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**PHARMACOLOGICAL AND PHYTOCHEMICAL STUDIES OF ETHANOLIC
EXTRACT OF *ZIZIPHUS OXYPHYLLA* ROOT**

**SHAIRBAZ KHAN¹, SHAFI MUHAMMAD¹, ABDUL JABBAR¹, TAUFIQUE AHMAD²,
AMJAD HUSSAIN² AND SULTAN AYAZ³**

1: Department of Pharmacognosy, Faculty Pharmacy and Health Sciences, University of Balochistan,
Quetta

2: Department of Eastern Medicine, University of Balochistan, Quetta

3: Department of Eastern Medicine, Government College University Faisalabad

***Corresponding Author: E Mail: shairbazhasni88@gmail.com**

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ABSTRACT

Ziziphus oxyphylla is locally known as gargola. It is found in district Barkhan, Balochistan. Current study involves phytochemical, neuropharmacological evaluation of root of *Z. oxyphylla*. In Phytochemical investigation alkaloids, flavanoids, glycosides, saponins, steroids and tannins were present. At 250 and 500 mg/kg oral doses of crude extract showed significant ($p < 0.05$) analgesic effects. In neuropharmacological activities plant showed significant CNS depressant activity. These Pharmacological activities confirm the traditional claim of the plant.

Keywords: Phytochemical, Neuropharmacological evaluation, *Ziziphus oxyphylla*

INTRODUCTION

Medicinal plants are well known source of drug for human being. Medicinal plant are traditionally use for the treatment of many diseases [1]. Plants are primary source of drugs [2]. Currently Asian countries use herbal medicines for healthcare purposes.

Medicinal plants consist of various kinds of chemical compounds that are divided into primary and secondary metabolites. Therapeutics properties are present in secondary metabolites [3]. Natural products

are studied to find out novel and beneficial therapeutic agents [4].

Rhamnaceae family consists of 55 genera [5]. *Z. oxyphylla* belongs to Rhamnaceae family [6, 7]. Traditionally this plant is used for the treatment of fever, diabetes, rheumatic pain, inflammatory conditions and sleep disorders [8]. Its fruits are used as expectorant and softening effect [9]. Current studies are carried out to rationalize the traditional uses of the plant.

MATERIAL AND METHOD

Plants material

The plant material is collected from Barkhan district of Balochistan, Pakistan. Plant was identified and voucher specimen no SB-139 was deposited at pharmacognosy Department, University of Balochistan Quetta.

Extraction procedure

Plant material were dried and soaked in ethanol for 7-15 days. The solvent was evaporated by using rotary evaporator having 40°C temperature at reduced vacuum pressure. Blackish brown semisolid residue was obtained.

Experimental animals

Albino mice (male & female) were used for study. The mice were kept according to standard protocol at animal house of Faculty

of Pharmacy, University of Balochistan Quetta.

Phytochemical tests

Test for saponin

In a test tube, 1 g of extract was mixed with 10 ml of distilled water. Mixture was stirred for 2 minutes. Formation of 2cm layer of froth confirms the presence of saponin [10].

Test for flavonoids

1. For this test 1g of plants extract was mixed with magnesium (Mg) and concentrated hydrochloric acid (conc HCl) was added drop wise. Presence of flavonoid was confirmed by appearance of pink colour in test tube [11].
2. 2g of plants extract and 10 ml of distilled water was mixed in a test tube and then few drops of lead acetate was added. Formation of yellow precipitates taken as evidence for the presence of flavonoids [12].

Test for alkaloids

In a test tube 2 ml of 1% hydrochloric acid (HCl) and 1 g of plant extract was mixed and this mixture was heated on burner. Wagner and Mayer reagent was added to this mixture. Turbidity of precipitates was regarded as positive for alkaloids [13].

Test for tannins

1g of plants extract and distilled water was mixed and then boiled. Few drops of ferric chloride were added. Appearance of Greenish brown precipitates was regarded as positive for the presence of tannins [14].

Test for glycosides

2g of plants extract and distilled water was mixed vigorously. Few drops of hydrochloric acid were added and then 2 ml of sodium-nitroprusides was added. Red colour confirms the presence of glycosides [15].

Test for steroids

2g of drug was dissolve in 20 ml of distilled water. Chloroform was dissolve in this mixture and concentrated sulphuric acid (conc H₂SO₄) was poured by side of test tube. Red colour was formed later on it was turned into yellowish green colour which confirmed the presence of steroids [16].

Central Nervous System (CNS) depressant activities

The ethanolic extract was evaluated for CNS depressant activities by using open field, cage cross method, rearing test, traction test and force swimming test. The animals were categorized into four groups (each group consist of 5 mice).

Group i: control

Group ii: *Z. oxyphylla* 250mg/kg

Group iii: *Z. oxyphylla* crude extracts 500mg/kg

Group iv: standard drug 2mg/kg (Diazepam)

Open field test

Open field test was used to determine the locomotory and exploratory activities. The plastic apparatus consist of four walled of thirty centimeter (30cm), floor of which has twenty five square of 10 cm width and length (10x10). After 30 minutes of oral administration of Vehicle, *Z. oxyphylla* extract (250 and 500 mg per kg) and diazepam, the mice were observed for 10 minutes in open field apparatus [17].

Rearing test

Central excitatory behavior was determined in this test. The apparatus made of open top clear plastic was used. After administration of plant extract (250& 500mg/kg) to mice its forelimb dragging against the wall of the cylinder was recorded for 10 mints [18].

Cage crossing test

Locomotor activity of mice is checked by cage crossing test. A standard cage (26x 26 x26 dimension,) made of plastic is used for this test [19]. Cage crossing by mice was recorded for 10 minutes.

Traction test

For this test non-slippery surfaced, 1 m long and 3 cm diameter rod and 15cm above the base was used [20.21]. Duration of rod crossed by mice was calculated in seconds after 30 minutes of dose administration.

Force swim test

Force swim test or behavior despair test was used to check the antidepressant activity. Cylindrical transparent apparatus with 25cm length and 10 cm width was used. 25 °C temperature was sustained depth of water was 18 cm [22]. Mice were allowed to swim in this water for 6 minutes. Mobility and immobility of mice was calculated with the help of stop watch after 30 minutes of administration of vehicle, sample drug and standard drug.

Analgesic activity

Acetic acid induced writhings

Pain was generated in mice through acetic acid. 0.6% acetic acid was injected intraperitoneally in all mice [23] after 30 minutes of administration of vehicle, sample drug and standard drug. After administration of acetic acid writhes were calculated for 30 minutes.

Formalin test

Formalin is a noxious stimulator to check the analgesic effect. 20 μ l 1% formalin in distilled water was injected dorsally through 100 μ syringe to right hind paw of all selected mice for this test [24.25]. After 30 minutes of administration of plant extract, standard drug i.e. diclofenac sodium and normal saline licking and biting of mice was calculated for 1st and 2nd Phases.

Statistical analysis

Statistical tests described by Jabbar *et al.*, (2016) were followed.

RESULTS

Phytochemical test

The phytochemical analysis of plant extract was positive for the presence of alkaloids, flavanoids, glycosides, saponins, steroids and tannins (table No1).

Open field test

In control group the open field activities were 164 \pm 1.48. After administration of 250mg/kg crude extract of *Z. oxyphylla* the open field activity were 78.6 \pm 1.91. After administration of 500mg/kg crude extract of *Z. oxyphylla* the open field activity were 58.4 \pm 1.07. After administration of standard drug the open field activity was 52 \pm 1.41 (Graph 1).

Rearing test

In control group the rearing activity was 35.4 \pm 1.63. After administration of 250mg/kg crude extract of *Z. oxyphylla* the rearing test activity was 18 \pm 1.26. After administration of 500mg/kg crude extract of *Z. oxyphylla* the rearing test activity was 12 \pm 1.3. After administration of standard drug the rearing test activity was 20 \pm 1.41 (Graph 2).

Cage crossing

In control group the cage cross activities were 31.2 \pm 0.91. After administration of

250mg/kg crude extract of *Z. oxyphylla* the cage cross activity was 16.8 ± 1.16 . After administration of 500mg/kg crude extract of *Z. oxyphylla* the cage cross activity was 13.4 ± 0.92 . . After administration of standard drug the cage cross activity was 11 ± 0.95 (Graph 3).

Traction

In control group results for traction activity was 8.4 ± 1.25 (sec). After administration of 250mg/kg crude extract of *Z. oxyphylla* the traction test activity was 9.6 ± 1.50 (sec). After administration of 500 mg/kg crude extract of *Z. oxyphylla* the traction test activity was 12.4 ± 1.33 (sec). Results for standard drugs were 9 ± 0.63 (sec) (Graph 4).

Force swim test

Mobility time

In control group the mobility time was 170 ± 1.02 seconds. After administration of 250mg/kg crude extract of *Z. oxyphylla* the mobility time was 194 ± 1.56 seconds. After administration of 500 mg/kg crude extract *Z. oxyphylla* the mobility time was 204 ± 0.89 seconds. After administration of standard drug mobility was 130 ± 1.30 seconds

Immobility time

In control group the immobility time was 190 ± 1.02 seconds. After administration of crude extract of 250mg/kg the immobility time was 166 ± 1.63 seconds. After administration of

500mg/kg crude extract of *Z. oxyphylla* the immobility time was 156 ± 0.89 seconds. After administration of standard drug the immobility time was 230 ± 1.30 seconds s(Graph 5).

Analgesic Activity

Acetic acid induced writhings

Analgesic effects are analyzed by using standard procedure, there were 124 ± 1.30 numbers of writhes of control group. After administration of 250mg/kg crude extract of *Z. oxyphylla* there was 91.8 ± 1.39 numbers of writhes. After administration of 500mg/kg crude extract of *Z. oxyphylla* there was 59 ± 1.41 numbers of writhes. After administration of standard drug there was 74 ± 1.41 numbers of writhes (table 2).

Formalin test

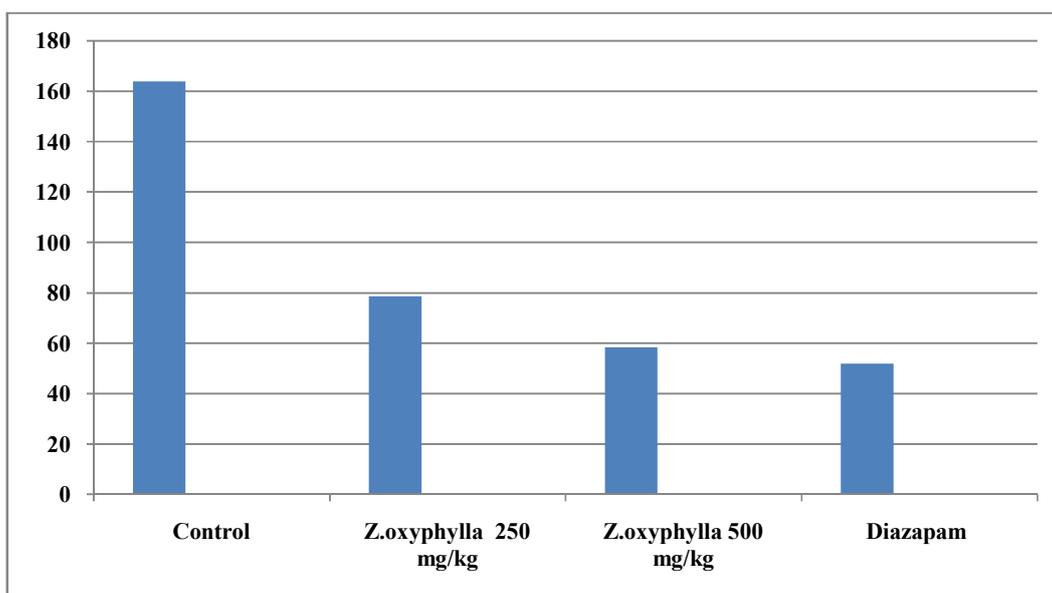
Numbers of licking and biting

Analgesic effects are analyzed by using standard procedures. For control group the number of licking and bites were 50 ± 1.14 and 71 ± 1.79 for first and second phase respectively. After administration of 250mg/kg crude extract the number of licking and bites were 41 ± 1.30 and 51 ± 1.22 for first and second phase respectively. After administration of 500mg/kg crude extract the number of licking and bites were 35 ± 1.52 and 49 ± 1.41 for first and second phase respectively. After administration of

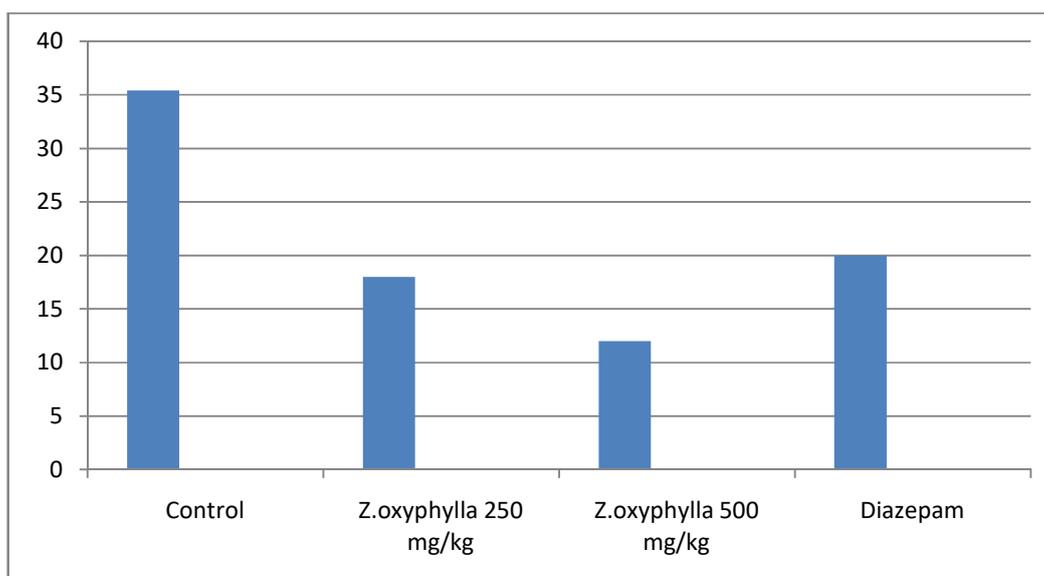
standard drug the number of licking and and second phase respectively (table 3). bites were 21 ± 1.58 and 17 ± 1.73 for first

Table 1: Results for phytochemical analysis Root extract of *Z.oxyphylla*

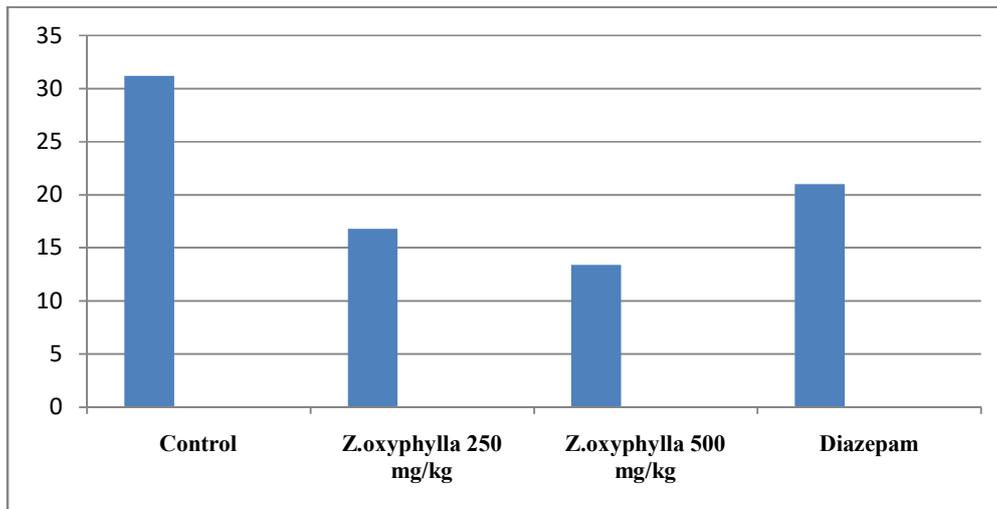
Chemical constituents	Detection
Alkaloids	Positive
Flavonoids	Positive
Glycoside	Positive
Saponins	Positive
Steroids	Positive
Tannins	Positive



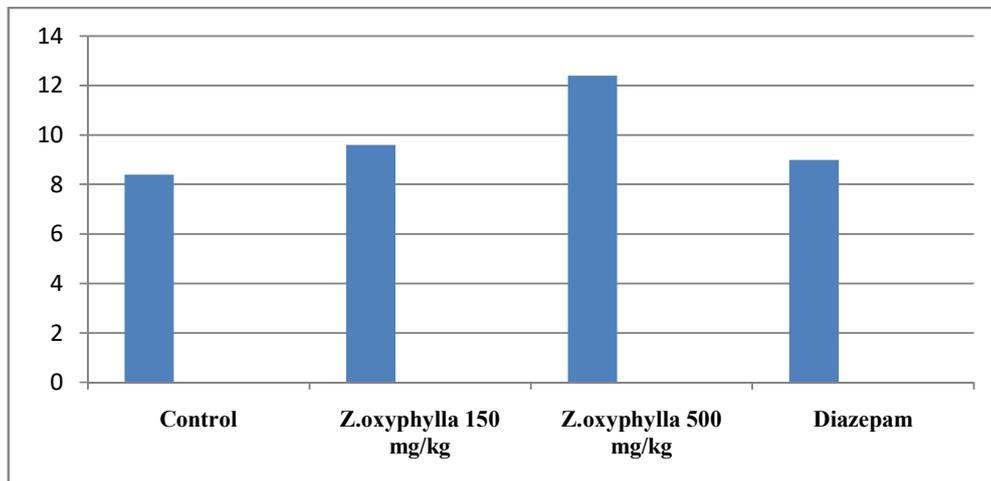
Graph No 1 open Field activity



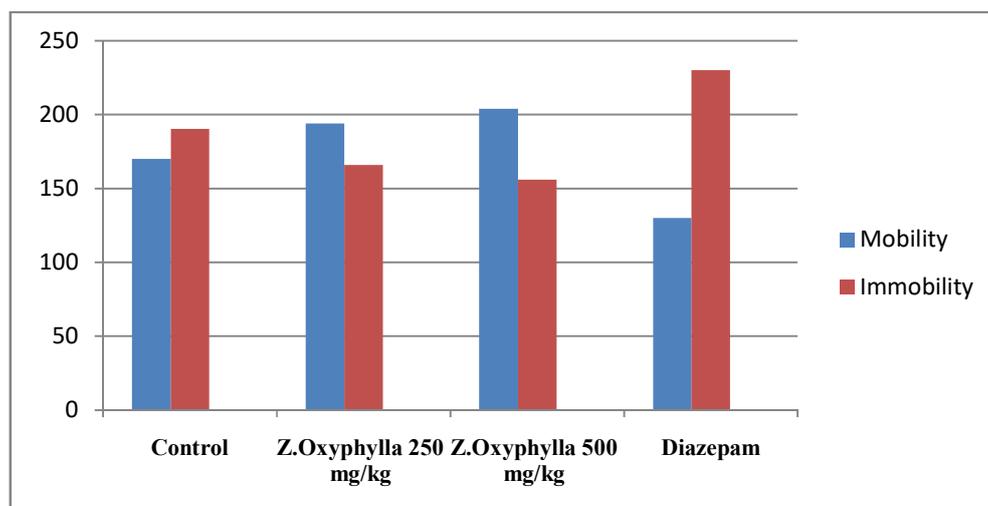
Graph No 2 rearing activity



Graph No 3 cage crossing activity



Graph No 4 traction activity



Graph No 5 Force swim activity

Table 2: Results for acetic acid induce analgesic effect

S#	Treatment	No. of writhes Mean±SEM
1	Control	124 ± 1.30
2	250mg	91 ± 1.39
3	500mg	59 ± 1.41
4	Standard drug	74 ± 1.41

Table 3: Results for Formalin test

S#	Treatment	Number of licking and biting Mean±SEM	
		1 st phase	2 nd phase
1	Control	50 ± 1.14	71 ± 1.79
2	250	41 ± 1.30	51 ± 1.22
3	500	35 ± 1.52	49 ± 1.41
4	Standard	21 ± 1.58	17 ± 1.73

DISCUSSION

Medicinal preparation of many plants is used in different ways and procedures for the treatment of different diseases. Many patient use natural herbs for their self treatment against many diseases [26]. The phytochemical screening of *Z. oxyphylla* extract of roots regarded as positive for the presence of saponin, alkaloids, glycosides, tannin, flavanoid and terpenoids. These all secondary metabolites are well known for having medicinal effects [27]. Many of the herbal products are used for the treatment of anxiousness, insomnia and other abnormalities. These preparations contain active ingredients and constituents that affect the ionotropic receptors neurotransmitters i.e GABA and serotonin in the brain. Depressive and anxiety disorders are the most common psychiatric disorders. CNS depressant agent from natural product is beneficial in these conditions [28]. There was significant

($p < 0.05$) decrease in cage crossing, tractions, force swimming, open field and rearing activities as compared with standard drug. Results reveals that *Z. Oxyphylla* have sedative effects. Recent studies reveal that sedative activity is due to presence of glycosides, alkaloids and flavonoids [29]. The studies showed that flavonoids have ability to bind to benzodiazepine site of GABA receptors. Phytochemical tests confirms the presence of Flavonoids along with other constituents, it is proposed that the sedative effect may be due to presence of flavonoids.

Decrease in the pain by ethanolic extract of the plant was dose dependent. Bradykinin, Histamine, Prostaglandin E2 and substance P are responsible for activation of nociceptive receptor to generate a pain. Pain is decreased when the release of prostaglandins and other mediators are blocked [30]. *Z. oxyphylla* extract showed significant result against

acetic acid induced pain and formalin induced licking of paws of mice. It is hypothesized that analgesic effect is may be due to presence of secondary metabolites.

CONCLUSION

The results show that crude extract of *Z. oxyphylla* has significant CNS depressant analgesic effects. However further studies are required on large number of animals models.

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