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EVALUATION OF NEUROPHARMACOLOGICAL AND ANALGESIC EFFECTS CONES OF *JUNIPERUS EXCELSA* (M. BIEB) OF ZIARAT, BALOCHISTAN

GHULAM SARWAR[1], SHAFI MUHAMMAD[1], MARVI[2], NISAR AHMAD[3], ABDUL QADIR[4], ABDUL JABBAR[1], MUHAMMAD ARSALAN[1], GHULAM MUSTAFA[6], MANZOOR AHMED KHATTAK[5]

1. Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan

2. Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan

3. Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan.

4. Department of Pharmaceutics, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan

5.Department of Chemistry, University of Balochistan, Quetta-Pakistan

6. Government College Quetta

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ABSTRACT

Juniperus excelsa (M. Bieb) found in Ziarat, Balochistan, Pakistan. Traditionally it is used for cure of various diseases. Current study was carried out to determine its neuropharmacological (open field, rearing, cage crossing and forced swimming tests) and analgesic activities of methanolic extract of *J. Excelsa* cones. In neuropharmacological studies Diazepam (2mg/kg) was used as standard drug and Diclofenac sodium (50 mg/kg) was used as standard drug in analgesic activity while methanolic extract of *J. Excelsa* cones at oral doses of 250 and 500 mg/Kg were administered orally. In neuropharmacological tests (open field, rearing, cage crossing, forced swimming) methanolic extract of *J. excelsa* reveals highly significant ($p<0.01$) decrease at both 250 and 500 mg/Kg oral doses. In analgesic activity methanolic extract of *J. excelsa* showed highly significant reduction in number of writhing and the paw licking & biting attempts in formalin test respectively. It is concluded

that the methanolic extract of cones of the *J. excelsa* have strong CNS sedative and analgesic effects.

Keywords: Analgesic, Balochistan, *Juniper excelsa*, Sedative, Ziarat

INTRODUCTION

There are nearly six thousand (6000) wild plant species in Pakistan [1] and amongst these, medicinally important are equal to one thousand and ten (1010) while nearly four hundred and fifty-six (456) medicinal plants are maintain and marketed in the local herbal industry [2]. Medicinal plants are being used for treating different diseases since the ancient time [3-5] and that plants have been used as folk medicine throughout the world [6] and the use of plant based medicines has been increasing gradually world-wide in recent years [7]. In the nineteenth century, necessary knowledge was already discovered to determine the chemical basis of medicinal plants for treating diseases [8]. The synergistic effects of phytochemicals in plants are responsible for their potent bioactive activities [9]. Herbal drugs have less side effects [3]. According to WHO Traditional Medicine Strategy 2002-2005 the 80% of Asian and African population basically relay on traditional medication for the primary health care [10].

There are 60–70 species of Genus *Juniperus* which are the aromatic evergreen plants are distributed all over world. *Juniperus excels* is belonging from Genus *Juniperus,* grows in Zairat Balochistan, its cones are used in traditional medicine [11]. Constituents of the fruit are limonene, terpene, caryophyllene, germacrene D, and α-humulin [12]. *J. excelsa* cone oil is known to be used for the treatment of plenty of diseases while playing the role as a sedative, antiseptic and as an analgesic [13]. It is continuously used to treat respiratory tract diseases, common cold to pneumonia, urinary tract inflammations, renal and gall bladder stones, and rheumatism. Moreover, the same ingredient is being used for the cure of so many ailments like the tuberculosis, jaundice, and eczema. The wood and barriers (cones) are also found to be used for producing incense where needed during pharmaceutical practices [14, 15, 16]. Current research work was carried out to determine analgesic and CNS sedative effects of the plant.

MATERIAL AND METHODS

Plant material

The plant material, fresh Juniper cones were collected from Juniper forests of Ziarat district of Balochistan, Pakistan. The cones of the plants were identified by Mrs. Bushra Aziz Khan, the

Chairperson/Assistant Professor Pharmacognosy Department and voucher specimen No. GS 34 issued from the Faculty of Pharmacy and Health Sciences, University of Balochistan Quetta, was submitted in herbarium of the same Department.

Extraction Procedure

Fresh Juniper cones were dried under shade for 30 days and the same was further crushed and turned in to thick granules, was further allowed to dry under previous conditions for another thirty days. The finally obtained material was soaked in 99.80 % (v/v) methanol solvent on room temperature for three weeks. Than the solvent was filtered and extracted by using a rotary evaporator. The dark brown coloured extract was obtained.

Animals

For this studies albino mice were purchased from the DUHS (Dow University of Health sciences) and were weighing about 25-27 gram. Standard environment was provided to the animals keeping the temperature 25±1 °C. The animals had access to standard laboratory chow and water and exposed to light and dark for 12 hours each.

Neuropharmacological studies

Mice were grouped in to 4 categories comprising 5 mice in each category i.e. Group I= Control (administered distilled water), Group II= administered Crude extract 250 mg/Kg, Group III= administered crude extract 500 mg/ Kg, Group IV= administered Standard Drug Diazepam 2mg/kg.To examine neuropharmacological effects of crude extract, rearing, traction, Open field and cage crossing and forced swimming tests were carried out.

Open Field Test

The open field test was performed on an apparatus comprised of four-sided area 76 cm each with impervious walls having height of 42 cm. The bottom of the device was marked by lines into 25 squares. Experiment was done in a silent area provided with white light as defined by Kennett et al. (1985) and Haleem et al. (1988). Oral drug administration route was used for dosing the test animals in this experiment. Only the squares crossed with all four paws were counted for a period of ten (10) minutes [17].

Rearing Test

For Rearing test mice were put in to a 1000 ml beaker. Oral drug administration route was used for dosing the test animals. Animals were observed for 10 minutes [18, 19]for sum of upward movements while having their front legs on the walls of beaker in an erect standing position [20, 21].

Cage Crossing Test

For the cage crossing a standard cage was used. Oral drug administration route was used for dosing to the test animals in this experiment. Number of Cage crossings were recorded for 10 minutes [6, 22].

Traction Test

In this test the mice travel with their forelimbs on an apparatus consisted an iron rod which was 1 meter stretched horizontally at a height of 35 cm. Oral drug administration route was used for dosing the test animals. The time duration was observed in term of crossing the iron pipe [23].

Forced swimming test.

The mice were put to perform forced swimming in to a transparent cylinder of 30 cm height and with15 cm diameter. The apparatus was filled with tap water and temperature of 35±2ºC was sustained. Mice were allowed for swimming for total 6-minute time. Oral drug administration route was used for dosing the test animals. The control, extract treated and standard drug treated groups mice were placed individually in a cylinder and swimming (mobility) period was measured with the help of a digital stopwatch. When the mice tried to swim or to escape from water was considered mobile and where they float in water without any movement, considered to be immobile [24-26].

Acetic Acid induced Writhing Test

In this experiment oral drug administration route was used for dosing the test animals with crude extract of *J. excelsa* before thirty minutes of 1% acetic acid injection, given by intraperitoneal route. After 5 min interval of acetic acid injection number of writhes were recorded for 15 minutes in three phases of 5-10, 11-15, 16-20 minutes [27].

Formalin Test

Oral drug administration route was used for dosing crude extract of *J. excelsa* to the test animals, thirty minutes before injecting twenty microliters of 2.5% formalin, in the right hind paw of the test animal and were analysed for thirty minutes for attempting licking and biting (number and time spent). This neurogenic pain (analysis) experiment was conducted in two phases i.e. mice were observed from 0 to 5 minutes (1^{st} phase) and 15-30 minutes (2^{nd} phase) of the experiment [28].

Statistical analysis

All the calculations were recorded as mean with $\pm$ SEM. The Dunnett's test was used to determine the significance of difference between the means and values of $P<0.01$ were considered as highly significant and $P<0.05$ significant [29].

RESULTS

Open Filed Test

The average results of the experiment signify that mean number of squares crossed for control group were 194.60 ±3.9, for crude extract of *J. excelsa* 250 mg/Kg 62.00 ±1.6, for 500 mg/Kg 46.80 ±2.3 and for Diazepam (standard drug) treated group 42.20 ±2.4. In this test, highly significant ($p<0.01$) results were proven in all the drug doses and so the open field activity greatly decreased likewise the standard drug diazepam (table 1).

Rearing Test

The average results of the experiment signify that mean number of upward moving attempts of control group was 34.40±4.3, for crude extract of *J. excelsa* 250 mg/Kg 26.80±1.9, for 500 mg/Kg 23.40±1.3 and for Diazepam (standard drug) group 16.00±2.3. Findings of experiment demonstrate that all the doses of *J. Excelsa* produced highly significant ($p<0.01$) results, which grounds for gradual decreased in rearing activity even effect of the high (500 mg/kg) dose of crude extract of *J. excelsa* proved comparative results to Diazepam (table 2).

Cage Crossing Test

The average results of the experiment signify that mean number of horizontal and vertical crossings were 37.20±1.4 for control group, for crude extract of *J. excelsa* 250 mg/Kg 37.40±3.1, for 500 mg/Kg 24.00±2.3 and for Diazepam (standard drug) treated group 23.80±1.5. Results show that 500 mg/K of *J. excelsa* produced highly significant results ($p<0.01$) which causes decreased cage crossing activity likewise the standard drug diazepam (table 3).

Traction Test

The average results of the experiment signify that mean time for crossings iron rod was 11.80±1.7 for control group, for crude extract of *J. excelsa* 250 mg/Kg 15.80±2.5, for 500 mg/Kg 18.20±1.0 and for Diazepam (standard drug) group 19.00±2.7. During the traction test administration of *J. excelsa* increased the traveling time, which shows that the drug has sedative effects, results were comparable with Diazepam (Table 4).

Forced Swimming (Mobility Time) Test

The results of the experiment signify that mean time spent for mobility was 3.48±0.04 minutes for control group, for crude extract of *J. excelsa* 250 mg/Kg 03.54±00.14 minutes, for 500 mg/Kg 2.51±00.25 minutes and for Diazepam (standard drug) group 02.36±00.12 minutes.

The results of the experiment signify that mean time spent for immobility was 2.12±0.04, for crude extract of *J. excelsa*

250 mg/Kg 2.06±0.08, for 500 mg/Kg 2.09±0.25 and for Diazepam (standard drug) treated group 2.56±0.14. All the doses of *J. excelsa* showed sedative effects as compared with the standard drug (Table 5).

Analgesic Activity

Writhing Test

The average results of Phase-I (6-10 minutes) of the experiment signify that mean number of writhing attempts were 18.60±0.5 for control group, for crude extract of *J. excelsa* 250 mg/Kg were 12.00±0.7, for 500 mg/Kg were 07.00±0.7 and for Diclofenac Sodium (standard drug) treated group 05.00±0.7.

The average results of Phase-II (11-15 minutes) of the experiment signify that mean number of writhing attempts were 12.80±0.6 for control group, for crude extract of *J. excelsa* 250 mg/Kg 08.60±0.5, for 500 mg/Kg 05.40 ±0.5 and for (Diclofenac Sodium) standard drug treated group 04.00±0.7.

The average results of Phase-III (16-20 minutes) of the experiment signify that mean number of writhing attempts were 09.00±0.7 for control group, for crude extract of *J. excelsa* 250 mg/Kg 06.00±0.7, for 500 mg/Kg 04.40 ±0.5 and for (Diclofenac sodium) standard drug treated group 02.20±0.4.Results reveal that all the doses of *J. Excelsa* showed highly significant ($p<0.01$) results as compared with standard drug (Table 6).

Formalin Test

The average results of the Phase-I (0-5 minutes) of the experiment signify that mean number of licking and biting attempts were 37.00±1.3 for control group, for crude extract of *J. excelsa* 250 mg/Kg 28.0±1.0, for 500 mg/Kg 19.20±0.6 and for (Diclofenac sodium) standard drug group also 19.00±0.7.

The average results of the Phase-II (16-30 minutes) experiment signify that mean number of paw licking and biting attempts were equal to 60.20±1.6 for control group, for crude extract of *J. excelsa* 250 mg/Kg 40.40±1.0, for 500 mg/Kg 22.00±1.8 and for Diazepam (standard drug) group 23.00±1.2.As per findings, all the doses of *J. Excelsa* showed highly significant results ($p<0.01$) as compared with (Diclofenac sodium) standard drug (Table 7).

Table 1: Open field activity crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg (Oral)	Mean No. of Observation (± S.E.M)
1.	Control	0.5 ml Distilled Water	194.60±3.9
2.	Crude Extract of *J. excelsa*	250	62.00±1.6**
		500	46.80±2.3**
3.	Diazepam	100	42.20±2.4**

Observations are Mean Values of 10 minute's Open Field Test, ± SEM where; n=5;
***= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)**

Table 2: Rearing test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg (Oral)	Mean No. of Observation (± S.E.M)
1.	Control	0.5 ml Distilled Water	50.40±4.3
2.	Crude Extract of *J. excelsa*	250	06.80±1.9**
		500	03.40±1.3**
3.	Diazepam	100	06.00±2.3**

Observations are Mean Values of 10 minute's Rearing test, ± SEM where; n=5;
***= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)**

Table 3: Cage crossing test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg (Oral)	Mean No. of Observation (± S.E.M)
1.	Control	0.5 ml Distilled Water	34.40±4.3
2.	Crude Extract of *J. excelsa*	250	26.80±1.9
		500	26.80±1.9**
3.	Diazepam	100	23.40±1.3 **

Observations are Mean Values of 10 minute's Cage Crossing experiment, ± SEM where; n=5; *= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)

Table 4: Traction test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg (Oral)	Mean No. of Observation (± S.E.M)
1.	Control	0.5	11.80±1.7
2.	Crude Extract of *J. excelsa*	250	15.80±2.5
		500	18.20±1.0
3.	Diazepam	100	19.00±2.7

Observations are Mean Values of 10 minute's Traction Test, ± SEM where; n=5;
***= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)**

Table 5: Forced Swimming Test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg (Oral)	Mean No. of Observation & ± S.E.M (Immobility) Min.	Mean No. of Observation & ± S.E.M (Mobility) Min
1.	Control	0.5 ml Distilled Water	3.48±00.04	2.12±0.04
2.	Crude Extract of *J. Excels*	250	03.54±00.14**	02.06±0.08**
		500	02.51±00.25**	02.09±0.25**
3.	Diazepam	100	01.36±00.12**	04.24±0.14**

Observations are Mean Values of 6minute's Forced Swimming Test, ± SEM where; n=5; *= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)

Table 6: Acetic acid induced writhing test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Oral Dose (mg/Kg)	No. of Writhes Phase-I (6-10 min)	No. of Writhes Phase-II (11-15 min)	No. of Writhes Phase-III (16-20 min)
1.	Control	0.5 ml Distilled Water	18.60±0.5	12.80±0.6	09.00±0.7
2.	Crude Extract of *J. excelsa*	250	12.00±0.7**	08.60±0.5**	06.00±0.7**
		500	07.00±0.7**	05.40 ±0.5**	04.40 ±0.5**
3.	Diazepam	100	05.00±0.7**	04.00±0.7**	02.20±0.4**

Observations are mean values acetic acid induced writhingtest, ± SEM where; n=5; *= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)

Table 7: Formalin test of crude extract of *J. excelsa* in mice

S. No.	Treatments	Dose mg/Kg orally	No. of Licking & Biting (Ph-I, 0-5 Min)	% Inhibition	No. of Licking & Biting (Ph-II, 16-30 Min)	% Inhibition
1.	Control	0.5 ml Distilled Water	37.00±1.3	-	60.20±1.6	-
2.	Crude Extract of J. *excelsa*	250	28.0±1.0**	75.67	40.40±1.0**	67.11
		500	19.20±0.6**	31.89	22.00±1.8**	78.57
3.	Diazepam	100	19.00±0.7**	-	23.00±1.2**	-

Observations are Mean Values of Formalin Test, ± SEM where; n=5; *= Significant results (*P*<0.05), ** = highly significant results (*P*<0.01)

DISCUSSION

In the current studies, findings of neuropharmacological tests indicate that barriers of *J. excelsa* have highly significant($p<0.01$) decreased values (using both 250 and 500 mg/Kg doses of crude extract) in open field, cage crossing, traction and rearing tests, results indicates that sedative effect is produced by crude extract of *J. excelsa* barriers.

J. excelsa is reported to be rich in α-pinene [30], cedrol, camphene, copaene, phyllocladane, ferruginol, podocarp-7-en-3-one, and pimara-8(14) 15-dien [31]. Abietanestotarol in berries [32] and monoterpenes; sesquiterpenes; and other compounds including alkanes are reported, among metabolites the g-elemene, germacrene-B, stenol, were reported in the leave extracts of either the 10 and 100-years old trees [33]. Locomotor activity is considered as an index of alertness, and the decreased activity indicates a sedative effect [34]. Cedrol, is a bioactive sesquiterpene, reported to exert antiseptic, anti-inflammatory, and sedative effects [35]. The presence of Cedrol and other compounds in the cones of *J. excelsa* are possibly responsible for the decreased locomotor activity.

The results of forced swimming test shows that *J. excelsa* crude extract produced highly significant ($p<0.01$) results in both the 250 and 500 mg/Kg doses. Previously it is reported that *J. excelsa* contain terpenoids [31-34] and terpenoids are reported to possess CNS sedative effects [36], so the sedative effect of *J. excelsa* crude extract may be due to terpenoids.

In the current studies while conducting the writhing test, all the doses of *J. excelsa* showed highly significant ($p<0.01$) results. The abdominal constriction response induced by acetic acid is common method to evaluate peripherally acting analgesics. Usually acetic acid induced pain is the result of liberating bradykinins, histamine and prostaglandins (PGs). Another assumption for abdominal constrictions response is the local peritoneal receptors being responsible [37].

Formalin test is conducted for the neurogenic pain analysis [25]and both phases of this test i.e. from 0 to 5 minutes and 15-30 minutes duration of the experiment results were highly significant ($p<0.01$)at both 250 and 500 mg/Kg doses of *J. excelsa* crude extract.

Methanolic crude extract of *J. excelsa* cones causes reduction in writhing and the paw licking and biting attempts. Essential oils and terpenoids are reported to possess analgesic activity [38, 39], So the analgesic activity is may be due to essential oils and terpenoids.

CONCLUSION

To summarize all the results, it can be said that cones of *J. excelsa* may have strong sedative and analgesic medicinal potentials but it still needs further studies to isolate the chemical constituents responsible for these effects.

REFERENCES

[1] Hamayun M (2015).Ethnobotanical profile of Utror and Gabral valleys, district Swat, Pakistan *Ethnobotanical Leaflets.* (1):9.

[2] Usmanghani K, Saeed A, Alam MT (1997): traditional medicine of herbal, animal, and mineral origin in Pakistan. *Indusyunic medicine*, 577.

[3] Qureshi H, Arshad M, Akram A, Raja NI, Fatima S, Amjad MS (2016) Ethnopharmacological and phytochemical account of paradise tree (Meliaazedarach L.: Meliaceae). *Pure and Applied Biology,*5(1): 5-14.

[4] Bashir T,Zahara K, Haider S, Tabassum S (2015), Chemistry, pharmacology and ethnomedicinal uses of Helianthus annuus (Sunflower): a review. *Pure and Applied Biology* 4(2):226-35.

[5] Wahid HA (2013) Antibacterial Activities of Plant Extracts and Honey on Local Bacterial Isolates. *Pure and Applied Biology*, 2(4):116-21.

[6] Ahmad I, Jan S, Begum A, Wali S (2015) Taxonomic diversity and ethnobotanical characteristics of the family Lamiaceae of Swat, Khyber Pakhtunkhwa, Pakistan. *Pure and Applied Biology,* 4(4): 465-70.

[7] Ali I, Ahmed W, Tariq M, Asghar R, Hussain MA (2016) Therapeutic potential of ethanolic extract of Solanumnigrum for lipofundin-induced hyperlipidemia in Rabbits. *Pure and Applied Biology*, 5(1):85-90.

[8] Singh RJ (2011) Genetic Resources, Chromosome Engineering, and Crop Improvement: *Medicinal Plants*, Volume 6. CRC press;

[9] Hussain MA, Khan MQ, Ali I, Dar ME, Habib T (2015) Antifungal potential of different parts of *Oleaeuropaea* and *Oleacuspidata* growing in Azad Jammu and Kashmir. *Pure and Applied Biology*. 4(2):204-16.

[10] WHO. Traditional Medicine Strategy. 2002-2005.

[11] Sarangzai AM, Ahmed A, Ahmed M (2012), Ethnobotany of *Juniperus excelsa* in ziarat, balochistan, pakistan. *FUUAST Journal of Biology*. 2(2):25.

[12] Abdel Ghany TM, Hakamy OM (2014). *Juniperusprocera* as food safe additive, their antioxidant, anticancer and antimicrobial activity against some food-borne bacteria. *J. Biol. Chem. Research. 31*(2):668-77.

[13] Sokovic M, Ristic M, Grubisic D (20014). Chemical composition and antifungal activity of the essential oil from *Juniperus excelsa* berries. *Pharmaceutical biology*. 42(4-5):328-31.

[14] De Medina FS, Gamez MJ, Jimenez I, Jimenez J, Osuna JI, Zarzuelo A (1994). Hypoglycemic activity of juniper "berries". *Planta Medica*. *60*(03):197-200.

[15] El Beyruthy M, Arnold N (2008). Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. *J. Ethnopharmacol*, *120*: 315-334.

[16] Ozturk M, Tümen I, Ugur A, Aydogmuş-Ozturk F, Topçu G (2011). Evaluation of fruit extracts of six Turkish Juniperus species for their antioxidant, anticholinesterase and antimicrobial activities. *J.Sci. Food and Agri, 91*(5): 867-76.

[17] Jabbar A, Muhammad S, Razaque G, Qadir A, Younis M, Ahmad N, Baloch I, Mustafa G (2016). Studies on neuropharmacological and analgesic effects of *Periploca aphylla* extract in mice. *Pure and Applied Biology*. 5(4):1207-1215.

[18] Ahmad M, Muhammed S, Jahan N, Jan SU, Qureshi ZU (2014) Anti-dermatitis, anxiolytic and analgesic effects of *Rhazyastricta* from Balochistan. *Pak. J. Pharm. Sci., 27* (3): 481-486.

[19] Qureshi M, Jahan N, Muhammad S, Mohani N, Wazir A, Baig IA and Ahmad M (2015). Evaluation of neuro-pharmacological, analgesic and anti-inflammatory effects of the extract of Centellaasiatica (Gotu kola) in mice. *Afr J. Pharm and Pharmacolo 9*(41):995-1001.

[20] Kasture VS, Deshmukh VK, Chopde CT (2002) Anxiolytic and anticonvulsive activity of Sesbaniagrandiflora leaves in experimental animals. *Phyto therapy Research*. *16*(5): 455-60.

[21] Sanchez-Mateo CC, Prado B, Rabanal RM (2002). Antidepressant effects of the methanol extract of several Hypericum species from the

Canary Islands. *Journal of Ethnopharmacology*. *79*(1):119-27.

[22] Ahmad M ,Rookh M , Rehman AB , Muhammad N , Amber , Younus M and Wazir A (2014). Assessment of anti-inflammatory, anti-ulcer and neuro-pharmacological activities of *Cyperusrotundus* Linn. *Pak J Pharm Sci27* (6) 2241-2246

[23] Boissier JR, Dermont C., Robins R and Pagny J (1961). Tentative de pharmacology previsionelledans la domain desneuroleptiques: Actions sedative Centrale et adrenolityque de la *N*-(dimethoxy-3,4 phenethyl) *N'*- (chloro-2 phenyl) piperazine. *Arch Int de PharmacoEt De Therap* 133: 29-32.

[24] Marvi, Iqbal J, Muhammad S and Ahmad M (2016) Evaluation of acute toxicity, sedative and analgesic effects of Taverniera glabra methanolic extract on mice. *Pakistan journal of pharmaceutical sciences*. 29(6) 2335.

[25] Jahan N, Ahmad M, Saeed F, Rehman AB, Muhammad S (2014). Anti-nociceptive activity of seed extract of *Vernoniaant helmintica* wild. *Pakistan journal of pharmaceutical sciences.* 27(6) 177-2181.

[26] Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Archives internationales de pharmacodynamie et de thérapie*. 29(2):327-36.

[27] Prabhu VV, NaliniG, Chidambaranathan N, Kisan SS (2011) Evaluation of anti-inflammatory and analgesic activity of Tridaxprocumbens Linn. against formalin, acetic acid and CFA induced pain models. *Int J Pharm Sci 3*(2):126-30.

[28] Ahmad M, Jahan N, Rehman AB, Muhammad S (2014) Antidiarrheal, anti-inflammatory and analgesic activities of *Symplocos racemesa* roxb. Bark. *Pakistan journal of pharmaceutical sciences*. *27*(6). 2221-222.

[29] Hossain MA, Rahman SM (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Research International*. *44*(3):672-6.

[30] Ehsani E,Akbari K, Teimouri M, Khadem A (2012). Chemical

composition and antibacterial activity of two Juniperus species essential oils. *African Journal of Microbiology Research. 6*(38):6704-10.

[31] Khajjak MH, Raza AM, Shawani MN, Ahmed F, Shaheen G, Saeed M (2012) Comparative analysis of essential oil contents of *Juniperus excelsa* (M. Bieb.) found in Balochistan, Pakistan. *African Journal of Biotechnology, 11*(32): 8154-9.

[32] Samoylenko V, Dunbar DC, Gafur M, Khan SI, Ross SA, Mossa JS, El-Feraly FS, Tekwani BL, Bosselaers J, Muhammad I (2008) Ant parasitic, nematocidal and antifouling constituents from Juniperus berries. *Phyto therapy research.22*(12):1570-6.

[33] Almaarri K, Alamir L, Junaid Y, Xie DY (2010). Volatile compounds from leaf extracts of *Juniperus excelsa* growing in Syria via gas chromatography mass spectrometry. *Analytical Methods, 2*(6): 673-7.

[34] Yadav AV, Kawale LA, Nade VS (2008). Effect of Morus alba L.(mulberry) leaves on anxiety in mice. *Indian journal of pharmacology,* 40(1): 32-36.

[35] Jeong HU, Kwon SS, Kong TY, Kim JH, Lee HS (2014) Inhibitory effects of cedrol, β-cedrene, and thujopsene on cytochrome P450 enzyme activities in human liver microsomes. *Journal of Toxicology and Environmental Health, Part A*. 77(22-24):1522-32.

[36] Subhan F, Karim N, Gilani A, Sewell R (2010). Terpenoid Content of Valerianawallichii Extracts and Antidepressant-likeResponse Profiles. *Phytotherapy Research*, 24(5), 686-691

[37] Mishra D, Ghosh GO, Kumar PS, Panda PK (2011). An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs. *Asian journal of Pharmaceutical and clinical research.* 4(1):78-81.

[38] Tumen I, Süntar I, Keleş H, Küpeli Akkol E (2011). A therapeutic approach for wound healing by using essential oils of cupressus and juniperus species growing in Turkey. *Evidence-Based Complementary and Alternative Medicine*. 18; 2012.

[39] Guimarães AG, Quintans JS, Quintans-Júnior LJ (2013), Monoterpenes with analgesic activity—a systematic review. *Phytotherapy Research*. *27*(1):1-5.

[40] Hunskaar S, Hole K (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain. 30*(1):103-14.