according to animal ethics committee Riphah International University Islamabad, Pakistan.

Collection and Extraction of Plant
The whole plant of “Opuntia monacantha Haw” was collected from District Malakand region of the Khyber Pukhtoon Khwa Province, and was identified by Dr. Ikramullah (Department of Botany Agriculture University, Peshawar) with voucher specimen no. BOT: 335. After washing the cladodes, covered with cloth and dried in shade for 20 days at room temperature. The cladodes were grinded through mechanical grinder to coarse of powder. The powdered of plant material (200gm) was extracted through maceration technique using 80% ethanol (1:5) as a solvent for 72 hours at room temperature with occasional manual shaking. After maceration, the mixture was then filter through Whatman #1 paper in a flask and tightly capped. The extract was then concentrated under reduce pressure through rotary evaporator (N-10000, EYELA, Japan) and then air dried.

Acute toxicity study
Acute toxicity study of the extract was determined on Balb C mice of either sex. The dose of extract was increased one to three folds to determine the safety level of the extract. The mice were divided into three groups each contained two mice. The first group received only normal saline and the second and third groups received i.p injection of tested drug at doses of 1000mg/kg and 3000mg/kg. After administration of doses mice were observed for 72 hrs for any toxic effect.

ANTIPYRETIC ACTIVITY
The antipyretic effect of the hydroethanolic extract of Opuntia Monacantha Haw was determined on Balb C mice (25-35gm). The animals were divided into five groups contained six mice in each. The normal body temperature of each mouse was recorded by using digital thermometer, inserted in rectum at predetermined interval. Fever was induced by subcutaneous injection of yeast 20% w/v in normal saline at the rate of 10ml/kg body weight. 17 hrs after the injection of yeast, the rectal temperature of each animal was again recorded.
by digital thermometer. Only those animals that show a minimum increased of 0.7°C in temperature after injection of yeast were taken for experiments. Aspirin (100mg/kg, i.p) was used as reference drug. Group 1st received only (10ml/kg) normal saline i.p, group 2nd received Aspirin (100mg/kg) as a reference drug. While group 3rd, 4th and 5th group received 200, 400 and 800mg/kg B/w of HEOM respectively. After drug administration rectal temperature of each animal were then recorded following at 0, 1, 2, 3 and 4 hrs by digital thermometer. Significant decrease in fever in tested animals was compared to control group (Adams, S.S., Hebborn, P. and Nicholson 1998).

Statistical analysis
Results were expressed as mean ± SEM. The statistical significance between control and treated groups were performed using analysis of variance ANOVA test. For multiple comparisons among the groups Bonferroni test was performed. A probability level of p < 0.05, p < 0.01 and p < 0.001 was accepted statistically significant

RESULTS
The present study was performed to find out the antipyretic effect of the hydroethanolic extract of Opuntia monacantha Haw. The result of the present study showed that the hydroethanolic extract of Opuntia monacantha Haw has significant antipyretic effect with a reasonable safe profile.

Acute toxicity Study
The hydroethanolic extract of Opuntia monacantha Haw was tested at two doses, 1000mg/kg and 3000mg/kg for toxicity and then compared with control (normal saline group). No major behavioral changes or mortality were noted post administration of the extract at dose of 1000mg/kg for 72 hrs. While mortality was observed at the dose of 3000mg/kg of extract for 72 hrs.

Antipyretic effect against yeast induced pyrexia
The antipyretic activity of the hydroethanolic extract of Opuntia monacantha Haw was determined by yeast induced pyrexia in mice. The result showed that tested drug at different doses caused lowering of the body temperature upto 4 hrs following its administration (Table). Seventeen hours after s/c injection of 20% of yeast; a significant increase in rectal temperature was observed in all animals. The effect of hydroethanolic extract on yeast induced pyrexia shows that rectal temperature was 38.10°C, 17 hrs after the s/c injection of
yeast suspension, further decrease to 37.31°C within 1 hr by the treatment of extract (800 mg/kg), and subside after 4 hrs showing a sizeable reduction in rectal temperature and was comparable to reference drug Aspirin. The extract at a dose of 200 mg/kg body weight showed reduction of pyrexia induced by yeast but it was not significant statistically p > 0.05 upto 3 hrs and showed significant p < 0.001 at 4 hrs when compared to control. Treatments with hydroethanolic extract of Opuntia Monacantha at a dose of 400 mg/kg and 800 mg/kg body weight decrease rectal temperature significantly (p < 0.05, p < 0.001 respectively) at 2, 3 and 4 hrs after administration compared to normal control. Treatment with aspirin at a dose of 100 mg/kg significantly p < 0.001 reduced pyrexia induced by yeast at 3 hrs after administration. Treatment with hydroethanolic extract of Opuntia Monacantha at dose of 400 and 800 mg/kg was nearly equally potent to reference compound Aspirin.

Table 1: Effect of Opuntia Monacantha (200, 400 and 800 mg/kg) i.p on Yeast induced pyrexia in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Rectal temperature (°C)</th>
<th>After administration of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal (X)</td>
<td>After 17 hrs (Y)</td>
</tr>
<tr>
<td>N.Saline</td>
<td>10ml/kg</td>
<td>35.73 ±0.178</td>
<td>37.88 ±0.111</td>
</tr>
<tr>
<td>Aspirin (STD)</td>
<td>100mg/kg</td>
<td>35.70 ±0.225</td>
<td>37.96 ±0.133</td>
</tr>
<tr>
<td>Extract</td>
<td>200mg/kg</td>
<td>35.66 ±0.254</td>
<td>37.73 ±0.199</td>
</tr>
<tr>
<td></td>
<td>400mg/kg</td>
<td>35.31 ±0.248</td>
<td>37.78 ±0.158</td>
</tr>
<tr>
<td></td>
<td>800mg/kg</td>
<td>35.80 ±0.188</td>
<td>38.10 ±0.086</td>
</tr>
</tbody>
</table>

Value are expressed as mean ± SEM, (number of mice N = 6), significant *P < 0.05, ** P< 0.01 and *** P < 0.001, when compared to control.
DISCUSSION

The aim of the present research was to validate the traditional uses of the extract of *Opuntia Monacantha* as antipyretic agent. The result of the present study showed that hydroethanolic extract exhibited significant effect against fever produced by s/c injection of yeast. The antipyretic activity produced by the hydroethanolic extract of *Opuntia Monacantha* is of considerable importance and justified its significance in fever as suggested in traditional medicines. Fever or pyrexia is a common medical sign associated primarily with abrupt increase in body temperature above normal and caused by certain illness related behavioral features like fatigue, depression, lethargy, anorexia, hyperalgesia (*Hossain, E., S.C. Mandal, and J. Gupta, 2011*). The elevation of body temperature occurs as a result of the release of certain chemical substances by immune system (*Indumathy.S et. al. 2011*). Infected or injured tissue enhances the formation of pro-inflammatory mediator i.e. cytokines like interleukin-1beta, alpha, beta and TNF- alpha which increase the synthesis of prostaglandin E2 (PGE2) and there by stimulating hypothalamus to raise body temperature (*Flier, J.S, et. Al.1986*).

Fever or pyrexia is a normal response against invading microorganisms to provide defense against infections (*Tonks Fawcett and R. Watson 2003*). Antipyretic drugs inhibit COX-2 enzyme expression and thus inhibiting biosynthesis of PGE2 to reduce high body temperature (*Cao, Y. and S.M. Prescott, 2002*). Cyclooxygenase (COX) also known as prostaglandin (PG) H synthetase catalyzes the conversion of Arachidonic acid into prostaglandin H2 (*Seibert, K*)
The common therapy for management and control of fever NSAIDs. They are used for relief of inflammation, headache, anti arthritis pain, heart attacks and stroke. (Buffum, M. and Buffum J.C.2000). NSAIDs drugs inhibit prostaglandin and its derivatives produced through cyclooxygenase enzyme that cause inflammation, fever, pain and related diseases (Fung, H.B. and H.L. Kirschenbaum, 1999, Fung, H.B. and H.L. Kirschenbaum, 2012). However, NSAIDS produces a number of side effects like gastrointestinal bleeding, mucosal erosion, hepatotoxicity, renal toxicity and nephropathy (Greisman, L.A. and Mackowiak P.A., 2012). Meanwhile, in order to avoid from side effect, there are development and introduction of new antipyretic agents that compete with NSAIDs. The use of natural remedies for the treatment of inflammatory and painful condition has long history starting with Ayurvedic treatment, extends to the Europe. Plant drugs are known to play a vital role in the management of inflammatory diseases.

Intraperitontial administrations of the yeast to mice significantly increase rectal temperature and the tested drug significantly reduced rectal temperature. Thus it can be hypothesized that the extract contained pharmacologically compounds that interfere with the release of prostaglandin. The present results shows that extract possess significant as well as dose dependent antipyretic effect in yeast induced pyrexia which is comparable to that of standard drug. The effect of hydroethanolic extract on yeast induced pyrexia shows decrease in rectal temperature within 1 hr of the extract (800mg/kg) treatment, and at 4 hrs a sizeable reduction in rectal temperature which was comparable to reference drug aspirin. The tested drug at a dose of 400mg/kg and 800mg/kg body weight decreased rectal temperature significantly (p < 0.001) respectively after administration as compared to negative control. Treatment of the tested drug at a dose of 400 and 800mg/kg produced a decrease in rectal temperature that was comparable to reference compound aspirin. The evaluated body temperature intensified the process of lipid per oxidation, which indicates that increase of oxidative stress causes pyrexia. The supplementation of antioxidant decreased the lipid per oxidation processes (Sehgal, Momekova D, Pencheva V, 2011). The flavonoids have antioxidant activity. Thus, antioxidant activity of Opuntia Monacantha may be one of the possible mechanisms to reduce the elevated body temperature. The lowering of body temperature observed can be recognized to the presence of flavonoids in Opuntia Monacantha that might be responsible to lowered body temperature (Nwafor, P.A., et.al.2012).The extract may reduce PGE2 by its action on cyclooxygenase (Cox2) or by increasing the production of the body’s own antipyretic substances like vasopressin and arginine (Saranya, R.et.al.2012).Various studies show that
formulation containing tannins, alkaloids, flavonoids and carbohydrates has been reported for their antipyretic potential.

CONCLUSION
The results of the present study showed that plant extract significantly reduced elevated body temperature in dose dependent manners. These results validate the basis for the traditional use of *Opuntia Monacantha* against fever. The preliminarily analysis shows the presence of phenols, flavanoids, alkaloids, saponins, tannins and terpenoids. The pharmacological activity of the plant extract may be due to presence of these phytochemicals. However, further detail studies are necessary for isolation of pure secondary metabolites from the plant to understand the exact mechanism responsible for pharmacological activities of the plant.

REFERENCES