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EVALUATION OF ANTI-NOCICEPTIVE ACTIVITY OF *PLEUROTUS SAJORCAJU* MYCELIUM BY EXPERIMENTAL ANIMAL MODELS

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ABSRACT

The present study was designed to investigate the analgesic activity of aqueous and methanolic extracts of *Pleurotus sajorcaju* mycelium in two experimental model, acetic acid-induced writhing and hot plate method in healthy mice. For this experiment two concentration of extract 500 mg/kg and 1000 mg/kg were used and given by oral route. Indomethacin (10 mg/kg) was used as a standard. Aqueous extract had significant analgesic activity at 1000 mg/kg in acetic acid induce writhing method but had no activity in hot plate method. Methanolic extract had significant activity in both test. Thus methanolic extract possess both centrally as well as peripheral activity may be due to presence of polysacchcharides and sterol as a constituent.

Key words: *Pleurotus sajorcaju,* Analgesic activity, Hot plate method, Acetic acid induce writhing method, Indomethacin.

INTRODUCTION

Inflammation and its consequence, pain is an unpleasant sensation no doubt; but pain is mainly a protective mechanism for the body. It occurs whenever any tissues are damaged, and it causes the individual to react to remove the pain stimulus. Uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses (Biswanath Das *et al*., 2010).

Many drugs used to relieve the pain and a few drugs like opiates (Brune K, 1990) and NSAIDs (Willete RE *et al*., 1987) have been significantly used for the last three decades. Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.

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Nature is the best source of medicinal constituents. From the vast natural resources, the plants are being used for therapeutic purposes from the beginning of the civilization (Kirtikar KR *et al*., 1980). The investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001).

Mushrooms are used in folk medicine throughout the world since ancient times as nutritionally functional food. *Pleurotus sajorcaju* is an edible and highly priced mushroom and *belonging to family* Tricholomataceae. So, objective of the present study to find out analgesic activity of *P. sajorcaju.*

MATERIAL AND METHOD

Collection of plant materials

Fresh fruiting body of *P. sajorcaju* was collected from Anand district, Gujarat, India and the plant was identified ,authentified by Prof. Shubhash J. Patel, Anand Agriculture University, Anand. Fruiting body was dried under shade and then stored in airtight container.

Preparation of fruiting body extract

The dry fruiting body was chopped and subjected to separately extraction with distilled water and methanol by Soxhlet apparatus for 8 hr. After filtration with cotton wool, the filtration were concentrated in rotavapor (Heidolph Instruments, Laborota 4000, Germany) under reduce pressure at 90ºC and 50ºC for aqueous and methanolic extract respectively. Nearly 85% solvents were recovered by distillation over a boiling water bath at atmospheric pressure and temperatures maintain at 95ºC and 65ºC for aqueous and methanolic extract respectively. The yield was 200mg/kg and 80.5 mg/kg for aqueous and methanolic extract respectively.

Animals used

Female albino mice of wistar strain weighing around 20-30gm were procured from Central Animal Facility, S. K. Patel College of Pharmaceutical Edu. and Research, Gujarat. The animals were housed in solid bottomed polypropylene cages under standard conditions of temperature (23±1°C), 12 h light/dark cycle. The mice were fed with standard pellet diet (Pranav Agro Industries Ltd., Sangali) and water *ad libitum.* The experiments were designed and conducted in accordance with guideline of CPCSEA and Institutional Animal Ethic Committee (Approved no. IAEC/2007/04).

Preparation of test solution

Dry extracts were dissolved in sterile saline solution and prepared final concentration of 500 mg/ml and 1000 mg/ml for determination of its analgesic activity. Standard drug taken was Indomethacin 10 mg/kg for hot plate method and acetic acid induced writhing method.

Phytochemical study

Phytochemical screening of the aqueous and methanol extracts were performed using qualitative chemical tests for the presence of polysaccharides, gum, lipid, protein, amino acid, fats and oils, alkaloid, flavones, sterols and saponins.

Short-term toxicity study

This was conducted by using the method described by Gayathri V *et al*., (Gayathri V *et al*., 2005).In the initial phase, mice were divided into 4 groups of six animals in each group and treated with the Aqueous and methanolic fruit extract of the plant at doses of 500 and 1000 mg extract/ kg body weight orally for 15 days. Body weight, food and water intake, and general behavior were monitored. The behavioral parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, convulsion and respiratory rate.

Acetic acid induced abdominal constriction

Female albino mice of wistar strain were divided into 6 groups of 6 mice in each group. The first group was given 1% CMC in distilled water 2 ml/kg orally and served as control.Groups 2 and 3 received aqueous extract 500mg/kg and 1000 mg/kg,orally respectively. Groups 4 and 5 received methanolic extract 500mg/kg and 1000 mg/kg, orally respectively. Group 6 received indomethacin 10 mg/kg orally as a positive control or standard. Thirty minutes after dosing, mice in all the groups were treated with Acetic acid (0.75%v/v, 0.1 ml/ 10g body weight orally). Five minutes after Acetic acid injection, mice were placed in individual cages and the number of abdominal contractions (writhing) was counted for each mouse for a period of 15 minutes (Nakamura H *et al*., 1986).

Hot-plate assay

Female albino mice of wistar strain were divided into 6 groups of 6 mice in each group. The first group was given 1% CMC in distilled water 2 ml/kg orally and served as control. Groups 2 and 3 received aqueous extract 500mg/kg and 1000 mg/kg, orally respectively. Groups 4 and 5 received methanolic extract 500mg/kg and 1000 mg/kg, orally respectively. Group 6 received indomethacin 10 mg/kg orally as a positive control or standard. Immediately after dosing mice were placed on a hot-plate (Instruments Mfg. Corpn., Ambala) maintain at 55 ± 1^0C. For both the control and test animals, the reaction time (in seconds) was taken as the time by the animals to lick the fore or hind paw or jump out of the plate was taken as the reaction time. This test was performed at 0, 30, 60, 90 and 120 min after the administration of dosing (Jacob *et al*., 1978).

Statistical analysis

The result was express as mean ±Standard Error of Mean (S.E.M.) statistical difference between two means was determine by one-way ANOVA followed by Dunnett multiple comparisons test by using InStat 3 statistical computer software. Only those mean values showing statistical difference $p\leq0.01$ or $p\leq0.05$ was considered as statistically significant.

RESULTS

Phytochemical test

Phytochemical screening of the aqueous extract indicated the presence of polysaccharides, gum, lipid, protein, amino acid, fats and oils, alkaloid, flavones and saponins and methanolic extract containing polysaccharides, gum, lipid, protein, amino acid, fats and oils, alkaloid, flavones and sterol.

Short-term toxicity study

The activity and feeding of the test mice appeared normal. There were no deaths. $LD_{sub.50}$>10 g/kg body weight was obtained by administration to both male and female mice via the oral route. The results demonstrate that the sampled *P. sajorcaju* contained nontoxic substances.

Acetic Acid Induced abdominal constriction

The aqueous and methanolic extracts of *P. sajorcaju* mycelium at concentration of 1000mg/kg and control group were reduce the number of mouse abdominal constrictions and which was 31±2.75, 29.6±2.87 and 57.2±2.764 respectively. Thus aqueous and

methanolic extract in the concentration of 1000mg/kg show significant ($p < 0.01$) analgesic activity against control group normal control group. Indomethacin (10 mg/kg) induced a protection against abdominal constriction which was 14.6±1.077 ($p \leq 0.01$). Methanolic extract show more analgesic activity against aqueous activity shown in fig. 1.

Hot Plate Method

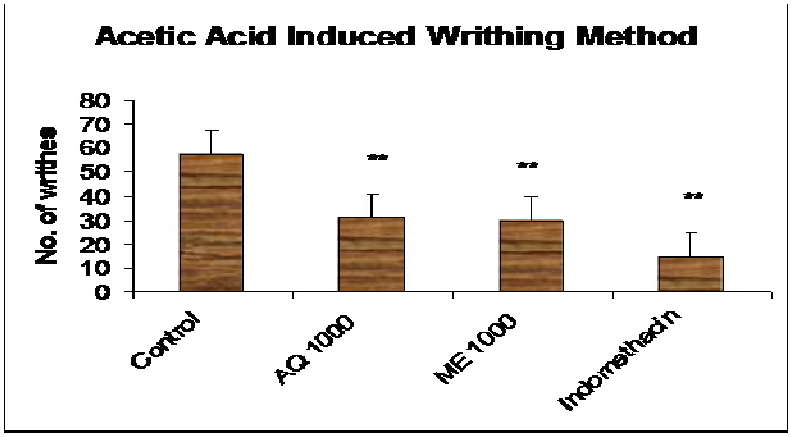
Aqueous extract of *P. sajorcaju* mycelium at concentration of 500 mg/kg and 1000 mg/kg had significant analgesic activity compared to control group which was shown in fig. 2. Methanolic extract of *P. sajorcaju* mycelium at concentration of 500 mg/kg and 1000 mg/kg had significant ($p \leq 0.01$) analgesic activity compared to control group which was shown in fig.2. Indomethacin (10 mg/kg, orally) had significant analgesic effect in the hot-plate test its beginning 30 minute after treatment ($p \leq 0.01$). Maximal effect of methanolic extract was observed after 1 hour(Fig.2).Table 1 shown average reaction time of each group at different time for all doses.

Table 1.Effect of aqueous and methanolic extract of *P. sajorcaju* on Hotplate assay

Group	0 minute	30 minute	60 minute	90 minute	120 minute
Control	11.86±0.86	12.28±0.19	11.83±0.35	12.25±0.45	12.33±0.86
Aqueous extract, 500 mg/kg	11.61±0.35	12.98±0.20	15.09±1.75	15.58±1.94	16.43±1.68
Aqueous extract, 1000 mg/kg	11.95 ±1.15	14.72±1.05	19.18±0.78	16.99±1.93	18.33±1.58
Methanolic extract, 500 mg/kg	12.02±0.63	12.64±0.39	14.11±0.64	14.07±0.28*	14.48±1.21**
Methanolic extract, 1000 mg/kg	12.17±0.31	14.4±1.40	17.43±1.68*	17.40±0.64**	17.65±1.79**
Indomethacin 10mg/kg	12.46 ± 1.06	15.41±0.61*	24.28±1.64**	23.57±1.75**	24.70±1.30**

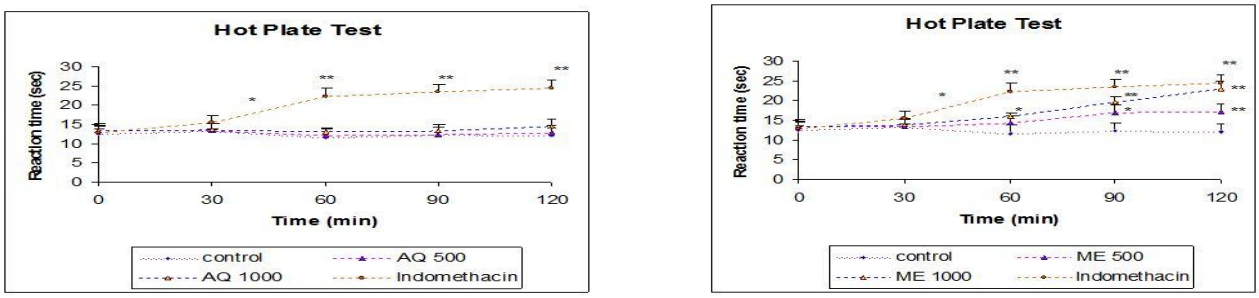
Values are expressed as mean±S.E.M. of six animals. * denotes significance at the level of $p \leq 0.05$, ** denotes significance at the level of $p \leq 0.01$ when compared with control group.

Fig. 1. Analgesic activities of aqueous and methanolic extract of *P. sajorcaju* mycelium and Indomethacin in mice (acetic acid induced writhing test)



Values are expressed as mean±S.E.M. of six animals. ** denotes significance at the level of $p \leq 0.01$ when compared with control group.

Fig. 2.Analgesic activities of aqueous and methanolic extract of *P. sajorcaju* mycelium and Indomethacin in mice (Hot plate test)



Values are expressed as mean±S.E.M. of six animals. * denotes significance at the level of $p \leq 0.05$, ** denotes significance at the level of $p \leq 0.01$ when compared with control group.

DISCUSSION AND CONCLUSION

Hot plate method and acetic acid induce writhing method produce noxious stimuli namely thermal and chemical stimuli respectively and so used as a models for screening of analgesic activity. Hot plate method and acetic acid induce writhing method are produced centrally acting and peripheral acting algesic activity respectively (Parkhouse *et al.*, 1979)**.**

In the present study, methanolic (500 and 1000 mg/kg, orally) mycelium extracts, produced an inhibitory effect on the nociceptive response in the hot plate test, while the aqueous extracts showed no analgesic activity in this test.

P. sajorcaju aqueous (500 and 1000 mg/kg, orally) and methanolic (500 and 1000 mg/kg, orally) extracts decrease stretching induced by acetic acid. Thus both have analgesic activity and reduce the inflammation which is produce by acetic acid.

Both analgesic activity and moderate anti-inflammatory effect observed with the extracts has also been shown in nonsteroidal anti-inflammatory drugs (NSAIDs). It is a well established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclo-oxygenase activity (Vane, 1971).

The aqueous extract may exert their activity via a peripheral mechanism while methanolic extract act through both pathway. It is concluded that aqueous and methanolic extracts of *P. sajorcaju* have antinociceptive effects in chemical pain tests may be due to their content of polysaccharides and methanolic extracts have analgesic effect in thermal pain test and chemical pain test may be due to their content of polysaccharides as well as sterol. However, the chemical constituents and mechanism(s) responsible for the pharmacological activities remain to be investigated.

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