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PHARMACOLOGICAL STUDIES ON ETHANOLIC EXTRACT OF COCCULUS PENDULUS COLLECTED FROM JHAL MAGSI DISTRICT, BALOCHISTAN, PAKISTAN

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ABSTRACT

Balochistan is the largest province of the country and native home of many medicinal plants. *Cocculus pendulus* (Family: Menispermaceae) is a medicinal plant used in the crude form by the local people for digestive,pain reliving and depressive disorders. Current study was carried out to ethanolic extract of whole plant to determine phytochemical tests, acute toxicity, analgesic activity, forced swimming test and neuropharmacological activities. Ethanolic extract of *C. pendulus* showed presence of saponins, tannins and flavonoids in phytochemical tests, Inanalgesic activity significant ($p<0.05$) results in writhing test, tail immersion test, formalin test were observed. Ethanolic extract of *C. pendulus* also showed significant ($p<0.05$) CNS depressant results in force swimming test and neuropharmacological activities.

Keywords: Analgesic, JhalMagsi, Cocculuspendulus

INTRODUCTION

Now a days the use of natural medicinal product and supplements as accelerated surprisingly over the previous three decade which shows its enormous potential and largely indicates the possibility of treating the disease **[1]**.

Nervous disorders are becoming major disorder and according to the Statistical data 20- 40% of the general population, comprising more than 7% group of adult population, A prevalence figure show that 14% of the population is suffering from nervous disease. There is another serious health problem of insomnia throughout the both geriatric and teenage population, the recent information established the prevalence associated with insomnia is equivalent to major psychiatric disorder such as depression. 88% of teenagers are suffering from chronic insomnia **[2]**.

Skeletal muscle relaxant act peripherally at the neuromuscular junction to reduce the muscle tone. By acting on cerebrospinal axis neuromuscular blockers minimize muscle firmness or initiate paralysis (cause dysfunction). For the purpose of surgery neuromuscular blocker are given which reduce the muscle tone and provide general aesthetic effect. Meanwhile for the pain full muscle spasm and spastic neurological condition centrally acting muscle relaxant are use. Although these drug result in un wanted effect so therefore searching or trying to find an effective alternative as always being essential in this regard **[3]**.

There are a variety of herbal preparations recommended for pain relieving effect (analgesic) and reduces the anxiety (anti-depressive drug). In search of new pain relieving effects and anti-depressive agent from a large number of medicinally significant plant, numerous research work has been carried out, around the globe **[4]**. Present study was carried out to find out the neuropharmacological and analgesic activities of the plant based on traditional claim.

Cocculus pendulus

The genus Cocculus belongs to the family Menispermaceae. It comprises about 35 species, it occurs in Pakistan, India, Iran, Iraq, Saudi Arab. Its Anti-inflammatory, wound healing and anti oxidant activities has been reported **[5]**.

MATERIAL AND METHOD

Plant specimen

Plant was collected from Jhal Magsi District of Balochistan, Pakistan, identified and voucher specimen No NG-143 were

deposited at department of Pharmacognosy, University of Balochistan, Quetta.

Extraction Procedure

C. pendulus whole plant was macerated in ethanol for 15 days, at reduce pressure the solvent was evaporated in rotary evaporator, and dark green color semisolid residue was obtained.

Test Animals

Albino Mice weigh upalmost 25- 28 g were utilized and housed in animal house of Faculty of Pharmacy and Health Sciences according to standard protocol.

Preliminary Qualitative Phytochemical Screening.

C. pendulus ethanolic extract of was subjected to confirm the presence of some important phytoorganic compounds such asflavonoids, alkaloids, polyphenols, saponins, anthraquinones, tannins, terpenes and sterols were determined by using standard protocols **[6]**.

Acute toxicity study

According to standard guideline (as per OECD 423 guidelines) the procedure was performed (OECD/OCDE. 2002). The oral dose of ethanolic extract of *C.pendulus* from 50 to 2000 mg/kg body mass was administered. Animal were held under observation for 14 days to enlist conceivable mortality. The weight and possible neurological lethality was observed**[6]**.

Analgesic activity

Writhing test with acetic acid

The pain releasingaction of *C. Pendulus* ethanolic extract were anticipated by utilizing acetic acid writhing model in mice. The animal were grouped into four groups i.e. Group I (control group), Group II & III (ethanolic extract of *C. pendulus* 250 and 500mg/kg treated group), Group VI (standard drug treatment). The 0.7% acetic acid administered intra peritoneal after 30 min of orally administration of test samples, standard drug and control vehicle. The writhing (constriction of abdomen, and extension of hind legs) was observed for half-hour **[7]**.

Tail Immersion Test

In this test,mice were separated into 4 groups (5mice in each group). Control group (treated with distil water1ml/kg), Group II & III (ethanolic extract of *C. pendulus* 250 and 500mg/kg treated group), Group VI (group of standard drug treatment).

After administration of each treatment, (about 2-3cm) tail of the each mice was immersed into a water bath (temperature maintained at 50 ± 1°C) and the time consumed for the mice to withdraw its tail from the warm water or flick it known as the

pain reaction time (PRT) was recorded for all the mice. 15 seconds were cut off time **[8]**.

Formalin test

In the right hind paw of the mice a formalin solution (2.5 % in 0.9 % sterile saline; 20 µl/paw sub plantar) was injected. Observation was the period of time spent on licking and biting the injected paw which is an indication of pain. After the 5 minutes of formalin injection the first phase (neurogenic pain) starts, than after 15–30 min of formalin injection second phase (inflammatory response) starts formalin injection were given 30 minutes after to oral treatment (n = 5 per group) with saline (control.), ethanolic extract of *C. pendulus* (250 and 500mg/kg) and Diclofenic sodium (50 mg/kg) treated groups **[9]**.

Forced swimming test

In this test mice were randomly selected and divided into 4 groups with five mice each. Saline 2ml/kg for control group, along with *C. Pendulus* ethanolic extract (250 and 500 mg/kg) and Diazepam 2mg/kg administered orally. The Mice are placed in an inevitableclearchamber that is filled with water and their escape related movementbehaviour is measured, mice were individually put in a round bottom tank (which was about 46 cm tall × 20 cm in breadth) filled up with tap water (temperature maintained at 25 ± 1°C) 20cm depth. Mice were placed for 6 minutes. Mice were observed immobile when remain suspended without struggling and generated only slight movements required to uphold the head above the water. Decrease in mobility time reflects the CNS depressant activity **[10]**.

Neuropharmacological activites

Open Field Activity Test (OFT)

The open field apparatus was consist of white Plexiglas and measured 72 × 72 cm with 36 cm walls. Red lines were drawn on the floor with a marker and were clearly visible through the clear floor. Mice were administered (orally) saline 2ml/kg for control group, with *C. pendulus* ethanolic extract (250 and 500 mg/kg) and Diazepam 2mg/kg. The test was performed 30 min after the administration of the test substance. The mice were placed in the open field box for 10 minutes, and their square crossed by the mice were recorded **[11].**

Hole-board test

This test is mainly used for measuring exploratory behaviours. This apparatus is comprises of a wooden, grey box, measuring (40 cm × 40 cm). The walls were 25 cm high, with sixteen equidistant holes (3 cm in diameter), the control group were administered *C. Pendulus* ethanolic extract

(250 and 500 mg/kg) and Diazepam 2mg/kg treated group. The observation was to count the all number of crosses through the hole from one chamber for 10 minutes **[12]**.

Stationary Rod Test

The Stationary Rod Test one of the most commonly used tools for testing motor coordination, balance and learning ability. The test apparatus consisted of horizontal stainless steel rods with two platforms at the ends. The mice first trained to walk on the rod at certain speed. The control group, *C. pendulus* ethanolic extract (250 and 500 mg/kg) and Diazepam 2mg/kg were administered 30 minutes before the test **[13]**.

Cage Crossing Test

Cage crossing test is one of the most commonly used tools for testing or for measuring of the Locomotor activity in specially designed cages that consist of transparent perplex cages (26x26x26 cm) with sawdust cover floor.Mice were placed individually in these cages the control group, *C. Pendulus* ethanolic extract (250 and 500 mg/kg) treated group and Diazepam 2mg/kg are observe number of cage crossing by mice for 10 minutes **[13]**.

Rearing Test

Rearing activity was performed by using 1 liter capacity glass beaker. Mice were placed in the beaker and number of upwards movements were observed for 10 minutes.

Statistical Analysis

Means ± standard deviation (SD) were used for expression of data. Level of significance between the means of treated groups and control group, was determined by one-way ANOVA. Differences were considered statistically significant at $p< 0.05$ **[6]**.

RESULTS

Phytochemical tests

In Phytochemical tests the positive results were obtained for Saponins, Tannins and flavonoids.

Acute Toxicity test

In acute toxicity test the *C. pendulus* ethanolic extract did not showed any significant toxicity up to the dose of 2g/kg oral dose.

ANALGESIC ACTIVITIES

Acetic acid induced Writhing test

In this test mean number of activities for control group was 69.8$\pm$0.80, for *C. pendulus* 250 mg crude ethanolic extract (CEE) were 61.6$\pm$0.40, for 500 mg 34.20$\pm$2.08, for standard drug 33.10$\pm$0.64.

Tail flick Test

The mean number of activities for control group were 3.05$\pm$0.05, for *C. pendulus*250mg CEEwere 5.18$\pm$0.37, for 500 mg5.46$\pm$0.19, for standard drug 6.78$\pm$0.24.

Formalin test

First phase

The number of biting's for control group were59.2±0.58, for *C. pendulus* 250mg CEE47.20±0.80, for *C. pendulus* 500mg CEE39.40±0.51 and for standard drug 33.06+0.66.

Second phase

The number of biting's for control group were 75.40±0.92, for *C. pendulus* 250mg CEE58.40±2.84, for *C. pendulus* 500mg CEE54.20±2.36and for standard drug 45.88±1.79.

Forced swimming test

The mean swimming time for control group was 231.71±1.39 seconds, for *C. pendulus* 250mg CEE179.65±1.63 seconds, for *C. pendulus* 500mg CEE 134.84±1.56 seconds and for standard drug 122.73±0.96 seconds.

Open field test

In this test mean number of square crossed for control group was73.20±1.93, For*C. pendulus* 250mg 66.46±2.45, for *C. pendulus* 500mg 56.89±0.90, standard drug 43.53±1.31.

Hole board Test

The mean number of activities for control groups were 30.84±0.69, for *C. pendulus* 250mg CEE were 25.44±0.74, for 500 mg 22.98±1.16, for standard drug 12.82±0.53

Stationary Rod Test

The meantime taken by iron rod for control group were 13.30±1.04 seconds, for *C. pendulus* 250mg CEE were 14.20±0.60 seconds, for 500 mg 24.20±0.34 seconds, for standard drug 27.38+0.39.

Cage Crossing Test

The mean number of activities for control groups were 31.78±1.66, for *C. pendulus* 250 mg CEE were 28.29±0.88, for 500 mg 28.97±0.54, for standard drug 23.02±0.72.

Rearing Test

The mean number of activities for control group were 37.99±0.70, for *C. pendulus* 250mg CEE were 32.04±0.72, for 500 mg 29.14±2.02, for standard drug 18.29+1.18

DISCUSSION

Compounds from plant sources are gaining importance for drug development. Analgesic drugs (narcotic and non narcotics) have sever adverse or toxic effects, whereas plant based medicines are easily available, have good absorption and less toxicity.Consequently efforts are made to find new medicinal plants, having less side effects and cheaper.The Writhing test (acetic acid induced abdominal constriction) is a most widely used method for the evaluation of peripheral antinociceptive activity **[14]**. *C. pendulus* ethanolic extract showed substantial analgesic effects in writhing test, as paralleled with control and standard drug.

The pathway responsible for the response are mediation ofprostaglandins, mast cells(peritoneal) and acid sensing ion channels **[15]**. Decrease in number of writhes in current study was probable anti-nociceptive action and the mechanism of analgesic effect might be due to inhibition of synthesis or action of the any of the above pathway.

For determination of antinociceptive activity, formalin test is widely used assay.For plant extracts formalin test is very effective model analgesic activity determination. Nociceptive impulses transmission is represented in early phase of test, while events of central activity are represented in the second phase of the test. Analgesics acting centrally shows effect in both phases whereas analgesics acting peripherally affect the only the first phase **[16]**. Current study has shown that ethanolic extract of *C. pendulus* has constituents that inhibit pain in both phases. Hence, the extract could be acting as a central analgesic activity and can be utilized as alternative analgesic agent.

Force swimming test is the test for muscle co-ordination and locomotor activity in mice. Ethanolic extract of *C. pendulus* revealed significant CNS depressant activity in forced swim test. Reduction in locomotion activity shows depressant effect on CNS. In Brain increased concentration of GABA leads to depressant effect on CNS **[17]**.

In neuropharmacological models the effect of *C. pendulus* on CNS was studied using open field, holeboard, stationary rod test, cage crossing and rearing tests. These models are extensively used standard models for screening neuropharmacological activity **[18]**. Ethaniolic extract of *C. pendulus* Showed significant CNS depressant activity in neuropharmacological tests.

The preliminary phytochemical screening of the ethanolic extract of *C. pendulus*showed the presence of Saponins, Tannins and Flavonoids. Many studies confirms the CNS depressant activity of flavonoids. In CNS for GABA type A receptors many flavonoids were found to be ligands, where it is hypothesised that, these ligands may act asmolecules like benzodiazepine. Reports suggests that, saponins possess potent sedative effect in similar animal models and saponins also inhibit impulsive motor activity in mice **[19]**. Hence it is hypothesized that, the flavonoids and saponins are responsible for the pharmacological activities.

Table No 1. Phytochemical tests

SNo.	Phytochemicals	Results
1	Saponins	+ve
2	Tannins	+ve
3	Flavonoids	+ve
4	Alkaloids	+ve
5	Steroid	-ve
6	Glycosides	-ve

Table No 2. Acute toxicity Test

SNo.	Dose	% mortality
1	50 mg/kg	0 %
2	100mg/kg	0 %
3	250mg/kg	0 %
4	500mg/kg	0 %
5	1500mg/kg	0 %
6	2g/kg	0 %

Table No. 03 Acetic acid induced Writhing test

SNo.	Drug	Number of writhes
1	Control	69.8±0.80
2	C. pendulous 250mg CEE	61.6±0.40
3	C. pendulous 500mg CEE	34.20±2.08
4	Standard drug (Diclofenac sodium)	33.10±0.64

Mean±SEM, N=05

Table No. 04 Tail Flick Test

SNo.	Drug	Number of Activities
1	Control	3.05±0.05
2	C. pendulous 250mg CEE	5.18±0.37
3	C. pendulous 500mg CEE	5.46±0.19
4	Standard drug	6.78±0.24

Mean±SEM, N=05

Table 05: Formalin Test

SNo.	Drug	First Phase	2nd phase
1	Control	59.2±0.58	75.40±0.92
2	C. pendulous 250mg CEE	47.20±0.80	58.40±2.84
3	C. pendulous 500mg CEE	39.40±0.51	54.20±2.36
4	Standard drug	33.06±0.66	45.88±1.79

Mean±SEM, N=05

Table 06: Forced swimming teat

SNo.	Treatment	Swimming time (seconds)
1	Control	231.71±1.39
2	C. pendulous 250mg CEE	179.65±1.63
3	C. pendulous 500mg CEE	134.84±1.56
4	Standard drug	122.73±0.96

Table 7: Open Field Activity

SNo.	Control	Number of open field activities
1	Control	73.20±1.93
2	C. pendulous 250mg CEE	66.46±2.45
3	C. pendulous 500mg CEE	56.89±0.90
4	Standard drug	43.53±1.31

Mean±SEM, N=05

Table 8: Hole Board Test

SNo.	Control	Number of activities
1	Control	30.84 ±0.69
2	C. pendulous 250mg CEE	25.44±0.74
3	C. pendulous 500mg CEE	22.98±1.16
4	Standard drug	12.82±0.53

Mean±SEM, N=05

Table 9: Stationary Rod Test

SNo.	Drug	Mean time (seconds)
1	Control	13.30±1.04
2	C. pendulous 250mg CEE	14.20±0.60
3	C. pendulous 500mg CEE	24.20±0.34
4	Standard drug	27.38±0.39

Mean±SEM, N=05

Table 10: Cage crossing test

SNo.	Drug	Mean Number of Cage crossings
1	Control	31.78±1.66
2	C. pendulous 250mg CEE	28.29±0.88
3	C. pendulous 500mg CEE	28.97±0.54
4	Standard drug	23.02±0.72

Mean±SEM, N=05

Table 11: Rearing Activities

SNo.	Drug	Number activities
1	Control	37.99±0.70
2	C. pendulous 250mg CEE	32.04±0.72
3	C. pendulous 500mg CEE	29.14±2.02
4	Standard drug	18.29±1.18

Mean±SEM, N=05

REFERENCES

[1] Wal, A., P. Wal, and R. Tiwari. "Neuropharmacological Effects Of The Ethanolic Extract Of Derivatives Of Lupeol In Rats". *Asian Journal of Pharmaceutical and Clinical Research*, Vol. 12, no. 1, Jan. 2019, pp. 222-6, doi: 10. 22159/ ajpcr. 2019. v12i1.28533.

[2] Rath M, Bhattacharya A, Santra S, Rath K, Ghosh G, Nanda BB. Neuropharmacological effects of methanolic extract of *Clerodendrum viscosum* leaves on wistar albino rats. Phcog Mag 2018; 14: 507-14.

[3] Madhuri AS, Roopa CR. Evaluation of skeletal muscle relaxant activity of methanolic extract of Hibiscus rosasinensis leaves in albino rats. Natl J Physiol Pharm Pharmacol 2019; 9(3): 206-209

[4] Uddin MJ, Ali Reza ASM, Abdullah-Al-Mamun M, Kabir MSH, Nasrin MS, Akhter S, Arman MSI and Rahman MA (2018) Antinociceptive and Anxiolytic and Sedative Effects of Methanol Extract of Anisomelesindica: An Experimental Assessment in Mice and Computer Aided Models. Front. Pharmacol. 9:246. doi: 10.3389/fphar.2018.00246

[5] Jangir S, Mathur K, Goyal M, Yadav SK. A Review on *Cocculus pendulus* (JR Forst. & G. Forst.) diels: traditional uses, phytochemistry and pharmacological properties. Indian Journal of Drugs. 2016; 4(2): 57-62.

[6] Bourhia, M., Haj Said, A. A., Chaanoun, A., El Gueddari, F., Naamane, A., Benbacer, L., &Khlil, N. (2019). Phytochemical Screening and Toxicological Study of *Aristolochia baetica* Linn Roots: Histopathological and Biochemical Evidence. Journal of Toxicology, 2019.

[7] Khanum S, Sarwar MS, Islam MS. In vivo Neurological, Analgesic and In vitro Antioxidant and Cytotoxic Activities of Ethanolic Extract of Leaf and Stem Bark of *Wedelia chinensis*. Bangladesh Pharmaceutical Journal. 2019 Jan 29;22(1):18-26.

[8] Ezeja, M. I., Omeh, Y. S., Ezeigbo, I. I., & Ekechukwu, A. (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). Annals of medical and health sciences research, 1(1), 55-62.

[9] Silva, J. C., de S Araújo, C., de Lima-Saraiva, S. R. G., de Oliveira-Junior,

R. G., Diniz, T. C., de S Wanderley, C. W., & da S Almeida, J. R. G. (2015). Antinociceptive and anti-inflammatory activities of the ethanolic extract of *Annona vepretorum* Mart. (Annonaceae) in rodents. BMC complementary and lternative medicine, 15(1), 197.

[10] Okokon, J. E., Obot, J., Amazu, L. U., &Ebinyo, N. (2018). Antidepressant activity of ethanol Doukkali, Z., Taghzouti, K. and Bouidida, E.L., 2016. Small Nettle: Evaluation of Its Anxiolytic Effect in the Elevated Plus-Maze and Open Field. Ann Clin Lab Res, 4, p.1.

[11] Foyet, H.S., Tsala, D.E., Bouba, A.A. and Hritcu, L., 2012. Anxiolytic and antidepressant-like effects of the aqueous extract of *Alafia multiflora* stem barks in rodents. Advances in pharmacological sciences, 2012.

[12] Anisuzzman, M., Hasan, M., Acharzo, A. K., Das, A. K., &Rahman, S. (2017). In Vivo and In Vitro evaluation of pharmacological potentials of secondary bioactive metabolites of *Dalbergia candenatensis* leaves. Evidence-Based Complementary and Alternative Medicine, 2017.

[13] Mahmood, R., Najam, R., Rizwani, G. H., &Khatoon, H. (2015). Evaluation of neuropharmacological activity of *cardiospermum halicacabum* (linn) leaf extract.

[14] Kumatia, E. K., Antwi, S., Brew-Daniels, H., Appiah, A. A., &Ocloo, A. (2019). In vivo Comparative Anti-Inflammatory and Analgesic Activities of Root Bark, Stem and Leaf Extracts of *Capparis erythrocarpus* (Capparaceae). Pharmacognosy Journal, 11(3).

[15] Khatoon, M. M., Khatun, M. H., Islam, M. E., & Parvin, M. S. (2014). Analgesic, antibacterial and central nervous system depressant activities of Albiziaprocera leaves. Asian Pacific journal of tropical biomedicine, 4(4), 279-284.

[16] Onzago, R. O., Kiama, S. G., Mbaria, J. M., Gakuya, D. W., Githiji, C. G., & Rukenya, Z. M. (2013). Analgesic activity of aqueous extract of *Vernonia hymenolepis* (A. Rich) a traditional medicine plant used in Kenya for toothache. The Journal of Phytopharmacology, 2(6), 41-45.

[17] Hoskeri, J. H., Venkatarangaiah, K., Hanumanthappa, S. K., Vootla, S. K., & Gadwala, M. (2011). CNS depressant activity of extracts from *Flaveria trinervia* Spring C. Mohr. Phytopharmacol, 1, 100-107.

[18] Sultana, T., Mannan, M. A., & Ahmed, T. (2018). Evaluation of central nervous system (CNS) depressant activity of methanolic extract of *Commelina diffusa Burm*. in mice. Clinical Phytoscience, 4(1), 5.