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**Research Article**

**Antipyretic Activities of Methanol Extract of the Ganoderma sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang on Albino Rats-An Experimental Study**

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**Abstract**

**Aim:** Antipyretic effect of the methanol extract of *Ganoderma sinense* against Brewer's yeast induced pyrexia model in albino rats of both sexes was investigated.

**Methods:** Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast suspension (20ml/kg) in the back below the nape of the neck of animals. Twenty four hours after the injection, the rectal temperature of each rat was measured. The temperature was measured at 30, 60, 90 and 120 min. after drug administration. Paracetamol (150mg/kg p. o.) was used as standard drug. The group received methanol extract 300mg/kg showed significant decrease in rectal temperature from 39.42± 0.24 to 35.50± 0.34 as compared with the group received standard drug. All experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). Differences at P < 0.05 were considered to be significant.

**Results:** The statistically processed results support the conclusion, that the methanol extract of *Ganoderma sinense* (300mg/kg) possesses dose dependent significant antipyretic activity.

**Conclusion:** From the study it was concluded that Wild Mushroom of *Ganoderma sinense* possess significant antipyretic activity.

**Key words:** *Ganoderma sinense*, Methanol extract, Paracetamol, Antipyretic activity.

**Introduction**

*Ganoderma* is a genus of polypore mushrooms which grow on wood and include about 80 species, many from tropical regions [1]. *Ganoderma* has been used in traditional Asian medicines for the prevention and treatment of various types of diseases, such as cancer, hepatopathy, arthritis, hypertension, neurasthenia and chronic hepatitis [2-4].
Among the genus, two species, *Ganoderma sinense* and *Ganoderma lucidum* are the key species for the production of medicinal materials because of their extensive use in traditional Asian medicines and their potential in bioremediation; they are a very important genus economically [5]. The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Pain, inflammation and fever are very common complications in human beings [6]. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Most of the antipyretic drugs inhibit Cox-2 expression to reduce the elevated body temperature by inhibiting prostaglandin E2 (PGE2) biosynthesis. Moreover these synthetic agents irreversibly inhibit Cox-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas the natural Cox-2 inhibitors have lower selectivity with fewer side effects. A natural antipyretic agent with reduced or no toxicity is therefore essential. The demand for herbal medicines is increasing rapidly due to their fewer side effects. Further as health care costs continue to escalate, the attraction for low cost remedies has stimulated consumers to re-evaluate the potential of alternatives [7-10]. Recent studies show that the triterpenoids or GAs from *G. sinense* and *G. lucidum* have various biological functions such as cytotoxicity to several cancer cells in vitro, or inhibition of tumor invasion in-vitro and in-vivo, inhibition of human immunodeficiency virus (HIV)-1 protease, inhibition of eukaryotic DNA polymerases, inhibition of cholesterol synthesis and absorption, regulation of osteoclastogenesis and inhibition of U46619-induced platelet aggregation [11-19]. An extensive search of the literature reveals no reports on the antipyretic activity of the mushrooms. Thus, present investigation was planned to find out the therapeutic level of methanol extract of *Ganoderma sinense* mushroom in antipyretic activity.

**Material and Methods**

The present study was conducted on adult Albino rat (150-200g) of either sex was procured from the central animal house, National College of Pharmacy, Shimoga district, Karnataka, India. The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The rats were acclimatized to laboratory condition for 5 days before commencement of the experiment. The animals (six per cage) were maintained under standard laboratory conditions (light period of 12 h/day and room temperature), with access to commercial pellet diets and water *ad libitum*. Food was withdrawn 12 hrs before and during the experimental hours. After the approval by the Institutional Animal Ethical Committee, an experimental study was undertaken according to their rules and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

**Collection of mushroom and authentication**

The *Ganoderma sinense* was collected from forest region which is located in Agumbe, Thirthahalli (T), Shimoga (D), Karnataka, India, during the month of June to August 2013. The *G. sinense* of mushroom was picked from the litter and decaying wood surface, with help of forceps and then they were cleaned and air dried in an oven at 40°C for 48h. Dried mushroom samples were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard literatures [20, 21]. The voucher specimen (KUABARN-74) has been deposited at the herbarium of mycology laboratory, Department of P. G. Studies and Research in Applied Botany,
Kuvempu University, Jnana Sahyadri, Shimoga district (Karnataka) for future reference.

**Chemicals**

Yeast was purchased from a local market. Paracetamol tablets 500mg were obtained from Remidex Pharma Pvt. Ltd.

**Extraction of mushroom**

The air dried powdered mushroom (500g) was extracted with 90% methanol in a Soxhlet apparatus at 60°C. The extract was filtered and concentrated to dryness at room temperature to avoid decomposition of natural metabolites. The yield (18.36%) was used for the experimental studies [22, 8, 23-28].

**Animals**

For this study healthy Wistar Albino rats (150-200g) were procured from the central animal house, National College of Pharmacy, Shimoga district, Karnataka, India was used. Rats were maintained under controlled condition at temperature in the experimental animal room was kept 25±3°C and humidity 55%. Lighting was artificial, the sequence of 12 hours light, 12 hours dark followed. For feeding, conventional laboratory diet was used with water *ad libitum*. The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Emulsion was prepared using 1% Tween 80 as surfactant and the concentrations were prepared according to 1.5ml/100g of body weight. The extracts were administered in a single dose by gavages using a stomach tube, after fasting the animals for 3-4 hours. In each step, six animals were used, starting dose of methanol extract of mushrooms was 100mg/kg body weight, then 200, 500, 1000, 1500, 2000 and 3000. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases death was observed within first 24 hours. Additional observations like changes in skin, fur, eyes, mucous membrane, respiratory, circulatory and autonomic and central nervous systems and somatomotor activity and behavior pattern. Attention was also given to observations of tremors and convulsions. Further the animals were under investigation up to a period of one week [29-32].

The LD$_{50}$ value obtained for methanol extracts of mushrooms extracts was 3000mg/kg body weight, Therefore 1/10$^{th}$ weight of maximum tolerated dose were found to be 300mg/kg body weight of mushroom extracts of *G. sinense*. However, 100mg/kg, 200mg/kg and 300mg/kg body weight were chosen for the pharmacological study.

**Antipyretic activity**

Antipyretic activity of alcoholic extracts of the whole fruiting body of mushroom of *G. sinense* was studied by Brewer’s yeast induced pyrexia method.

**Evaluation of antipyretic activity:**
Hyperthermia was induced in mice by s.c. injection of 20ml/kg of a 20% aqueous suspension of brewer’s yeast in the back below the nape of the neck. The animals were then fasted for the duration of the experiment (approximately 24 hrs), water was made available ad lib. Control temperatures were taken 24 hrs after the yeast injection to determine the pyretic response to yeast [33, 34]. Pyrexia was measured by Digital thermometer. Temperature measured 1 hr prior to drug administration in fevered animals served as a pre-drug control. Rectal temperature of each rat was measured using a digital thermometer after 24 hrs of administration of yeast suspension. Rats those shown an increase in temperature of at least 0.7°C used for the experiments. After it animals were divided into five groups (5 groups) and each group containing six animals. Group-I acted as control which received vehicle (distilled water 5ml/kg-1 b.w., p.o.). Group-II, III, IV and V acted as study groups. Group-II received standard drug (Paracetamol 150mg/kg b.w., p.o.) was given 24 hrs after the yeast injection, p.o. and Group-III, IV and V were treated with graded doses of methanol extract 100, 200 and 300 mg/kg b.w., per oral respectively. The rectal temperature was recorded at 30, 60, 90, and 120 min after its administration [34].

**Statistical Analysis:**
The results of statistical analysis for animal experiment were expressed as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). The results obtained were compared with the vehicle control group. The results were considered significant at p<0.05.

**Results**
In acute toxicity study, it was found that all the animals were safe at a dose of 3000mg/kg body weight and there was no abnormal behavior. The 1/10th and 1/5th tolerated dose i.e. 200mg/kg body weight and 300mg/kg body weight were selected as a therapeutic dose for antipyretic studies. In the present study the antipyretic effect of various concentrations of methanol extract, with that of Paracetamol at different times are compared. This anti-pyretic effect appears to be dose dependent as 100mg/kg b.w. and 200mg/kg b.w. dose did not produce any pyrexia lowering effect but at the 300mg/kg b.w. dose showed (Table-1) the anti-pyretic effect on and after 90 min, comparable to that of standard drug (Paracetamol) and was then sustained during the whole observation period (Figure-1). This may be due to ineffective content of flavanoids in extract dose (100 and 200mg/kg b.w.).

**Table 1: Antipyretic activity of methanol extract of Ganoderma sinense on rats**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg) oral</th>
<th>Temperature °C±SEM</th>
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<tbody>
<tr>
<td>Normal vehicle</td>
<td>1 ml</td>
<td>Initial</td>
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<tr>
<td></td>
<td></td>
<td>Pyretic</td>
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<tr>
<td></td>
<td></td>
<td>30 min</td>
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<td>60 min</td>
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<td></td>
<td>90 min</td>
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<tr>
<td></td>
<td></td>
<td>120 min</td>
</tr>
<tr>
<td>Standard (Paracetamol)</td>
<td>150</td>
<td>37.4±0.09</td>
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<tr>
<td></td>
<td></td>
<td>37.33±0.29</td>
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<td></td>
<td></td>
<td>37.89±0.16</td>
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<td></td>
<td></td>
<td>37.80±0.07</td>
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<td></td>
<td></td>
<td>37.93±0.04</td>
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<tr>
<td></td>
<td></td>
<td>37.78±0.12</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>100</td>
<td>37.3±0.29</td>
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<td></td>
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<td>39.45±0.19</td>
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<td>38.88±0.43</td>
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<td></td>
<td></td>
<td>38.58±0.29</td>
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<td>38.27±0.21*</td>
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<td></td>
<td></td>
<td>38.31±0.24*</td>
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<tr>
<td></td>
<td>200</td>
<td>37.4±0.06</td>
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<td>39.24±0.34</td>
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<td>36.64±0.34*</td>
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<td>36.04±0.53*</td>
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<td>36.17±0.13*</td>
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<td></td>
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<td>36.51±0.21*</td>
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<tr>
<td></td>
<td>300</td>
<td>37.3±0.05</td>
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<td></td>
<td></td>
<td>39.42±0.24</td>
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<td>35.05±0.40*</td>
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<td>35.62±0.28*</td>
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<td>35.41±0.40*</td>
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<td>35.50±0.34*</td>
</tr>
</tbody>
</table>

Values are mean± SEM (n=6), *p<0.05, **p<0.01 (p value as compared to control group)
Discussion

In general non steroidal anti inflammatory drugs produce their antipyretic action, through inhibition of prostaglandin synthesis within the hypothalamus [35]. Therefore it appears that antipyretic action of methanol extract of Ganoderma sinense may be related to the inhibition of prostaglandin synthesis in hypothalamus. Fever may be a result of infection or one of the sequel of tissue damage, inflammation, graft infection or other diseases states. Regulation of body temperature requires a delicate balance between the production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and drugs like Paracetamol don't influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature [36]. Yeast-induced pyrexia is called pathogenic fever and it's a etiology involves production of prostaglandins. The effect of the drugs may be due to inhibition of prostaglandin synthesis [34].

The present study reveals that the methanol extract of Ganoderma sinense causes a significant antipyretic effect in yeast provoked elevation of body temperature. In the cases, the ethanol extract caused a significant lowering of body temperature, with the effect being comparable to that of Paracetamol. Thus the present pharmacological evidence provides support for the folklore claim as an antipyretic agent. Flavonoids are known to target prostoglandins which are involved in the late phase of acute inflammation, pyrexia and pain perception. Flavonoids reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and by increasing the vascularity. Hence the presence of flavanoids in the methanol extract of Ganoderma sinense may be contributory to its antipyretic activity [37].

Conclusion

In the present pharmacological evaluation the methanol extract of Ganoderma sinense mushroom was extensively investigated for its antipyretic
activity against Brewer’s yeast induced pyrexia model in rats. At the end of our study, a strong conclusion can be drawn that, the methanol extract of *Ganoderma sinense* possess Antipyretic activity more or less depending on the dose levels. The methanol extract of *Ganoderma sinense* has antipyretic effect supporting the ethno pharmacological use as antipyretics. The effect may be explored in the use of the plant in the management of some other diseases.

**Acknowledgment**

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**References**