OPEN ACCESS
Scholars International Journal of Traditional and Complementary Medicine
Abbreviated Key Title: Sch Int J Tradit Complement Med
ISSN 2616-8634 (Print) |ISSN 2617-3891 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://scholarsmepub.com/sijtcm/

Original Research Article

In Vivo Assessment of Neuropharmacological Activity of Methanol Leaves Extract of *Callicarpa arborea* (Family: Verbenaceae) In Swiss Albino Mice

Aliza[1], Sharmin Sultana[2*], Md. Lokman Hossain[1]

[1]Department of Pharmacy, Stamford University Bangladesh, Dhaka-1217, Bangladesh
[2]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

DOI:10.21276/sijtcm.2019.2.6.1 | **Received:** 28.07.2019 | **Accepted:** 04.08.2019 | **Published:** 18.08.2019

***Corresponding author:** Sharmin Sultana

Abstract

Callicarpa arborea is a shrub or tree (Family: Verbenaceae) that has been well studied for its antioxidant and anti-inflammatory effects. Nevertheless, scientific information on *Callicarpa arborea* regarding the neuropharmacological effect is limited. The present study was performed to investigate the phytochemical nature and neuropharmacological activities of methanol extract of the *Callicarpa arborea* (MECA). Phytochemical analysis of *Callicarpa arborea* extract indicated the presence of flavonoids, tannins, steroid, terpenoid, alkaloids and saponins. The neuropharmacological activity was determined by hole cross and open field test using Swiss albino mice as experimental animal at the doses of 200 and 400 mg/kg body weight. The extract showed significant neuropharmacological activity compared to standard drug. Diazepam at the dose of 1 mg/kg body weight was used as a reference drug in all the experiments. We found that MECA produced a significant dose-dependent inhibition of locomotor activity of mice in both hole cross and open field tests ($P<0.05$). Our exploration suggests that *Callicarpa arborea* contains sedative bioactive principle(s).

Keywords: *Callicarpa arborea*, Neuropharmacological activity, Phytochemical analysis, Swiss albino mice, Hole cross test, Open field test.

INTRODUCTION

Callcarpa arborea, belonging to verbenaceae family is locally known as Bormala and Beauty berry. It grows well in different environments and mainly found in Bangladesh, China, India, Indonesia, Nepal, Papua New Guinea, Thailand, United States and Vietnam [1]. The bark is carminative, digestive and tonic [2]. It is used to stimulate the digestion and also for the treatment of skin complaints [3]. The powdered bark, combined with a pinch of turmeric (*Curcuma longa*) is used to relieve fevers. A decoction of the leaves is used to treat stomachache and cutaneous diseases [4]. The leaves are used as a poultice to heal sores [3]. The root is used as astringent and decoction to treat internal bleeding. Externally, the dried powdered roots are used to treat cuts, wounds, bleeding piles etc. The root is chewed to treat boils on the tongue [4]. The whole plant is used to treat influenza [5]. Previous studies revealed that the bark of the plant contains bauerenol, β-sitosterol and betulinic acid, while the leaves contain β-sitosterol, epilupeol, ursolic acid [6-7]. The bark of the plant has been traditionally used in the treatment of various painful and anti-inflammatory conditions, and infectious disease. There is no significant reports on neuropharmacological activities and other bioactivity studies of this valuable medicinal plant. Therefore, the present study was designed to investigate the neuropharmacological activities of the MECA.

MATERIAL AND METHODS

Plant collection and extraction of plant material

The plant *Callicarpa arborea* was collected from Coxbazar, Bangladesh on February 16, 2018. The sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No.: 45827 DACB). *Callicarpa arborea* leaves were separated from undesirable materials and dried in a shaded place. After drying, the plant part was grinded by Blender Machine (NOWAKE, JAPAN). 300 gm coarse powdered plant material was soaked in 800 mL Methanol. After ten days the maceration was filtered through fresh cotton bed and finally with Whatman no.-1 filters paper. The volume of the filtrate was concentrated by air drying (approximate yield value 0.867%). After proper drying crude extract was obtained and stored in cool, dark and dry place [8].

Animal

For the experiment, Swiss Albino mice of both sex having 3-4 weeks of age, weighing between 22 to 28 gm were collected from the Jahangirnagar University, Saver, Dhaka, Bangladesh. Soft wood shavings were used as bedding of cages. Animals were maintained under Standard Environmental conditions of temperature (24.0±1.00 C), Relative humidity (55-65% and 12 hrs. light /12 hrs. dark cycle). Husk and excreta were removed from the cages every day. Pellets of mice food, provided by ICDDRB were given to the fresh water. The newly bought mice were given a week rest to get over the food and water restrictions incurred during transit and to get themselves adapted with the new environment of the laboratory, before being employed in any experiment [8].

Drug and Chemicals

The standard drugs Diazepam was collected from local Pharmacy in Dhaka, Bangladesh.

Phytochemical screening

The methanol extract of *Callicarpa arborea* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using standard procedures [9]. The tests carried out on *Callicarpa arborea* extract revealed the presence of several important phytochemicals, which might be responsible for its medicinal properties.

Neuropharmacological Activity Test

Drug acting on the central nervous system (CNS) was first discovered by primitive human and are still the most widely used group of pharmacologic agents CNS action. The effect of drugs on the central nervous system (CNS) with reference to the neurotransmitters for specific circuits, attenuation should be developed to general organizational principals of neurons. The view that synapses represent drug modifiable control points within neuronal networks. It requires explicit delineation of the sites at which given neurotransmitters may operate and the degree of specificity with which such site that may be affected. The neuropharmacological activity of C. *arborea* extract was determined by widely used hole cross and open field test [10].

Study Design

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV, consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

Hole Cross Test

The method was carried out as described by Akeda H, *et al.* [11]. A steel partion was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into control, positive control, and test groups. The test groups were given *C. arborea* extract at the doses of 200 and 400 mg/kg body weight whereas the control group was provided vehicle (1% Tween 80 in water) and the positive control group was treated with standard drug, Diazepam (1mg/kg body weight). The number of passage of mouse through the hole from one chamber to another was counted for a period of 3 min at 0, 30, 60, and 90 minute after oral administration.

Open Field Test

In open field test, the test groups were given *C. arborea* extract at the doses of 200 and 400 mg/kg body weight orally while the control group received vehicle (1% Tween 80 in water) and positive control group received Diazepam ate the dose of 1 mg/kg body weight. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration.

Statistical analysis

SPSS for WINDOWS™ (version 12.0) was applied for the data analysis and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett t-test (2-sided). Data were presented as mean ± Standard error of the mean (SEM). $P<0.05$ was considered to be statistically significant.

RESULT

Preliminary Phytochemical analysis revealed the presence of flavonoids, tannins, steroid, terpenoid, alkaloids and saponins in the leaves extract of *C. arborea* (Table 1).

Table-1: Result of Phytochemical group test

Constituents	Result
Reducing sugar	-
Combined reducing sugar	-
Tannins	+
Flavonoids	+
Saponin	+
Gums	-
Steroids	+
Alkaloids	+
Glycoside	-
Proteins	-
Terpenoids	+
Acidic compounds	-

Here, + indicates Presence, - indicates Absence

In the open field test, the methanol leaves extract of *C. arborea* significantly suppressed the number of squares traveled by mice from its initial score at the doses level of 200 and 400 mg/kg body weight which is comparable to the reference drug Diazepam (Table 2). The maximum suppression was exhibited at 90 min after standard/extract administration.

Table-2: Effect of *Callicarpa arborea* in open field test on mice

Treatment	Dose (mg/kg b.w.)	Number of Movement			
		0 min	30 min	60 min	90 min
Control	0.1 mL/mouse	99.00±5.57	86.66±6.69	78.00±7.09	67.00±7.81
Diazepam	1	59.00±3.78	45.33±4.91	33.33±4.97	28.00±4.04
Group-I	200	38.66±2.20	26.66±2.18	19.33±3.38	13.66±2.33
Group-II	400	24.66± 3.75	16.66±2.96	11.66±1.15	7.00±1.15

Control: Distilled Water (0.1 mL/mouse), Positive Control: Diazepam (1 mg/kg b. w.)
Group-1: Extract (200 mg/kg b. w.) and Group-2: Extract (400 mg/kg b. w.)
Values are expressed as Mean ± SEM (n=5)

The sample significantly decreased the number of movement of mice compared to control group at the doses of 200 and 400 mg/kg body weight in hole cross experiment (Table 3). The maximum decrease in movement was observed at 90 min after drug/extract administration.

Table-3: Effect of *Callicarpa arborea* in hole cross test on mice

Treatment	Dose (mg/kg b.w.)	Number of Movement			
		0 min	30 min	60 min	90 min
Control	0.1 mL/mouse	84.66±8.56	74.00±7.83	74.66 ±8.00	74.00±8.14
Diazepam	1	85.00±4.58	75.33±3.84	67.00±4.35	57.33±4.48
Group-I	200	68.33±3.75	63.66±5.23	53.00±5.50	42.66±2.90
Group-II	400	59.66±2.33	52.33±2.60	45.33±4.40	31.66±5.81

Control: Distilled Water (0.1 mL/mouse), Positive Control: Diazepam (1 mg/kg b. w.)
Group-I: Extract (200 mg/kg b. w.) and Group-2: Extract (400 mg/kg b. w.)
Values are expressed as Mean ± SEM (n=5)

DISCUSSION

From the ancient times, medicinal plants provide remedies of various ailments for their therapeutic effects [12]. Traditionally, anxiety and numerous behavioral disorders have been managed from the medicinal plants of verbenaceae genus. Our rudimentary screening for phytochemicals suggests that the availability of flavonoids, tannins, steroid, terpenoid, alkaloids, saponins may exert neuropharmacological activity. Many phytochemical studies well tried that tannins exert sedative activity. Saponins even have potent sedative activity [13]. Moreover, it's already reportable that flavonoids have potent sedative and anxiolytic effects [13].

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system [14-17].

The locomotor activity lowering effect of *C. arborea* extract was evident in Swiss albino mice for the both doses of 200 and 400 mg/kg body weight at the 2nd observation (30 min) and continued up to 3rd and 4th observation (60 and 90 min) period (Table 2 and Table 3). Moreover, the validation of anxiety was performed by measuring external signs through hole-cross tests.

Open field test exhibited that the depressing action of the extract was evident from the second observation period in the test animals at the doses of 200 and 400 mg/kg body weight (Table 2). Although evaluating the neuropharmacological activities of *Callicarpa arborea*, it was found that the extract possesses central nervous system depressant activity as indicated by decreased exploratory behavior of mice in both open field and hole cross test. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 4th observation period (90 min). Both hole cross and open field test showed that the depressing acting potential of the sample was dose-dependent (evident from the 2nd observation period in the test animals at the doses of 200 and 400 mg/kg body weight). Maximum depressant effect was observed from 2nd (30 min) to 4th (90 min) observation period at the dose of 400 mg/kg body weight. The result obtained in our present study, indicates that the leaves of *Callicarpa arborea* might possess depressant action on CNS.

The presence of flavonoids, alkaloids, terpenoids and tannins in the methanol extract of *C. arborea* may be accountable for neuropharmacological activity, because it is well established that these phytochemicals may be responsible for a wide range of bioactivities [18-20].

The sedative effect of the extract may be due to hyperpolarization of the CNS via interaction with gamma-amino-butyric acid (GABAA) receptor or benzodiazepine receptor sited adjacent to the GABA receptor. GABA is the key inhibitory neurotransmitter

in the CNS and CNS depressant drugs mainly exert their action through GABAA receptor [21].

CONCLUSION

The results of the pharmacological investigation rationalize the uses of leaves of the plant in folk medicine. Based on the result of the present study, it can be concluded that methanol extract of leaves of *Callicarpa arborea* possesses significant neuropharmacological effect. Hence, further studies are required to isolate bioactive compound(s) and elucidate the specific molecular mechanisms responsible for the pharmacological activities of the plant.

List of abbreviations

MECA = Methanol extract of *Callicarpa arborea*
ICDDR, B = International Center for Diarrhoeal Disease and Research, Bangladesh
GABA = Gamma-Amino Butyric Acid
Min = Minute
SEM = Standard error of the mean
b. w. = Body weight
CNS = Central Nervous System

Acknowledgements

The authors are thankful to the Department of Pharmacy, Stamford University Bangladesh to permit to use the facilities of the Pharmacology and Phytochemistry Laboratory.

Ethics Approval and Consent to Participate

All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) postulated by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/TAEC/11.01) of Stamford University Bangladesh permitted all experimental rules.

Competing Interests

The authors declare that they have no conflict of interests.

Consent for Publication

Not applicable

Funding

This research wasn't funded by any funding agencies. All the studies had been self-funded by author and co-authors.

Authors' Contributions

Aliza conceived, planned, did literature searches, performed the experiments and wrote the manuscript. Sharmin Sultana contributed to data analysis, interpreted results, manuscript writing. Md. Lokman Hossain was the supervisor and finally edited the manuscript. All authors read and approved the final manuscript.

REFERENCE

1. Asolkar, L. V., Kakkar, K. K., & Chakre, O. J. (1992). Second supplement to Glossary of Indian Medicinal Plants with Active Principles Part-I (AK). *Council of scientific and industrial research (PID)(part-I), New Delhi*, 217-218.
2. Usher, G. (1974). *A dictionary of plants used by man*. Constable and Company Ltd..
3. Wiart, C. (2006). *Medicinal plants of Asia and the Pacific*. CRC Press.
4. Manandhar, N. P., & Plants, S. M. (2002). People of Nepal Timber Press Inc..
5. Tu, Y., Sun, L., Guo, M., & Chen, W. (2013). The medicinal uses of Callicarpa L. in traditional Chinese medicine: An ethnopharmacological, phytochemical and pharmacological review. *Journal of ethnopharmacology*, *146*(2), 465-481.
6. Sen, M., & Pal, B. C. (1974). Chemical investigation of the bark of Calicarpa arborea (Verbenaceae). *Journal*.
7. Heywood, V. H., Brummitt, R. K., Culham, A., & Seberg, O. (2007). *Flowering plant families of the world* (No. Sirsi) i9781554072064). Ontario: Firefly Books.
8. Hossain, M. L., & Sultana, S. (2019). *In vivo* Assessment of Neuropharmacological Activity of Methanol Bark Extract of *Mimosa pudica* in Mice. Adv. Pharmacol Pharm, 7(2), 33-37.
9. Karmakar, U. K., Sultana, S., Nishi, S., Biswas, N. N., Hossain, L., & Sheikh, S. (2018). Antioxidant, Analgesic, Antimicrobial, and Anthelmintic Activity of the Dried Seeds of *Bixa orellana* (L). *Int. J. Pharm*, 8 (1), 150-163.
10. Basak, A., Hossain, M. L., & Parvin, M. N. (2016). Evaluation of phytochemical and pharmacological activities of *Bacopa monnieri* (L.). Int. J. Sci. Rep, 2 (10), 242-247.
11. Akeda, H., Tsuji., M, & Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. European Journal of Pharmacology, 350(1):21-29.
12. Wagner, H., Ott, S., Jurcic, K., Morton, J., & Neszmelyi, A. (1983). Chemistry, 13C-NMR study and pharmacology of two saponins from *Colubrina asiatica*. Planta medica, 48, 136-141.
13. Häberlein, H., Tschiersch, K., & Schäfer, H. (1994). Flavonoids from Leptospermum scoparium with affinity to the benzodiazepine receptor characterized by structure activity relationships and *in vivo* studies of a plant extract. Die Pharmazie, 49, 912-922.
14. Leeratiwong, C., Chantaranothai, P. & Paton, A.J. (2009). A synopsis of the genus *Callicarpa* L. (Lamiaceae) in Thailand. Thai Forest Bulletin (Botany), 37: 36-58.
15. Bramley, G. L. (2009). The genus *Callicarpa* (Lamiaceae) on Borneo.

Botanical Journal of the Linnean Society, 159, 416-455.

16. Jones, W. P., & Kinghorn, A. D. (2008). Biologically active natural products of the genus callicarpa. Curr Bioact Compd, 4(1), 15-32.
17. Tu, Y., Sun, L., Guo, M., & Chen, W. J. (2013). The medicinal uses of Callicarpa L. in traditional Chinese medicine: an ethnopharmacological, phytochemical and pharmacological review. Ethnopharmacol, 146(2), 465-81.
18. Adedapo, A. A., Sofidiya, M. O., Maphosa, V., Moyo, B., Masika, P. J., & Afolayan, A. J. (2008). Anti-inflammatory and analgesic activities of the aqueous extract of Cussonia paniculata stem Bark. *Records of Natural Products*, *2*(2), 46.
19. Akkol, E. K., Tatli, I. I., & Akdemir, Z. S. (2007). Antinociceptive and anti-inflammatory effects of saponin and iridoid glycosides from Verbascum pterocalycinum var. mutense Hub.-Mor. *Zeitschrift für Naturforschung C*, *62*(11-12), 813-820..
20. Narayana, K. R., Reddy, M. S., Chaluvadi, M. R., & Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian journal of pharmacology*, *33*(1), 2-16..
21. Dolai, N., Karmakar, I., Kumar, R. B. S., & Haldar, P. K. (2012). CNS depressant activity of Castanopsis indica leaves. *Oriental Pharmacy and Experimental Medicine*, *12*(2), 135-140.