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NEURO AND ANALGESIC ACTIVITIES OF OLIVE LEAF METHANOLIC CRUDE EXTRACT

Sami Ullah[1*], Javeid Iqbal [2], Aman Ullah Khan [3], Tajala Aman [4], Ghulam Razaque [5]

[1]PhD Scholar, Pharmacology, University of Balochistan, Quetta
[2]Professor, Pharmacology, University of Balochistan, Quetta
[3]Director, Drug Testing Laboratory, Quetta
[4]Medical Student, Quetta
[5]Assistant Professor, University of Balochistan Quetta

Abstract

Genus Olea (Oleaceae) are prominent for its therapeutic value and are comprised in British and Indian pharmacopeias. In Pakistan Olea europaea leaves widely spread in Baluchistan province, NWFP, Punjab and in northerly areas at increase of 900 to 2900 m. In Balochistan it is found in sherani and zirat districts. Olea europaea leaves have multipotential remedial properties Present study was conducted for the investigation of some neurologic, properties of medicinal plant olea europea (oleaceae). The crude methanolic herbal extract was evaluated for its Neuropharacological aspects by forced swimming, cage crossing, rearing, traction and open field activity. The extract showed considerable Neuropharacological property. Moreover the crude methanolic extract was tested for its anti-pain activity by formalin induced pain and acetic acid induced pain test. The crude extract was sufficiently and significantly active to induce analgesia.

Key words: ***Methanolic, Crude Extract,*** *Olea europaea leaves*

Corresponding author:

Sami Ullah,
PhD scholar,
Pharmacology,
University of Balochistan,
Quetta.
samiullah9000@gmail.com

QR code

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INTRODUCTION:

Remedial plants are familiar for the ameliorative purposes or for the synthesis of beneficial medications. Therapeutic plants are effortlessly open precursor for wellbeing reason in rural and associated area.[1]. These days, foodstuff is not the foundation of nutrients but also a potent medication [2]. Even as methods of medications, during the centuries plants assume an essential part in the wellbeing issue .[3]. Around (30%) yields existing in the market are set up from plants [4]. The American consumer paid 3 million dollars for drugs obtained from higher plants [5]. In China old treatment are focused for treating 90% of country and 40% urban patients [6, 7]. n 1991 around (700,000) tons of plants accustomed in pharmaceuticals out of which 80% were gained from the land whereas [8] In India, 400,000 enrolled worthy medicinal specialist, contrasted with (332,000) enlisted specialists [9]. Pakistan is a nation loved with exhibit of atmosphere, environmental zones and land area and have distinctive biodiversity, wide-ranging of plant species. About 6000 plants species with therapeutic properties are found in Pakistan [10]. The plants species are still commonly utilized for helpful purposes by indigenous people in their routine lives[11]. A total of 1572 genera and 5521 species recognized in Pakistan, out of which 400–600 are therapeutically significant [12]. About 400 species are endemic to Pakistan [13]. In Pakistan the bounded communities of altered regions accept centuries old awareness and traditional practices of a lot of the plants. This native knowledge of medicinal plants has been conveyed from generation through articulate advice and personal acquaintance [14-17]. Baluchistan is the main province expressive 43.6 per cent of the land accumulation of Pakistan [18]. The rural residents of the Baluchistan are very much abased on biological assets for their nourishment. Among these rural populaces, remedial plants are the most suitable solution for plentiful of the health problems [19].

The highlands of N. Baluchistan (North) are the warm spots of healing and widespread plant in Pakistan, In Balochistan and numerous remedial plants have been collected and flogged in the native marketplace by local public so healing plants are limited only in endangered areas[20].Moreover, over misuse of medicinal plants affected serious risk to the existence and re-generation of countless curative plant species[21]The widespread species that which are used in traditional medication in the region lack in pharmacological studies. In this regard present study is designed to examine the pharmacological properties of *Olea europaea leaves [22]*

Genus Olea (Oleaceae) are prominent for its therapeutic value and are comprised in British and Indian pharmacopeias[23]. In Pakistan *Olea europaea leaves* widely spread in Baluchistan province, NWFP, Punjab and in northerly areas at increase of 900 to 2900 m[24]:.[12] In Balochistan it is found in sherani and zirat districts (Ref…….). *Olea europaea leaves* have multipotential remedial properties and for the treatment of eye illnesses menorrhagia, chronic diarrhea, febrifuge and piles while root for jaundice and stem for diabetes, broken bones wounds, ulcers and sore eyes, stomachic antihistaminic, antipyretic, astringent, diaphoretic, antispasmodic and properties [25]. Its fruit is used as a tonic against liver and heart diseases [26].*Olea europaea leaves* is reported to contain various types of plant secondary metabolites including alkaloids diarylheptanoids, flavonoids terpenoids, steroids, phenols and tannins [27].

MATERIAL AND METHOD:

Plant Material

The plant's fresh leaves were collected from the Ahmadedergah (village) near to Tahkhta Suleiman District Sherani Balochistan Pakistan.

Preparation of Extract

The leaves were wash with tap water kept for drying under shade then powdered in fine particles and extracted with methanol by maceration process at room temperature for (15) consecutives days with periodically shaking as well as stirring. The Methanolic extracts(ME) were filtered through wattman filter paper and concentrated on rotary evaporator, dark brown semisolid extract was obtained.

Animals

The Male mice of weight about (23-30 g) were used from Center for advance studies vaccinology and Biotechnology research (CASVAB), University of Balochistan Quetta. The animals were kept under standard protocol of relative, temperature (22±1ºC) humidity, 12 hrs light/dark cycles and provided with water and food *ad libitum* at the Laboratory (CASVAB).

Drugs and Chemicals

Diazepam (Martin Dow Pakistan) and Diclofenac Sodium (Hisun Pharma, Pakistan) Acetic Acid (Merck) and Formalin (Merck) were used in experiments. All drugs and chemicals used were of analytical reagent grade.

Neuro-pharmacological activities

The (CME) was assessed for its Neuropharacological activities by using cage cross, traction test, open field, swimming test, and rearing test. Animals were arranged in four groups (control group treated with 0.5 ml/kg saline, B. lyceum (CME) 250 mg and 500mg/kg and standard drug treated groups).

Open field test

The (OFT) is classically used to evaluate anxiety and also be used for common assessment of animal basal locomotors activity and examination in rodent was carried out through methods designated by Buccafusco [28]:[29] The (OFT) of square meter was alienated into some squares. The device was surrounded a with 40 cm top wall. After thirty minutes of oral administration of crude extract, the number of squares visited by the mice was noted 30 min.

Rearing test

A behavior test. 1000 ml glass beaker was used in this study. The observation was evaluated to count the upward movements of the mice locating the body in an erect posture in the beaker. The [30]:[31]observation were analyzed for 10 minutes (Qureshi *et al*., 2015).

Traction test

This test was to calculate the time spend by the mice to covered an iron rod of 1 meter length. At first, the mice were skilled to covered iron rod. Any difference in time taken by the treated mice compared to the control mice to travel the iron rod describes the calming or stimulating result of the drug, respectively[32]: [33]

Cage crossing movement test

The study was made on animal in an accurately advised container of rectangular form. Both treated and control animal were kept in cage and their cage crossing travels were commended in (10 min). The investigation is significant for the motor action in an animals. This study was accomplished that is reported by [34]

Force induced-swimming test (FST)

This test was done as directed by [35].This test is used to examine CNS and muscle activity of the crude extract. Animals were positioned alone for period of six (Min) in the glass tub full with water at room Temp. Mice were placed alone for six minutes up to the level marked. Mouse when placed in the tub quick starts to move its front and hind paws. The action time period of the animal is evaluated by a stop watch out of a total observation time of 6 minutes.

Analgesic activity

Acetic acid-induced writhing test

The peripheral Analgesic activity of the (MCE) was evaluated by acetic acid-induced writhing test. The animals were distributed into, reference drug, control group and test groups (n = 5). Acetic acid (0.7%) was intraperitoneally (i.p) introduced to each mouse after 30 min of oral administration of the extract , vehicle and standard drug(Diclofenac sodium). Mice were observed for writhing (abdominal tightening, elongation of the body, and extension of the hind limb) for the 30 minutes[36].

Formalin test

The test was performed as reported by (Dubuisson & Dennis, 1977:[37, 38].30 minutes after administration of vehicle, crude extract and standard drug, 20 µl of 5% formalin was introduced subcutaneously into the right hind paw of mice. The time spent (seconds) on biting and licking and number of biting and licking of the inserted paw for first phase (0-5 minutes) and second phase (16-30 min) were observed (refe>>>>)

RESULTS:

Open Filed Test (OP)

The average outcome of the test indicate that mean number of squares blocks crossed for control group were 215.2±4.54, for crude extract of *Olea europaea leaves* 250mg/Kg 193.6±4.43, for 500 mg/Kg 199.8±3.22 and for standard drug (Diazepam) treated group 98.2±4.13. In this test significant ($p<0.05$) result were observed in all of the drug doses and results were significant as compared with control group.

Rearing Test

The results of the test suggest that mean number of rising moving efforts of control group was 45 ±16.4, for *Olea europaea leaves* 250 mg/kg 15±1.79, for 500 mg/Kg 11.16±1.72 and for standard drug group (Diazepam) 5.4±2.75. Results of experiment reveal that all the doses of *Olea europaea leaves* produced significant ($p<0.05$) results, which grounds for steady declined in rearing activity at both doses and the results were comparable with standard drug diazepam.

Cage Crossing Test

The result of the test indicate that mean number of parallel and upright crossings were 29±1.41 for control group, for of *Olea europaea leaves* 250 mg/Kg 12.6±0.81, for 500mg/Kg 17.6±1.97and for standard drug (Diazepam) treated group 15.8±1.39. Results show that 500 mg/K of *Olea europaea leaves*

250 produced significant results ($p<0.05$) that causes reduced cage crossing activity as compared with the standard drug

Traction Test

The result of the test indicate that mean time for crossings steel rod was 8±9.06 for control group, for *Olea europaea leaves* 250 mg/Kg value was 11±1.7, for 500 mg/Kg 13.6±1.29 and for standard drug (Diazepam) treated group 18.4±1.07, Throughout the traction test administration of *Olea europaea leaves* increased the traveling time, which shows that the drug has sedative effects, results were comparable with standard drug.

Forced Swimming (Mobility Time) Test

The results of the experimentation signify that, for control group mean time consumed for mobility was3.25±0.009 minutes, and mean time consumed for immobility was 2.35±0.009, for *Olea europaea leaves* 250 mg/Kg mobility time was 3.38±0.01 minutes and immobility time was 2.22±0.014, for 500 mg/Kg mobility time was 3.39±0.02 and immobility time was 2.21±0.01 . For Diazepam (standard drug) group mobility time was 2.19±0.02 minutes immobility time was 3.41±0.02 The results of the experiment signify that, for of *Olea europaea leaves* 250 and 500 mg/Kg produced anxiolytic effect.

Table 1: Effect of *Olea europaea leaves* on Neuropharmacological activities of mice

Treatment	Dose	Open field activities Mean±SEM	Cage crossing activities Mean±SEM	Rearing activities Mean±SEM	Traction activities Mean±SEM
Control	0.5ml saline/kg	210.2±3.34	31±1.31	49 ±13.4	9±7.04
Olea europaea leaves Crude extract	250mg/kg	184.6±4.43	11.8±0.92	13±1.69	12±1.4,
	500mg/kg	187.5±2.83	15.9±1.94	10.19±1.83	12.9±1.45
Diazepam	2mg/kg	101.0±3.65	16.1±1.45	6.1±1.99	17.3±1.87

Table 2: Effect of *Olea europaea leaves* on forced swimming test of mice.

Treatment	Dose	mobility time Mean ±SEM (minutes)	Immobility time Mean ±SEM (minutes)
Control	0.5ml/kg	3.25±0.009	2.35±0.009
Olea europaea leaves	250mg/kg	3.30±0.014	2.30±0.014
	500mg/kg	3.42±0.02	2.18±0.01
Diazepam	2mg/kg	2.10±0.02	3.50±0.02

Table 3: Effect of *Olea europaea leaves* on formalin test of mice.

Treatment	Does mg/Kg	1st Phase (Mean ±S.E.M)		2nd Phase (Mean ±S.E.M	
		No of Licking	Time spent sec	No of Licking	Time spent sec
Control	0.5ml/kg saline	35.1+9.10	42+4.14	50.3+3.99	13.0+12.99
Olea europaea leaves	250mg/kg	33.2+2.7	45.2+2.14	44.6+3.00	81.3+14.22
Olea europaea leaves	500mg/kg	21.6+3.00	46+3.31	58+5.39	42.2+7.7
Sodium declofenic acid	10 mg/kg, oral	33+1.76	38+1.54	60.0+0.6	52.7+0.56

Table 4: Effect of *Olea europaea leaves* on acetic acid induced writhing test of mice.

Treatment	Dose (mg/kg)	Mean Number of writhing
Control	0.5ml/kg saline	75.0±2.55
Olea europaea leaves	250 mg/kg	29.2±2.43
Olea europaea leaves	500 mg/kg	27.4±1.97
Sodium Declofenic acid	50 mg/kg,.)	36.5±0.83

Analgesic Activity

Formalin Test

The results of Phase-One (0-5 Min) of the test show for control group, mean number of biting and licking efforts were 40.2+8.33 and time consumed on licking and biting was 48+5.39 and in 2nd phase number of biting and licking were 60.6+3.86 and time consumed on licking and biting was 124+14.87 (seconds), for *Olea europaea leaves* 250 mg/Kg number of biting and licking were 22.2+3.4 and time consumed on licking and biting was 55.2+3.11 (seconds), and in 2nd phase number of biting and licking were 37.6+3.96 time consumed on licking and biting was 71.6+12.59(seconds) for 500 mg/Kg number of biting and licking were 20.8+3.22 and time consumed on licking and biting was 43+4.42 (seconds) and in 2nd phase number of biting and licking 57+6.47 and time consumed on licking and biting was 43.2+6.5 (seconds) for standard drug (Diclofenac Sodium)treated group number of biting and licking 30+2.16 and time consumed on licking and biting was 36+1.03(seconds), in 2nd phase number of biting and licking were 57.2+0.8 and time consumed on licking and biting was 58.8+0.37 (seconds). Results reveal that all the doses of *Olea europaea leaves* showed highly significant ($p<0.05$) results as compared with standard drug.

Writhing Test

The average number of writhes for control group was 76.2±2.645, for *Olea europaea leaves* 250 mg/Kg were 26.2±2.38, for 500 mg/Kg were 23.4±1.86 and for standard drug (Diclofenac Sodium) treated group 21.8±0.49. Results reveal that *Olea europaea leaves* showed significant analgesic effects.

DISCUSSION:

This work represents the first step toward the understanding of the effects in the central nervous system of the crude extract obtained from the leavess of the *Olea europaea* on experimental animals. The plant showed anxiolytic, CNS depressant and analgesic effects. Phytochemical literature reveals that *Olea europaea leaves* contain alkaloids flavonoids, terpenoids, phenols, diarylheptanoids, tannins and steroids, [39].Anxiolytic, CNS depressant and analgesic effects This effect is probably may be due to presence of alkaloids, flavonoids [40] alkaloids are reported to produce CNS depressant effects [41].Many neuro-active steroids and flavonoids are GABAA receptors ligands in the CNS [42]. that may act as benzodiazepine-like agent[43] Current study shows that *Olea europaea leaves* produced anxiolytic and sedative effects and this may be due to the phyto-constituents to the GABAA-BZD complex. In provision of this, it has been found that flavones bind with great affinity BZD location of the GABAA receptor [44]The locomotor test is a measure of the level of nervousness of the (CNS) and calm resulting from sadness of the (CNS) The result showed that the extract pointedly reduced the locomotor activity as presented by the results of the open field test, cage crossing, rearing and traction test[45, 46] Immobility or despair behavior produced in forced swimming test were hypothesized to display animal's hopelessness and low mood (behavioral despair), and are taken as paradigm of depression. This simple behavioral procedure is widely used test for screening novel antidepressants [47]The results reveals that immobility period with *Olea europaea leaves* crude extract was markedly short as compared to that of control at both (250 and 500mg/kg) doses. Phytochemical literature reveals that *Olea europaea* contain phenols[48] .previous reports indicated that polyphenolic compounds possess comparable antidepressant test with that of standard antidepressant by increasing the level of noradrenaline and serotonergic transmission in brain of antidepressant models. The present study showed that plant *Olea europaea* might have antidepressant and anxiolytic activity due to the existence of polyphenolic agents (Afsar *et al*., 2017).

The acetic acid induced abdominal shrinking test issued commonly for peripherally acting drugs[49]. The pain generation arises by liberating endogenous constituents and some other pain compound such as arachidonic acid metabolites via cyclooxygenases, such as prostaglandins.[50] Thus, *Olea europaea leaves* comprises two sorts of components have analgesic mechanisms. Specific components in the *Olea europaea leaves* act peripherally by preventing endogenous aching constituents deprived of any interruption in onset .Thus, the analgesic activity

(peripherally) of *Olea europaea leaves* (MCE) may be recognized by the existence of alkaloids probably belonging to various groups of polyphenol compounds[51].

Therefore, it could be suggested that *Olea europaea leaves* might comprise pharmacologically dynamic constituents that can stop the release or the effect of endogenous constituents responsible for the excitation of nerve terminations[52]. These constituents may be qualitatively, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarone, triterpenes, sterols, anthraquinones and phenolic compounds are classes of compounds thate known to have analgesic properties in various models of pain [53, 54] Therefore, the phytochemicals found in the extract might have antagonized peripheral mediators of pain and thereby blocking transmission of pain [55]

Intense pain immediately starts after formalin injection (early phase). Besides inhibiting the production of prostaglandin, the findings suggest that the extract inhibited the activation of primary afferent fibers by formalin.[56] The formalin test was used to the discriminate whether the analgesic effect of this extract occurs at the central or peripheral level. Formalin Injection has been reported to produce a distinct biphasic analgesic response.[57] The 1st phase (0 to 5 min) results from direct stimulation of nociceptors. The 2nd phase (15 to 30 min) is thought to be an inflammatory response related with inflammatory pain, a process in which several inflammatory mediators are involved, including histamine, serotonin, bradykinin and prostaglandins [58]

The inhibitory effects of *Olea europaea leaves* extracts were observed in both phases, The literature shows that centrally acting drugs inhibit both phases of pain, while peripheral acting drugs mainly inhibit the second phase Therefore, the results here in point to a predominantly both peripheral and centrally action of *Olea europaea leaves* The analgesic effects showed by the extracts might be attributed of phytochemicals present in the plant, including tannins and flavonoids possess analgesic activity[59] .

CONCLUSION:

In light of the results of this study, it may be concluded that plant extract of *Olea europaea leaves* has, analgesic and Neuro-pharmacological activities, which may be assisted through central mechanism of pain. The current Study also confirm the outdated usage of plant in pain. Extract may have several advantages such as cost-effectiveness and compatibility for biomedical and pharmaceutical applications so it need further investigation for quantitative and qualitative isolation of active constituents.

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