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# Analgesic and antipyretic activity of aqueous and alcoholic extrcts of fruits of *Zizyphus Oenoplia*

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## ABSTRACT

The present study was aimed to investigate the analgesic and antipyretic activity of alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* in standard experimental models in mice and albino rats following oral route of administration. The centrally acting analgesic activity of the extracts was evaluated using Eddy's hot plate method, where as peripheral analgesic activity by acetic acid induced writhing Method. The extracts were studied for antipyretic activity by yeast induced hyperthermia in rats. Here the alcoholic extract of fruits of *Zizyphus Oenoplia* was found to have more significant analgesic effect in both centrally and peripherally than aqueous extract when compared to control. Diclofenac sodium was used as standard reference drug. Similarly the alcoholic extract showed a significant antipyretic activity than aqueous extract when compared to control. Paracetamol was used as standard reference drug.



## KEYWORDS

*Zizyphus Oenoplia*;
Antipyretic;
Analgesic.

## INTRODUCTION

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 mainly depends on the plant based drugs.

*Zizyphus Oenoplia* belonging to the family Rhamnaceae has been used both for its food as well as for medicinal value. It is usually grown in dry deciduous forests and in waste lands region. This fruits have been found to be believed some of the pharmacological properties and commonly used as Blood purifier, febrifuge, abdominal pain, etc[1-3]. The earlier results shows that the chloroform and methanol extracts have been used to show antibacterial activity against few bacterial strains[4]. Preliminary phytochemical studies revealed that both the extracts have the presence of phytochemical constituents like sterols, carbohydrates, Saponins and flavonoids, where as in alcoholic extract there are some traces of alkaloids. Literature survey revealed that no systemic work has been carried out to establish the analgesic and antipyretic activity on the *Zizyphus Oenoplia* fruits. In view of this, the present study was aimed to evaluate the analgesic and antipyretic activity of aqueous and alcoholic extracts of *Zizyphus Oenoplia* fruits.

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## EXPERIMENTAL

### Preparation of extracts

*Zizyphus Oenoplia* fruits were collected from the surrounding of shivamogga, Karnataka, India. The fruits were shade dried and powdered mechanically. About 250 g of the powdered material was subjected for extraction with alcohol using soxhlet apparatus and the other 250 g of powder was macerated with distilled water which gives aqueous extract. The percentage yield of alcoholic and aqueous extract was found to be 26% w/w and19% w/w respectively. Both the extracts were subjected to preliminary phytochemical study according to the standard procedures[5] and the extracts were screened for pharmacological activities.

### Pharmacological screening

### Acute toxicity studies[6]

The experiment was initiated only after approval of Institutional Animal Ethical Committee. Acute toxicity study was carried out for both the extracts by stair case or Up and down method. The $LD_{50}$ of aqueous and alcoholic extracts of fruits was found to be 3500mg/kg b.w and 2000mg/kg b.w respectively. One tenth of $LD_{50}$ was taken for the evaluation of analgesic and anti pyretic activity.

### Animals

Adult male mice (20-25 g) were used for analgesic experiments. Adult male Wistar rats weighing 150-200g were used for antipyretic activity and this were procured from Central animal house, National College of Pharmacy, shivamogga. The animals were housed in polypropylene cages and were maintained at $27\pm 2^0$, relative humidity 60 ± 5 % and 12H light/dark cycle; they were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *adlibitum*. The mice were acclimated to laboratory condition for 10 days before commencement of experiment.

### Screening of analgesic activity[7,8]

### Analgesic activity by acetic acid induced writhing method

Animals were divided into four groups containing six animals in each. Group one served as control. Second group received Diclofenac Sodium 10mg/kg b.w. third and fourth groups received the extracts of fruits of *Zizyphus Oenoplia* of alcoholic and aqueous respectively. One hour after administration of the standard and test, each animal intraperitoneally received 1% acetic acid injection with a volume of 1ml/100g body weight. After the administration of acetic acid injection, the numbers of stretching or writhing response per animal were recorded during the subsequent 10 min.

**TABLE 1 : Effect of alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* on acetic acid induced writhing method in mice.**

| Groups | Treatment | Dose, Route | No. of Writhings | %inhibition |
|---|---|---|---|---|
| Group-I | Vehicle | 0.4ml, *p.o* | 44.333 ± 1.944 | ---------- |
| Group-II | Diclofenacsodium | 10mg/kg, *i.p* | 8.333 ± .7149** | 81.20 |
| Group-III | Alcoholic extract | 200mg/kg, *p.o* | 20.333 ± 1.054** | 54.14 |
| Group-IV | Aqueous Extract | 350mg/kg, *p.o* | 37.333 ± 1.726** | 15.80 |

**Results are presented as mean ± SEM, (n=6), *= p<0.05, ** = p<0.01**

### Analgesic activity by Eddy's hot plate method

The paws of mice and rat are very sensitive to temperature 55±0.5ºC, which are not damaging to the skin. The response in the form of jumping, withdrawal or the licking of paws. Male Swiss albino mice were divided in it four groups of five each. The animals were removed from the holding room and randomly assigned to treatment groups. Animals received the vehicle, (Water) extracts of fruits of *Zizyphus Oenoplia* of alcoholic and aqueous (200mg/kg b.w and 350mg/kg b.w) and Diclofenac sodium (10mg/kg b.w.). Following the 30 min of habituation period, the animals were placed in Eddy's hot plate maintained at a temperature of 55±0.5ºC. A cut off period of 15 seconds was observed to avoid the damage to the paw. Reaction time and the type of response were noted using a stop watch. Response latency was recorded at 30, 60, and 90 minutes after administration of the test drugs.

TABLE 2 : Effect of alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* on latency to hot plate test in mice.

| Groups | Dose | Mean latency(s) before and after durg administration | | | | % inhibition | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 30 min | 60min | 90min |
| Group-I | Vehicle, p.o | 2.442 ± 0.160 | 2.642 ± 0.226 | 2.580 ± 0.276 | 2.598 ± 0.233 | ------- | ------ | ------ |
| Group-II | 10mg/kg, i.p | 2.204 ± 0.022 | 4.556 ± 0.267** | 8.614 ± 0.0272** | 11.662 ± 0.260** | 42.01 | 70.05 | 77.72 |
| Group-III | 200mg/kg, p.o | 2.394 ± 0.261 | 4.380 ± 0.196** | 6.392 ± 0.256** | 6.108 ± 0.294** | 39.68 | 57.47 | 59.64 |
| Group-IV | 350mg/kg, p.o | 2.388 ± 0.050 | 3.580 ± 0.240* | 4.076 ± 0.133** | 4.668 ± 0.339** | 26.20 | 36.70 | 44.34 |

**Results are presented as mean ± SEM, (n=5), * p<0.05, ** = p<0.01**

## Antipyretic activity[9]

The rats of either sex were divided in to four groups comprising six in each. The normal body temperature of each animal was measured rectally at 1hr. interval on a telethermometer and recorded. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10ml/kg of 15%w/v yeast suspended in 0.5%w/v methyl cellulose solution and the rats were then returned to their housing cages. After 18hrs of yeast injection, water was administered orally to the first group which is control group. The second group of animals received the standard drug paracetamol (150 mg/kg) orally. Whereas the third and fourth group receives alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* orally at doses of 200mg/kg and 350mg/kg respectively. Rats were restrained for recording of their rectal temperature at 1hr just before water, paracetamol and alcoholic and aqueous extracts administration and again at 1hr. interval up to 22 hours after yeast injection.

TABLE 3 : Antipyretic effect of alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* on rats.

| Group | Treatment | Dose | Initial Rectal Temp. in $0^C$ before Yeast Injection | Rectal Temperature in 0C after 18hrs of Yeast Injection (Mean± SEM) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0 hr | 1hr | 2hr | 3hr | 4hr |
| I | Vehicle | 5 ml/kg | 38.195 ± 0.024 | 39.196 ± 0.026 | 39.198 ± 0.026 | 39.180 ± 0.008 | 39.088 ± 0.017 | 39.088 ± 0.017 |
| II | Standard Paracetamol | 150 mg/kg | 38.283 ± 0.023 | 39.296 ± 0.017 | 38.908 ± 0.011** | 38.680 ± 0.011** | 38.490 ± 0.012** | 38.418 ± 0.012** |
| III | Alcoholic Extract | 200 mg/kg | 38.3 ± 0.024 | 39.28 ± 0.027 | 38.90 ± 0.018** | 38.808 ± 0.011** | 38.606 ± 0.012** | 38.521 ± 0.013** |
| IV | Aqueous Extract | 350 mg/kg | 38.48 ± 0.017 | 39.465 ± 0.019 | 39.141 ± 0.031 | 39.08 ± 0.039* | 38.970 ± 0.045* | 38.811 ± 0.046** |

**Results are presented as mean ±SEM, (n=6), *= p<0.05, **=p<0.01**

## Statistical analysis

The results were expressed as mean ± S.E.M. The data was subjected to one way ANOVA followed by Dunnet's multiple comparison tests.

## RESULTS

The alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* was found to be non toxic up to the dose of 2000mg/kg b.w and 3500mg/kg b.w respectively and did not cause any death of the tested animals. The phytochemical tests revealed that both the extracts have the presence of phytochemical constituents like sterols, carbohydrates, Saponins and flavonoids, where as in alcoholic extract alkaloids are present.

The oral administration of extracts of alcoholic and aqueous of fruits of *Zizyphus Oenoplia* significantly inhibited the writhing reaction induced by acetic acid. Both the extracts inhibit the writhing; however the alcoholic extract was more significantly reduced the writhing reaction than the aqueous extract. The data of writhing test is presented in TABLE 1.

The extracts of alcoholic and aqueous of fruits of *Zizyphus Oenoplia* administered in mice have shown a significant analgesic activity in hot plate method as supported by increase in latency time ; however the alcoholic extract was more significant than aqueous extract. The results are tabulated in TABLE 2.

In the anti pyretic activity the fractions of alcoholic and aqueous extracts produced significant reductions in the temperature at the entire interval at which the rectal temperature were measured. However the alcoholic extract was more significant than the aqueous extract, when compared with that of the standard paracetamol. The results were tabulated in TABLE 3.

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## DISCUSSION

Pain is a subjective experience which is very difficult to express or define it properly even though we all experienced it. Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipids[10] via cyclooxygenase (COX), and prostaglandin biosynthesis[11]. In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The significant pain reduction of both the extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. It was found that the observed analgesia in *Zizyphus Oenoplia* was demonstrated by the active constituents, isolated from the plant extract through a peripherally acting mechanism. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, carbohydrates, saponins, steroids, flavonoids. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins[12]. Besides these alkaloids are also well known for their ability to inhibit pain perception[13].

The alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* and diclofenac sodium also presented longer latency time than the control group in the hot plate test in a dose related manner. At 90 minutes administration of the alcoholic and aqueous extracts the percent inhibition was found 59.64% and 44.34% for respectively. The hot plate method is considered to be selective for the drugs acting centrally. The hot plate method is considered to be selective for screening the compound acting through the opoid receptor. Therefore, the alcoholic and aqueous extracts of fruits of Zizyphus Oenoplia must have a central activity. Again, narcotic analgesics inhibit both peripheral and central mechanism of pain. Thus analgesic effect of the alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* suggests that they have been acting through central and peripheral mechanism.

Fever may be due to infection or one of the sequele of tissue damage, inflammation. Antipyretic are the agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[14]. The present results show that *Zizyphus Oenoplia* possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is Comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis[15] could be the possible mechanism of antipyretic action as that of paracetamol.

## CONCLUSION

In conclusion, we can confirm that the alcoholic and aqueous extracts of fruits of *Zizyphus Oenoplia* fruits are endowed with both central and peripheral analgesic and antipyretic properties. In both the activities alcoholic extract was more significant than the aqueous. This could provide a rationale for the use of this fruit in fever and pain disorders in folk medicine. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological Characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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