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PHARMACOLOGICAL INVESTIGATIONS OF *Ziziphus mucronata*

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Abstract

The aim of this project work was to investigate the pharmacological activity of the *Ziziphus mucronata* fruits (Family: Rhamnaceae). Fruits are the natural source of many medicine.*Ziziphus mucronata* are the most common plant fruits is used as a source of vitamine,minerals,carbohydrate,and antioxidant etc. Ziziphus fruits commonly known as kul or boroi or jujube.Ziziphus fruits are also used are the source of herbal medicine and the treatment of many diseases as anxiety ,anti-inflammatory,anti-insomnic,antidiabetic.anti-cancer etc. The present study primarily describes the analgesics activities and anti-inflammatory activities of ethanolic extracts of *Ziziphus mucronata*. The presence of analgesic and anti-inflammatory activity were examined. All the test samples in our study showed positive result.Analgesic activity of the plant extracts against pain by inhibit the cox-1 and cox-2 was investigate. Diclofenac was used as standard. In All three method the extract of *ziziphus mucronata* showed positive result such as in formalin induce paw licking test reduce 65.9091% inflammatory pain and 60% neurogenic pain at the dose of 200mg/kg.In tail immersion method the analgesic activity showed 75% at 500 mg/kg and the last method was hot plate method that showed analgesic activity is 54.16%.The ethanolic extract of *ziziphus mucronata* fruits showed some positive result in anti-inflammatory test, at the dose of 250mg, the percent of inhibition is 2.41935 at 2hour and 200mg it is 1.6129%.So,the extract of the experimental plant have considerable analgesic and anti-inflammatory activities which indicates that the plant have bioactive principles.

Key words: *Ziziphus mucronata*, anti-inflammatory, analgesic.

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INTRODUCTION

The Buffalo thorn is a small to medium sized tree, reaching a height of about 10m (33ft). It can survive in a variety of soil types, occurring in many habitats, mostly open woodlands, often on soils deposited by rivers, and grows frequently on termite mounds. Buffalo thorn has distinctive zigzag branch lets, and hooked and straight thorns. The bark is a red-brown (on young stems) or roughly mottled grey which is cracked in small rectangular blocks revealing a stringy red under bark. The fruit are roughly grape size, and ripen into a deep brown-red. The main stem is green and hairy when young; year old branches often zigzag; the bark is reddish brown or roughly mottled grey, cracked into small rectangular blocks, revealing a red and stringy under-surface. Young stems are reddish brown [1].

Fig. 1: Plants of *Ziziphus mucronata*

Leaves are simple, alternate; ovate or broadly ovate; vary enormously in size from tree to tree, 30-90 x 20-50 mm, tapering or often mucronate apex, base strongly asymmetrical, cordate to rounded on one side; margin finely serrated, often badly eaten by insects, glossy green above, slightly hairy and paler below; 3- to 5-veined from the base; veins covered with fine hairs when young; petiole up to 20 mm long; stipules, when present, take the form of small thorns at the nodes, one straight and one hooked. Leaves turn golden yellow in autumn. Flowers are borne in dense clusters in leaf axils; green to yellow; ± 4mm in diameter; inconspicuous (October-February). The fruit is a smooth, shiny, leathery, spherical drupe, 12-20 mm in diameter, reddish-brown or deep red when ripe, slightly sweet, the pulp is dry. The fruit sometimes stays on the plant long after the leaves have fallen (March-August). The seeds are usually solitary, elliptic and compressed [2].

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Fig. 2: Plants, fruits and bark of *Ziziphus mucronata*

Name of the plant: Ziziphus Mucronata

Scientific classification

Kingdom: Plantae

Order: Rosales

Family: Rhamnaceae

Genus: *Ziziphus*

Species: *Z. mucronata*

Medicinal use

The leaves are edible, and can be cooked into tasty spinach; the fruit are also very nutritional, though not very tasty. The leaves can be used as an aphrodisiac, either by being chewed or used in dishes. During the Anglo-Boer war the stones were roasted and ground as a substitute for coffee. A beer can be made from the fruit. The Ovambo people call the fruit of the Buffalo thorn eenghekete and use it to distill Ombike, their traditional liquor. The Buffalo thorn also has medicinal properties, an extract of the roots is given as a painkiller and a solution of the bark and leaves in water is used for chest complaints. A decoction of the glutinous roots is commonly administered as a painkiller for all sorts of pains as well as dysentery. A concoction of the bark and the leaves is used for respiratory ailments and other septic swellings of the skin. Pastes of the root and leaves can be applied to treat boils, swollen glands, wounds and sores. Steam baths from the bark are used to purify and improve the complexion [3]. In East Africa, roots are used for treating snake bites [4]. All of the above can be attributed to the peptide alkaloids and antifungal properties isolated from the bark and leaves. The berries are edible and were used by residents in the former Transvaal in making porridge or as a coffee substitute. The fruit can also make a beer

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if fermented properly. During the Anglo-Boer war, the seeds were ground and used as a coffee substitute. Africans have many beliefs and superstitions attached to this tree. Zulus and Swazis use the buffalo thorn in connection with burial rites. It was once customary that when a Zulu chief died, the tree was planted on his grave as a reminder or symbol of where the chief lies. Hence the name *umLahlankosi*- that which buries the chief. A twig from the tree was and is still used to attract and carry the spirit of the deceased from the place of death to the new resting place. When a stock owner died, and was buried according to custom, within the cattle or goat kraal, some branches were placed on the grave so that the animals nibbled on leaves and twigs, and so understood that their master had died. In other parts, Africans drag a branch round the village to protect it from evil spirits, as it is believed to keep evil spirits away. Wood from this tree is used for timber, wagon making and fence posts as it yields a yellow, fine-grained, heavy wood which contains 12.2-15.7% tanning matter [5].The elasticity of the shoots makes it suitable for bows and whip sticks. Some African tribes use the thorny branches to make kraals or hedges. This protects their livestock from lions and other predators. [6]

Fig.3: Fruits of *Ziziphus mucronata*

Some other Ziziphus speceis and their medicinal activity are given below:

Zizyphus jujuba. - Fruit is edible; honeybees visit flower, analgesic, anti-diabetic and antipyretic.

Zizyphus mauritiana, - Tonic, antibacterial, antipyretic, analgesic, bronchodilator, emollient, anti-vomiting, sedative and abdominal pain in pregnancy [7].

Ziziyphus nummularia -Tonic, antipyretic, antibacterial and analgesic and anti-diabetic.

Zizyphus oxyphylla - Digestive disorders, weakness, liver complaints, obesity, urinary troubles, Diabetes, skin infections, fever, diarrhea and insomnia [8].

Zizyphus rugose – Body ache [9]

Zizyphus spina-christi - It is used in traditional folk medicine for treatment of some diseases such as (Baluchistan) stomach pain and other gastrointestinal tract ailments, diabetes and diarrhea. It is believed that its leaves have blood pressure reduction properties as well [10].

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Zizyphus sativa - Fruit is edible, anti-diabetic, used for cough and fever, honeybees visit flowers.

Protocol of the study

The study protocol consists of the following steps:

Collection of the fruits drying and pulverization.

Extraction of the fruits parts with ethanol solvent.

Filtration of the fruits by using filter paper and cotton and subsequently through solvent evaporation.

Test for analgesics activity.

Test for anti-inflammatory activity.

MATERIALS AND METHODS

In vivo Analgesic investigation

Formalin - induced paw licking in mice:

Principle:

In this method, formalin is given to the sub plantar region of the experimental animals. As a result, paw licking occurred to the animals. Any substance that has got analgesic activity is supposed to lessen the paw licking of animals within in a given time frame and with respect to the control group. The paw licking inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Diclofenac was used to serve the purpose.

Procedure of formalin induced paw licking test:

At zero hour saline water to Group I, Diclofenac to Group II & test samples to Group III were administered orally by means of a long needle with a ball-shaped end.

After 30 minutes formalin (2%) was administered to each of the animals of all the groups.

The thirty minutes interval between the oral administration of test materials and administration of formalin was given to assure proper absorption of the administered samples.

The number of paw licking was measured in each mouse from 0-5 minutes and 20-30 minutes.

The number of paw licking in first 5 minutes indicate response to neurogenic pain and The number of paw licking in first 20-30 minutes indicate inflammatory pain.

Counting of Licking:

Each mouse of all groups were observed individually for counting the number of licking they made in first 5 minutes and 20-30 minutes after the administration of formalin .

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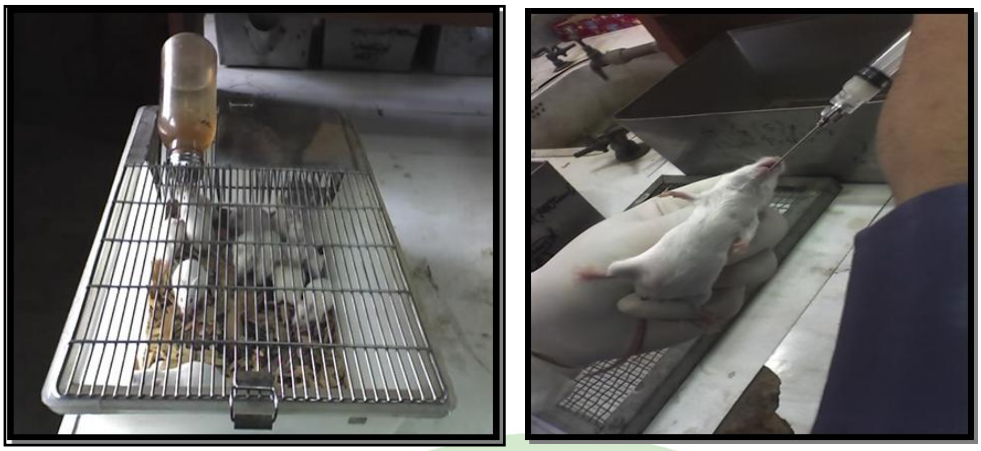

Fig. 4: Some Experimental Work in Lab

Eddy's hot plate method

Principle:

In this method, the experimental animal is placed on the hot plate at 55˚C. As a result, Mice will lick the paw or jump from the hot plate. Any substance that has got analgesic activity is supposed to lessen the paw licking or jumping of animals within in a given time frame and with respect to the control group. The paw licking or jumping inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, diclofenac was used to serve the purpose.

Counting of Licking or jumping:

Each mouse of all groups were observed individually for counting the number of licking or jumping they made at 0, 1, 2, 3, 4 and 5 hour after the respective treatment

Tail immersion method:

Principle:

In this method, the tip of tail of the mice is dipped up to 5 cm in hot water maintained at 58˚C. As a result, mice will withdraw its tail from the hot water and it is noted as the reaction time. Any substance that has got analgesic activity is supposed to lessen the withdrawal time within in a given time frame and with respect to the control group. The withdrawal time inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Diclofenac was used to serve the purpose.

Designing of the Experiment and Identification of Animal

Twelve experimental animals were randomly selected and divided into three groups denoted as,

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- Group-I: Normal Control group
- Group-II: Standard control group &
- Group-III: Drug control group

Consisting of 4 mice in each. Prior to any treatment, each mouse was weighed properly. Each group received a particular treatment i.e. Group-I was served as the control and received only saline water. Group-II was received the standard drug (diclofenac) for comparison of potencies. The last group i.e. Group-III was administered orally with the crude extract suspensions.

Each group consisted of 4 mice. As it was difficult to observe the biologic response of 4 mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were marked as M-1= Mice 1, M-2= Mice 2, M-3= Mice 3 & M-4= Mice 4.

Procedure of Anti-inflammatory activity:

At zero hour saline water to Group I, Diclofenac to Group II & test samples to Group III were administered orally by means of a long needle with a ball-shaped end.

⇩

After one hour all the animals were received carrageenan 0.1 ml/ mice.

⇩

The animals were kept in separate transparent plastic container for observation.

⇩

The inhibition of paw volume was assessed each hour for 5 hour.

RESULT AND DISCUSSION

Result of anti-inflammatory activity test of *Ziziphus mucronata*

The result of anti-inflammatory activity of *Ziziphus mucronata* Fruit is shown. The ethanolic extract of *Putranjiva Ziziphus mucronata* fruit at the doses 250mg/kg, 200 mg/kg-body weight& 100mg/kg body weight showed highly significant anti-inflammatory effects compared to control on carrageenan induce paw mice edema method in mice.

Table 1: Anti-inflammatory effects of ethanolic extracts of *Ziziphus mucronata* fruit by carrageenan induce paw mice edema method in mice

Animal group	Dose	Number of mice	edema				
Normal control group	1ml/kg	Mice	0hr(mm)	1hr(mm)	2hr(mm)	3hr(mm)	4hr(mm)
		1	31	31	32	30	31
		2	28	28	30	31	30
		3	31	31	30	32	29
		4	33	33	32	30	29

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		Mean	30.75	30.75	31	30.75	29.75
		SD	2.06155	2.06155	1.1547	0.95743	0.95743
		SEM	1.19024	1.19024	0.66667	0.55277	0.55277

Animal group	**Dose**	**Number of mice**	**Edema**				
Standard control group(received diclofenac)	**10mg/kg body**	**Mice**	**ohr(mm)**	**1hr(mm)**	**2hr(mm)**	**3hr(mm)**	**4hr(mm)**
		1	32	27	28	28	28
		2	33	24	32	31	30
		3	35	25	33	27	28
		4	31	31	31	28	28
		Mean	32.75	26.75	31	28.5	28.5
		SD	1.70783	3.0957	2.16025	1.73205	1
		SEM	0.98601	1.7873	1.24722	1	0.57735
			-6.5041	13.0081	0	7.31707	4.20168

Animal group	**Dose**	**Number of mice**	**Edema**					
control group(received fruit extract of ziziphus mucronata	200mg/ kg body	**Mice**	**0hr(mm)**	**1hr(mm)**	**2hr(mm)**	**3hr(mm)**	**4hr(mm)**	**5h(mm)**
		1	39	32	29	29	29	28
		2	39	36	30	31	31	30
		3	37	37	32	32	31	30
		4	31	31	31	30	30	29
		Mean	39.5	34	30.5	30.5	30.25	29.25
		SD	2.87228	2.94322	1.29099	1.29099	0.95743	0.82916
		SEM	1.65831	1.69967	0.74536	0.74536	0.55277	0.40825

Discussion on Analgesic activity test

The ethanolic extracts of plant (*Ziziphus mucronata)* were evaluated in Formalin induce paw licking method, Tail immersion method & Eddy's hot plate method for its analgesic activity.

In Formalin induce paw licking method *Ziziphus mucronata* reduces 65.9091%inflammatory pain at dose 200mg/kg body weight & 60% neurogenic pain at the same dose .But at dose 100mg/kg body weight shows the same analgesic effect (reduces 65.9091% of inflammatory & 40% of neurogenic pain) compared to control group.

In tail immersion method the analgesic activity of Ziziphus *mucronata* starts after 1 hr & continued till 2 hr. Its activity gradually decrease after 2 hr at dose 250mg/kg. At 500mg/kg dose analgesic activity shows peaks in the 2nd hr (75%) & decreases after 2 hr.

In Eddy's hot plate method *Ziziphus mucronata* maximum analgesic activity shows at 2nd hr(54.16%) then decreases at dose 500mg/kg body weight & at 250mg/kg body weight analgesic activity starts from 2nd hr (50) & continued in the 3rd hr, after 3hr its activity gradually decreases.

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So, the results found in the present study demonstrated that the Fruit extracts of *ziziphus mucronata* has analgesic effects.

Discussion on Anti-inflammatory test:

Anti-inflammatory activity of *Ziziphus mucronata* shows in significance anti-inflammatory activity evaluated by Carrageenan induced paw edema in mice.

It has anti-inflammatory activity compared to the normal group. It gives its highest peaks (2.41935) at 2hour at 250 mg/kg-body weight. At 200 mg/kg start to shows activity (1.6129%) also from 2nd hour & become to reduce from the next hour compared to the normal group.

CONCLUSION

Based on the results of our study it can be concluded that the ethanolic extract of *Ziziphus mucronata* Fruit possess significant analgesic effects in all Three methods (Formalin induced paw licking method, Eddy's hot plate and Tail immersion method) done for analgesic activity. Further studies have to be carried out to identify the phyto-constituent responsible for the exact and detailed mechanism of action responsible for this activity. The results of the present study indicates that ethanolic extract of *Ziziphus mucronata* Fruits has insignificant anti-inflammatory activity evaluated by Carrageenan induced paw edema in mice only about 2.41935% at 250mg and 1.6129% at 200mg/kg body weight, which deserves further studies to establish its therapeutic value. All of the experiments were performed in multiple dose & further studies have to be carried out know its constituents responsible for activity & to evaluate other therapeutic effects.

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