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"Evaluation of anti oxidant and CNS stimulant activity of hydro alcoholic extract of "*Moringa oleifera*"on SD rats"

Sujata Kushwaha
Associate Professor
Aryavart University, Sehore (M.P.)

ABSTRACT

In the present study Moringa oleiferaextract was prepared in two different solvents depending on polarity and was tested for the presence of different phytoconstituents. Phytochemical analysis of Moringa oleiferarevealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. Moringa oleiferahas rich presence of phenols and flavanoids.

Antioxidant, antiviral, antifungal, diuretic, laxative, anti-diarrhoeal activities are reported in Moringa oleifera. The phenols are the centrally active class of phytoconstituents which are responsible for many pharmacological activities. Acute toxicity study was carried out on the total phenols extract of Moringa oleifera. The estimated LD_{50} of HEAS was 290.00 mg/kg indicating its moderately toxic properties.

This study not only substantiate central depressant and muscle relaxant activity of Cissus quadrangularis *L*, it also attempts to establish phyto-pharmacological co-relation with presence of phenols are known for their central effect. Ethnobotanically similar Vitaceae plant contains triterpenes and phytosterol like βamyrin, 24-metaylene cycloartenol and β-sitosterol has reported sedative, anti-anxiety and anti-depressant activity. Further studies are suggested to explore mechanism of action of *Moringa oleiferalaris*phenols in concern to interaction with central receptors.

KEY WORDS

Moringa oleifera, CNS Activity, Antioxidant, Antiviral, Antifungal, Diuretic.

INTRODUCTION

Antioxidants became a vital part of our lives today since antioxidants neutralizes or destroys "reactive oxygen species" (ROS) or free radicals before they damage cells. The oxidation induced by ROS results in cell membrane disintegration, membrane protein damage, and DNA mutations, which results in aging and further initiates or propagates the development of many diseases such as arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis.[1]

Central nervous system (CNS) stimulation is the primary action of a diverse group of pharmacological agents and an adverse effect associated with the administration of an even larger group of drugs. CNS stimulation consists of a range of behaviors including mild elevation in alertness, increased nervousness and anxiety and convulsions.[2]

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In general, any hyper excitability associated with drug administration results from an alteration in the fine balance normally maintained in the CNS between excitatory and inhibitory influences. Thus, the bases for CNS stimulation by the class of drugs reside in adjusting the integration of excitatory and inhibitory influences at the level of the individual neuron.[3]

Cissus quadrangularis is an annual or perennial herb with huge medicinal properties distributed throughout the tropical world. It requires warm tropical climate and propagated by stem cuttings in months of June and July. It is found throughout the hotter parts of Bangladesh, Thailand, Java, Philippines also India, West Africa and Ceylon. [4]

The plant contains potassium, calcium, zinc, sodium, iron, lead, cadmium, copper, calcium oxalate and magnesium. Other constituents of the plant are resveratrol, piceatannol, pallidol, parthenocissus, 31 methyl triacontanoic acid, taraxeryl acetate, taraxerol, iso-pentadecanoic acid, phenol, tannin, carotene and vitamin. It also contains 31 methyl tritiacontanoic acid and 7 –Oxo onocer-8-ene-3 β 21 α diol.[5]

Traditionally, the roots and stems are most useful for healing of fracture of the bones. The plant has been documented in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis. The stem juice of plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis. The herb is fed to cattle to induce flow of milk. The stout fleshy quadrangular stem is traditionally used for treatment of gastritis constipation, eye diseases, piles and anemia. [6]

MATERIAL AND METHODS

Collection, Authentication Plant Drugs

It would be possible to identify crude drugs using organoleptic characteristics, morphological characteristics, and microscopic investigation. Herbariums and renowned botanical gardens are very useful for identifying unknown medications. *Cissus quadrangularis L*, the chosen plant, was obtained at the Moolchand Phoolchand herbal shop in Bhopal, Madhya Pradesh, for its leaves. Expert botanists from the Department of Botany at Safiya College in Bhopal verified the authenticity of the complete plant medicine. For later usage, every plant medication that was gathered was cleansed, shade-dried, ground into a reasonably coarse powder, and then stored in an airtight container.

SUCCESSIVE SOLVENT EXTRACTION OF PLANT DRUGS

Soxhlet extraction.[7]

Water (40°–60°C) was used to remove the plant material (leaves) for around 12 hours. A tarred conical flask was used to collect the hydroalcoholic extracts. The solvent was eliminated by heating it up. The % weight-to-weight basis was calculated after the extracts produced by each solvent were weighed to a fixed weight.

Preliminary Phytochemical Screening

To analyze the plant material in terms of its active ingredients, one must first perform a preliminary phytochemical screening. Excoecaria agallocha extract samples were put through routine phytochemical assays to identify the various compounds they contained. Alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins, and amino acids were identified using phytochemical screening.[8]

Determination of Total Phenolic Content (TPC):

The total phenolic content of the extracts was determined by the modified Folin-Ciocaltu method (Imran and Khan, 2014). In short, 1.0 mL of each extract (1 mg/mL) was mixed with 5 mL Folin-Ciocaltu reagent (1:10 v/v in distilled water) and 4 mL of 7.5% Sodium carbonate. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Then the absorbance was measured at 765 nm with a spectrophotometer (Specord 205, Germany). Gallic acid was used as a standard for the calibration curve. The estimation was carried out in triplicate then the results were averaged and expressed as GAE (mg/gm of dry extract).[9]

DPPH (1,1-diphenyl-2-picrylhydrazyl)

Radical Scavenging Activity:[10]

The stable DPPH radicalscavenging activity was measured using the modified method described by Ukwueze et al. (2014). In this assay, 2 mL of 0.1 mM methanolic DPPH solutions was added to 2 mL of acetone and ethanol extracts solution at different concentrations and the contents were stirred vigorously for 15 sec. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. Absorbance was measured against a blank at 517 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Germany). The percentage of DPPH radical-scavenging activity was calculated as:

$$\frac{A0 - A}{A} x100$$

Where, A0 is the absorbance of the control solution (containing all reagents except seed extracts); A is the absorbance of the DPPH solution containing seed extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration to determine the concentration of extract necessary to decrease DPPHradical-scavenging by 50% (called IC_{50}). Ascorbic acid and BHA (Butylated Hydroxyanisole) were used as positive control standards.

Total Antioxidant Capacity Determination:

Total antioxidant activity of the extract was calculated by the phosphomolybdenum assay method which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition (Matthias et al., 2015). The

extract (2.0 mg/mL, 0.3 mL) was allowed to mix up with 3.0 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate) and the reaction mixture was incubated at 950C for 90 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm using a UV-Visible spectrophotometer against an appropriate blank. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.[11]

Reducing Power Assay:

This assay was determined according to the method reported by Dehpour et al. (2009). Briefly, 10 mL of acetone and ethanol extracts solution of different concentrations was mixed with 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%; w/v) and 2.5 mL of phosphate buffer (0.2 M; pH 6.6) . The mixture was incubated at 50°C for 20 min. After that the reaction was terminated by adding 2.5 mL of trichloroacetic acid (10%; w/v), then the mixture was centrifuged at 3000 rpm for 10 min. Finally the supernatant solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL; 0.1%, w/v) solution. Then the absorbance was measured at 700 nm against a blank reading. Increased absorbance value of the reaction mixture indicates increased of reducing power of the extracts.[12]

Animal Studies

Male and female breed Swiss Albino mice weighing between 25-32 gm were used in the experiments. All the experiments were performed between 9:30 to 16:30 hr to overcome diurnal and circadian variations. All the animals were housed at a temperature of 24 ± 2^0C and in a relative humidity of 65 ± 5%. A 12:12 hr L: D cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of RKDF College of Pharmacy (Proposal number IAEC/RKDFCP/2023/09) and were in accordance with the guidelines of the IAEC.

The acute toxicity study was performed *Moringa oleifera*using Swiss Albino mice. The animals were fasted for 12 hr prior to the experiment and were administered i.p with different dose of HEAS and observed for mortality up to 48 hr (short term toxicity). Based on the short term toxicity, the dose of next animal was determined as per OECD guideline 425, a limit test was performed to categorize the toxicity class of the compound. The limit test was performed at 2000 mg/kg, which showed 40.0% mortality. A main test was performed to determine the exact LD50 value following OECD up and down method. LD50 was calculated as 283.00 mg/kg from graphical representation. A dose range of 25, 50 and 75 mg/kg was selected for evaluation of pharmacological activities. All the animals were also observed for long term toxicity up to 14 days [13]

Effect of HEAS on thiopental sodium induced hypnosis on mice After 30 min of i.p injection of ETTS, vehicle and standard drug (Diazepam, 2 mg/kg), all the animals were given thiopental sodium in doses of 25 mg/kg. The animals were placed on their backs on a warmed pad and duration of loss of righting reflex was measured until they regain their righting reflex.[14] Effect of HEAQ on locomotor activity of mice on Actophotometer Locomotor activity was recorded with a digital activity cage (Dolphin, India). In a pre-test session mouse was individually placed in the actophotometer for 10 min to score the basal reading. All the animals were treated with vehicle, different doses of HEAQ and standard drug diazepam (2 mg/kg) then placed individually in the actophotometer to score locomotor activity after 30 min. Mean change in the locomotor activity was calculated for each group.[15]

Effect of HEAQ on muscle grip performance of mice on rotarod apparatus Digital rotarod apparatus (Jyoti Scientific, India) was used to evaluate the muscle relaxing and sedative effects of HEAQ. The animals were placed individually on the rotarod, rotating at a speed of 25 rpm to score the fall off time. Respective groups of animals were treated with vehicle, different doses of HEAQ and reference standard diazepam (2 mg/kg), and were subsequently assessed for their performance on the rota rod after 30 min of drug treatment. Percentage changes in fall off time were calculated for each group [15]

Effect of HEAQ on parameters of anxiety on elevated plus-maze in mice Locally fabricated apparatus consisting of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) elevated to the height of 25 cm was used. Animals of all five groups were treated with vehicle, different doses of HEAQ and diazepam (2 mg/kg) respectively, 30 min before the test. Animals were placed individually at the centre of the Plus-maze with their head facing towards the open arm. First preference of mice to open or enclosed arm, number of entries in open and enclosed arms and average time of each animal spends in each arm was recorded for next five minutes [15]

Effect of HEAQ on tail suspension induced immobility on mice the animals were suspended by a plastic string 75 cm long, about 20 cm above a table top. The duration of immobility was recorded for a period of 6 min (after discarding activity in the first 2 min because animals try to escape during this period). Mice were considered immobile only when they hung passively and remain motionless. The same procedure was followed with animals treated with vehicle, standard drug (diazepam and imipramine in doses of 2 and 20 mg/kg, i.p respectively) and HEAQ 30 min before the test, and percentage change in immobility was calculated [16]

Effect of HEAQ on forced swimming induced immobility in mice Animal were forced to swim individually, for 15 min, in glass beaker (11 cm diameter, 5 cm height) containing fresh

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water up to a height of 6 cm, at a temperature of 22 ± C. This constitutes the "pre-test" session. Twenty four°1 hours later, the animal were administered the vehicle, HEAQ, standard drugs imipramine (20 mg/kg) and diazepam (2 mg/kg) 30 min before test and each animal was once again forced to swim in a similar environment for a period of 6 min in a "test session". The animals' attempts to get out of the beaker were interspersed with periods of the immobility signifying "behavioral despair". The total duration of the immobility during the last 4 min of the 6 min test was recorded [16].

Statistical analysis

All the values were expressed as mean ± SEM. The data was analyzed by using one way and two way analysis of variance (ANOVA) as appropriate followed by Turkey's test. p value

RESULTS AND DISCUSSION

Extraction of Plant Drugs

250g of the leaves of the plant *Moringa oleifera*were ground into a relatively coarse powder, and water was used as the solvent in a Soxhlet extraction. The extracted material was weighted and dried. The plant's percentage yield was estimated using a conventional formula.

The measured plant medicine extract was kept in desiccators for further use. The yields were found to be (12.6% w/w of crude drug) of hydroalcoholic extract with Bark brown colour semisolid mass, for *Moringa oleifera*Leaves.

EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY

Total phenolic content:

The TPC were obtained from Folin-Ciocalteu's reagents with correction. The calibration curve of gallic acid has the equation y=6.9103x–0.0936 (R2=0.997), where y is absorbance at 765 nm and x is the concentration of gallic acid in µg/mL.

Results revealed that the best solvent and extraction method for extracting phenolic compounds followed by HEAS. From Table it is seen that HEAS gave the highest phenolic content 72.77 mg GAE/gm of dry extract.This may be due to the fact that, phenolics are often extracted in better amounts in more polar solvents such methanol.

Table: 1 Amount of total phenolic content in hydroalcoholic extract extracts of *Cissus quadrangularis L*

Cissus quadrangularis L	**Total phenolic content (mg GAE/ gm of dry extract)**
HEAS	72.77±1.35

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DPPH Scavenging Activity:

The total antioxidant capacity was measured as the cumulative capacity of the compounds present in the sample to scavenge stable organic free radicals with a deep violet color by giving the absorbance ranging from 515-528 nm, using the DPPH reaction. Presence of antioxidant in the sample leads to disappearance of DPPH radical chromogens which can be identified spectrophotometrically at 517 nm.

The radical scavenging effects of tamarind seed are represented in Figure 1, where hot methanol extracts, the higher the concentration of extracts the higher is the %inhibition (DPPH scavenging activity). In the present study, the scavenging activity of extracts is as follows:
Here % inhibition for ascorbic acid is 96.90 compared to IC_{50} value was 98.77±0.94 (HEAS).

Total antioxidant capacity determination:

The calibration equation for ascorbic acid for the determination of total antioxidant capacity in different solvent extracts of *Moringa shay*was determined to be y=0.002x+0.015 (R2=0.997) where y is absorbance at 695 nm and x is concentration in µg/ml. Using the equation TAC values are summarized in Table 3 showed that highest TAC was obtained from HEAS (44.60 AAE/g). These results are in concordance with the previous findings of Soong and Barlow (2004)

Ferric reducing power assay (FRAP):

FRAP assay is one of the most simple, rapid, very useful and inexpensive test for analysis. The FRAP assay was developed for direct antioxidant power of a sample. The FRAP measures the antioxidant effect of any substance in the reaction medium as reducing ability.

Antioxidant potential of the *Moringa shay*extracts of tamarind was estimated from their ability to reduce Fe3+ complex to Fe2+ complex. The results of antioxidant capacities of raw and processed seed coat extracts are given in Figure 2. The FRAP of *Moringa shay*extracts increased with increasing concentration. Here HEAS showed higher ability to reduce Fe3+ to Fe2+ with absorbance 0.7802 and can be compared with ascorbic acid whose absorbance was 1.4885. The plant extracts with higher levels of total phenolic content also exhibited greater reducing power. Bushra et al. (2007) found reducing power (at absorbance 700nm) of Methanol extract of *Moringa shay*1.73 under hot extraction

Animal Studies

Acute toxicity

The estimated LD50 of HEAS was found to be 290.00 mg/kg, i.p.

Effect of HEAS on thiopental sodium induced hypnosis in mice

Table 2 showed that the group treated with 150 mg/kg HEAS showed extremely significant increase ($p < 0.001$) in average sleeping time (73.40%).

Table: 2 Effect of Moringa oleiferaon thiopental sodium induced hypnosis in mice.

Groups	Dose (mg/kg, i.p)	Onset of action in min (M ± SEM)	Average sleep time in min (M±SEM)	% Change in sleeping time
Thiopental	25	6.49 ± 1.32	21.50 ± 2.13	-
Diazepam+ Thiopental	2+25	11.33 ± 1.07	53.23 ± 2.72	+ 161.66
HEAS	50	8.27 ± 1.41	17.12 ± 1.42	-22.47
HEAS	100	6.13 ± 1.01	23.70 ± 1.18	+ 10.48
HEAS	150	9.47 ± 1.46	36.24 ± 1.35	+ 73.40

Values are expressed as Mean ± SEM with (n = 6) per group.

Effect of HEAS on locomotor activity of mice

The HEAS significantly decreased the locomotor activity in a dose dependent manner. The depression of locomotor activity was found to be maximum at a dose of 150 mg/kg (70.45%).

Table: 3 Effect of *Moringa oleifera*on locomotor activity on mice.

Groups	Dose (mg/kg, i.p)	Locomotion score (M ± SEM)		% Change in locations
		Basal	After drug administration	
Vehical control	0.5ml/100mg	493 ± 11.62	-	-
Diazepam	25	417 ± 12.54	17.25 ± 10.47	- 96.58
HEAS	50	473.21 ± 25.87	178.19 ± 10.28	-63.78
HEAS	100	556.11± 49.87	171.04 ± 30.47	-68.74
HEAS	150	513.21 ± 11.67	147.68 ± 10.84	-70.45

Effect of HEAS on muscle grip performance of mice

HEAS at 150 mg/kg dose caused significant ($p < 0.05$) decrease in fall off time with 81.22% decrement while the standard drug diazepam had fall off time sec with 92.55% decrease (Table 4).

Table: 4 Effect of Moringa oleiferaon muscle grip performance of mice on rotarod apparatus

Groups	Dose (mg/kg, i.p)	Locomotion score (M ± SEM)		% Change in locations
		Basal	After drug administration	
Vehicle control	0.5ml/100mg	199.50 ± 6.41	-	-
Diazepam	25	144 ± 12.41	75.11 ± 11.41	- 92.55
HEAS	50	117.14± 11.47	190.47 ± 11.54	-66.33
HEAS	100	179.21± 9.54	93.57 ± 4.77	-49.04
HEAS	150	168.16 ± 13.55	33.91 ± 2.10	-81.27

Effect of HEAS parameters of anxiety in mice

The HEAS treated groups at dose of 50, 100 and 150 mg/kg showed 51.47, 51.88 and 49.21 % respective preference to open arm which was nearly same in vehicle treated group (53.04%). Whereas in standard drug diazepam treated group the percent preference to open arm was 61.54 %. HEAS treatment showed decrease in percentage time spent in open arm in a dose dependent manner and increased the % open arm entries comparable to diazepam (Table 6.7)

Table: 5 Effect of Moringa oleiferaon parameters of anxiety on elevated plus–maze in mice

Groups	Dose (mg/kg, i.p)	% Preference to open arm	Total no. of entries (M ± SEM)	% Open arm entries	% Time spent in open arm
Vehicle control	0.5ml/100mg	53.04	14.87 ± 3.22	31.24	17.35
Diazepam	2	61.54	12.52 ± 2.22	69.85	53.74
HEAS	50	51.47	13.47± 1.81	51.14	16.58
HEAS	100	51.88	13.11± 2.74	50.24	14.47
HEAS	150	49.21	12.01 ± 1.51	53.08	13.66

Effect of HEAS on tail suspension induced immobility on mice

Treatment of diazepam showed extremely significant increase (97.11%) in duration of immobility while Imipramine decreased the duration of immobility (90.47%) as tabulated in Table 6.8. HEAS at dose of 150 mg/kg showed highly significant increase ($p < 0.01$) in duration of immobility (48.**43%).**

Table: 6 Effect of *Moringa oleifera*on tail suspension induced immobility on mice

Groups	Dose (mg/kg, i.p)	Duration of immobility in sec (M ± SEM)	% Change in immobility duration
Vehicle control	0.5ml/100mg	126.78 ± 7.14	-
Diazepam	2	249.81 ± 15.47	+97.11
Imipramine	20	13.41± 4.44	-90.47
HEAS	50	83.21± 7.89	-34.58
HEAS	100	151.61± 3.91	+21.45
HEAS	150	187.74 ± 11.81	+48.43

Effect of HEAS on forced swimming induced immobility in mice

Diazepam and HEAS treated animals in all the tested doses showed non-significant change in duration of immobility compared to control. Imipramine significantly decreased the immobility duration as presented in Table 9.6.

Table: 7 Effect of *Moringa oleifera*on forced swimming induced immobility in mice

Groups	Dose (mg/kg, i.p)	Duration of immobility in sec (M ± SEM)
Vehicle control	0.5ml/100mg	160.35 ± 12.47
Diazepam	2	189.11 ± 14.88
Imipramine	20	36.71± 3.85
HEAS	50	138.22± 22.87
HEAS	100	131.62± 14.78
HEAS	150	128.52 ± 11.77

The use of herbal items or natural therapies to treat anxiety and depression has attracted a lot of public interest. Recently, it was shown that a number of plants exhibit anxiolytic properties in several anxiety-prone animal models. There has also been speculation that a number of conventional herbal remedies have anxiolytic properties.

This study examined the potential central effects of Cissus quadrangularis L. using a variety of experimental models relevant to the central nervous system, such as rotarod, elevated Plus-maze, tail immersion test, and forced swim test.

These tests serve as traditional models for screening CNS action and provide data on anxiolytic, myorelaxant, psychomotor, and depressive or stimulant properties.

CONCLUSION

In the current study, the extract of *Moringa shay*was made in several solvents based on their polarity, and the presence of various phytoconstituents was assessed. Alkaloids, saponins, flavonoids, triterpenes, tannins, and steroids were found in *Moringa shay*after a phytochemical

examination. With a yield of 10.05 percent, *Moringa shay*has a high concentration of phytosterol. Cissus quadrangularis L. has been shown to have antioxidant, antiviral, antifungal, diuretic, laxative, and anti-diarrheal properties.

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