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EVALUATION OF NEUROPROTECTIVE PROPERTY OF *TECOMA STANS* FLOWERS EXTRACT

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ABSTRACT

Objective: The present work was designed to investigate the neuroprotective efficacy of methanolic extract of *Tecoma stans* flowers (METSF) in laboratory animal models. **Methodology:** The extract of *Tecoma stans* flowers was investigated against motorcordation test, elevated plus maze and by morris water maze. The standard drugs, various doses of METSF was administered to experimental animals to assess the motorcordation test using rotarod apparatus, retention of memory in mice using elevated plus maze and learning & memory study by morris water maze. **Results:** The animals administered with drug and METSF showed a increase in muscle grip action on the 0 day, 7th day, 14th day and 21st day compared to positive control animals. Pretreatment with drug and test extract at different doses showed a reduction of LPO and nitrite levels in brain when compared to positive control animals. On administration of standard and test extract at different doses showed a marked increase in levels of GSH and Total protein when matched to positive control animals. In elevated plus maze model the Donepezil and METSF of various doses significantly reversed (decreased the TL) scopolamine-induced memory impairment in mice as compared to scopolamine treated groups. The scopolamine administered mice showed significant increase in the ELT as compared to normal control group animals. However, the administration of METSF at different doses and standard drug Donepezil was found to reduce the ELT significantly as compared to that of scopolamine treated animals. **Conclusion:** The protective property of METSF may be due to preventing the oxidative stress and potent anti-oxidant property.

KEYWORDS: Neuroprotective, *Tecoma stans*, scopolamine, chlorpromazine, rotarod.

INTRODUCTION

The Alzheimer's and Parkinson's diseases (PD) producing slow death of neuron producing loss sensory and cognitive functions.[1] Pathological processes such as oxidative stress, apoptosis, and mitochondrial dysfunction lead to neuronal deterioration in Alzheimer's disease (AD).[2] The research reports documented that lipid peroxidation may cause destruction of cholinergic neurons in AD[3] and dopaminergic neurons in PD.[4] The low antioxidant activity of the brain tissue liable to oxidative damage because the brain contains high level of polyunsaturated fatty acids and is more sensitive to peroxidation reactions.[5] Clinically the PD may cause slowness of movement, muscle rigidity, and rest tremor.[6] The PD can be treated with levodopa and carbidopa which act by reversing the symptoms of PD but the long term usage of these drugs produces side effects such as hallucinations, convulsions, anxiety and transient dizziness.[7]

The use of herbal drugs in the present scenario is increasing day by day because the allopathic drugs have more side effects and the presence of chemical constituents in herbal drugs have contributed significantly to the development of new drugs.[8]

Tecoma stans Linn is belonging to the family Bignoniaceae originate in India and commonly known as yellow bells and trumpet flower. It is an important medicinal plant exhibited anticancer[9], antioxidant[10], antimicrobial[11], anti-proliferative[10] effects. Different parts of the plant have been used in a variety of diseases in traditional medicinal system. The different parts of plant contain alkaloids namely tecomine and tecostamine.[12] In traditional system of practice the flower and bark were used as anti proliferative, wound healing, cytotoxic, antimicrobial, antifungal and for treating various cancers.

However, the neuroprotective efficacy of flowers of title plant in respect to learning, loss of memory, motor coordination and muscle grip strength in animal models

resembling to Alzheimer's disease and Parkinson's syndrome has not been validated scientifically till date. Hence, the present work is designed.

MATERIALS AND METHODS

Authentication of plant materials by the Botanist

The flower of *Tecoma stans* was identified and authenticated by Mrs. Gangambika Biradar, Professor of Botany Dept., KCP Science College, Vijayapur – 586103. Later the sufficient amount of *Tecoma stans* flowers were collected in the BLDE University garden, Vijayapur.

Extraction of the plant materials

For this study, the collected flowers were shade dried and ground to course powder. By Soxhlet's extraction method the coarse powder was then extracted with methanol. Later, with the help of rotary flash evaporator the extract was concentrated. The yield of the extract was 20.8%.

Preliminary phytochemical[13]

The screening of phytochemicals present in crude extract was determined by the reported methods mentioned in Practical Pharmacognosy by Kandelwal.

Institutional Animals Ethics Committee (IAEC) clearance

The research work was approved from IAEC before initiation of the experiment bearing approval number IAEC No.: BLDE/BPC/2019-20/645 Dated 21.09.2019.

Acute toxicity study[14]

The guideline No.423 of OECD was adapted for acute toxicity study of METSF in female albino mice weighing 20-30g. The METSF at dose of 2000 mg/kg i.p. did not cause any mortality of the animals. Hence, 2500 mg/kg was taken as LD50 cutoff value.

Evaluation of plant extract for neuroprotective property in experimental animals

The neuroprotective activity of METSF was evaluated against various experimental models in animals as mentioned under

1. Motorcordation test using Rotarod Apparatus (RA)
2. Memory improving activity using Elevated Plus Maze (EPM)
3. Learning and memory study using Morris Water Maze (MWM)

Motorcordation test by rotarod apparatus[15,16]

Rats were allocated into six groups of 6 animals each.

G - 1: Vehicle 1% gum acacia
G - 2: Chlorpromazine 3 mg/kg, i.p. for a period of 21 days
G - 3: Carbidopa + Levodopa (1:10 ratio) (10 mg/kg, i.p.).
G - 4: METSF 100 mg/kg orally 21 days
G - 5: METSF 250 mg/kg orally 21 days
G - 6: METSF 500 mg/kg orally 21 days

Chlorpromazine 3 mg/kg, i.p. was administered 30 minutes before the administration standard and METSF for a period of 21 days.

The muscle grip strength was recorded on 0 day, 7th day, 14th day and 21st day using rotarod test. After the 21st day, the brains were removed and weighed from sacrificed animals. The LPO, GSH, nitrites and total protein were measured in the brain tissue homogenate.

Assessment of brain LPO[17]

The LPO content was determined as per the method described in Ohkawa H et.al.

Assessment of brain GSH[18]

The brain content of GSH was estimated by procedure described in Moron MS et.al.

Assessment of brain Nitrites[19]

The brain content of *Nitrites* was estimated by procedure described Lidija R et.al.

Estimation of brain Total protein[20]

Lowry method was applied for the measurement of protein content of brain.

Memory improving activity in EPM[19-21]

Mice were allocated into six groups comprising of 6 in each group

G 1: Normal saline for 10 days
G 2: Scopolamine (SCP) 10 days
G 3: SCP and Donepezil for 10 days
G 4: SCP and METSF for 10 days
G 5: SCP and METSF for 10 days
G 6: SCP and METSF for 10 days

Dose: SCP 0.3 mg/kg, i.p., Donepezil 1 mg/kg, p.o., METSF G 4 100, G 5 250 and G 6 500 mg/kg, p.o. respectively.

Behavioral study

EPM serves as the exteroceptive behavioral model to evaluate acquisition and retention of memory. On the day 1, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was recorded on the first day (i.e.10th day of drug administration) for each animal. If the animal did not enter into one of the closed arm within 90 sec, it is gently pushed into one of the two closed arms and TL is assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h (11th day) after the first day trial.

Memory retention was calculated after 24 hrs of acquisition trial (on the day 1) as inflation ratio using the following formula:

$$\text{Inflation ratio (IR)} = L_1 - L_0 / L_0$$

Where, L_0 is initial LT on day 1 (i.e. 10th day of drug administration) in seconds and L1 is the LT after 24 hrs (11th day) of acquisition trial (11 day).

Estimation of Acetylcholinesterase[22]
After behavioral assessment, the mice were sacrificed and brain was isolated and weight was recorded. Than brain tissue was homogenized in phosphate buffer and was centrifuged at 3000 rpm for 10 min later the supernatant was separated and used for the estimation of acetylcholinesterase in brain tissue using Ellman et.al method.

Scopolamine employed amnesia in MWM test
Mice were allocated into six groups containing six animals each.
G 1: Normal saline only
G 2: Scopolamine (SCP) 0.3 mg/kg, i.p.
G 3: SCP + Donepezil
G 4: SCP + METSF
G 5: SCP + METSF
G 6: SCP + METSF

Dose: SCP 0.3 mg/kg, i.p., Donepezil 1 mg/kg, p.o., METSF G 4 100, G 5 250 and G 6 500 mg/kg, p.o. respectively.

After one week of training period (first seven days), from 8th day to 21st day the G 2 animals received SCP, i.p., G 3 animals received SCP + Donepezil, G 4 to G 6 received SCP and different doses of METSF. Later the learning and memory was evaluated by the MWM test.

On 7th day the results were documented in which the mice was placed into the maze 180° from the platform always. The time engaged by the mice to reach the platform was recorded initial acquisition latency (IAL). On 14th and 21st day the retention transfer latency (RTL) was recorder. The actual trial was conducted 30 min after treatment. On 21st day after recording of RTL the mice were sacrificed and brains were isolated. The isolated brains were further homogenized in phosphate buffer at pH 7.4. The homogenates obtained was centrifuged at for 15 min. The supernatant was used for the estimation of Brain acetylcholinesterase.[22]

Lipid peroxidation (LPO-MDA)[17]
The LPO content was determined as per the method described in Ohkawa H et.al.

Statistical analysis
The results from the research subjected to statistical analysis using one-way ANOVA followed by Turkey Kramer Multiple Comparison Test.

RESULTS

Phytochemical screening
The METSF showed the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins and phenolic compounds.

Acute toxicity
The METSF did not cause any mortality of the animals at dose of 2000 mg/kg. Hence, 2500 mg/kg was taken as LD_{50} cutoff value. Screening doses chosen for the activity were:
125 mg/kg - 1/20th of 2500 mg/kg b.w.
250 mg/kg - 1/10th of 2500 mg/kg b.w.
500 mg/kg - 1/5th of 2500 mg/kg b.w.

METSF effect on muscle rigidity in rotarod test
Muscle rigidity was determined in the rotarod apparatus. The positive control animals showed decreased mean fall off time on 0 day, 7th day, 14th day and 21st day as compared to that of normal control animals. The rats treated with combination of Carbidopa + Levodopa showed a significant increase in muscle grip activity on the 0 day, 7th day, 14th day and 21st day when compared to G 2 animals, whereas pretreatment with METSF at different doses also showed significant increase in the muscle grip activity on 0 day, 7th day, 14th day and 21st day as compared to G 2 animals. The results are presented in table 1.

METSF effect on brain LPO and Nitrites in rotarod test
The G 2 animals treated with CPZ showed increase in LPO and nitrite levels in brain (nM/mg protein) as compared to G 1 animals. Pretreatment with standard and test extract at different doses showed a significant reduction in level of LPO in brain and nitrite levels when compared to G 2 animals. The results are represented in table 2.

METSF effect on brain GSH and Total protein in rotarod test
The animals administered with CPZ (G 2) showed a substantial decrease in GSH and Total protein levels in brain (nM/mg protein) as compared to G 1 animals. Pretreatment with Carbidopa + Levodopa and METSF at different doses showed a substantial increase in levels of GSH and Total protein when compared to G 2 animals. The results are indicated in table 3.

METSF effect on memory performance in EPM
The administration of SCP increased TL in animals, representing its amnesia as compared to G 1 animals. The administration of standard Donepezil and METSF of various doses for 10 days did not affect TL of mice on 10th day (learning) as matched to the G 1 animals. But on 11th day Donepezil and METSF of various doses significantly reversed (decreased the TL) SCP induced memory impairment in mice as matched to G 2 animals. The results are tabulated in table 4.

METSF effect on brain Acetylcholinesterase in EPM
The augmented level of brain Acetylcholinesterase was seen in SCP treated animals as compared to that of G 1 animals. Treatment with different doses of METSF and Donepezil produced a decrease in brain Acetylcholinesterase as matched to G 2 animals. The results are indicated in table 5.

METSF effect on SCP induced impairment of learning and memory in MWM test

The SCP administered mice exhibited significant increase in the ELT as compared to G 1 animals. However, the treatment of various doses of METSF and Donepezil was found to decrease the ELT as compared to that of G 2 animals. The results are shown in the table 6.

Effect of METSF on brain Acetylcholinesterase

Administration of SCP caused increased level of the brain Acetylcholinesterase when matched to G 1 mice. The brain Acetylcholinesterase level was found to be decreased in standard and METSF treated groups as matched with the SCP administered group. The results are indicated in table 7.

METSF effects on brain MDA

SCP treated animals suggestively increased the brain MDA levels matched to the G 1 animals, indicating increased oxidative stress. The administration of standard and different doses of METSF significantly restored the brain MDA level as compared with G 2 animals. The results presented in table 7.

DISCUSSION

The work was aimed to evaluate the neuroprotective efficacy of METSF in cholopromazine induced muscle rigidity using rotarod apparatus and SCP produced memory alterations using EPM and MWM in investigational animals.

Chlorpromazine is an antipsychotic drug discovered after II World War. Administration of Chlorpromazine blocks dopamine D_2 receptors in the brain, the mechanism believed to relieve the positive symptoms of schizophrenia. The blockade of nigrostriatal dopamine receptors causes movement disorders, collectively known as extrapyramidal side effects.[18]

In the present research work the administration of Cholopromazine in the experimental causes muscle rigidity and increased level of oxidative stress in brain, which may cause the altered level of LPO, GSH, nitrites and total protein. The muscle grip strength was determined by rotarod apparatus in which the treatment of METSF at different doses and standard drug increased the mean fall of time. The increased levels of LPO and nitrates and decreased levels of GSH and total protein in brain are considered to be indication of neuronal damage and oxidative stress. The administration of different doses of METSF and standard drug reversed the chloropromazine induced changes which suggest the protective effect of METSF by preventing the oxidative stress and potent anti-oxidant property.

Elevated plus maze is used as exteroceptive behavioral model to evaluate acquisition and retention of memory. The administration of SCP in experimental animals causes the detrimental effects on short term memory, memory acquisition, learning and psychomotor speed. SCP produced amnesia has been projected as a model for dementia where reduced cholinergic function is the suspected cause. The dysfunctions are reversible by physostigmine which suggests that the memory impairment is specifically related to reduced cholinergic transmission caused by SCP.

The administration of different doses of METSF and Donepezil decreases the transfer latency in experimental animals indicating that plant extract is exhibiting the anti-amnestic activity. The anti-amnestic activity of different doses of METSF may be due to facilitation of cholinergic transmission.

Acetylcholine, an important neurotransmitter helps in the regulation of cognitive functions. Acetylcholinesterase (AChE) is an enzyme of brain cholinergic system that hydrolyses the neurotransmitter acetylcholine to choline and acetate in the synaptic cleft. The evidences have shown reduced activity of AChE in several brain disorders. The loss of cholinergic neurons in brain is the characteristic feature dementia. The administration of SCP in experimental animals causes the increased level of AChE whereas treatment with METSF at different doses and Donepezil reduce the AChE level indicating improved memory due to the potent drug METSF.

It has been documented that SCP alters short memory and acquisition of new knowledge and increases AChE activity and oxidative stress in the brain.[19] Scopolamine significantly increased the levels of MDA, a marker of cellular degeneration and AChE. Administration of METSF at different doses restored the MDA levels and AChE compared to G 1 animals. The neuroprotective activity of the METSF may be due to by inhibiting brain acetylcholinesterase activity and antioxidant property.

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Table 1: METSF Effect on muscle rigidity in rotarod test.

	Fall of time in Sec			
	0 Day	**7th Day**	**14th Day**	**21st Day**
G 1	123.1 ± 2.9	130.3 ± 2.4	114.4 ± 2.7	117.2 ± 3.1
G 2	95.2 ± 3.1@	65.5 ± 2.0@	53.8 ± 3.3@	35.9 ± 2.6@
G 3	115.5 ± 2.9***	115.5 ± 2.9***	112.5 ± 2.6***	101.6 ± 2.3***

G 4	117.5 ± 2.3*	70.5 ± 2.1*	75.6 ± 3.4***	76.2 ± 4.1***
G 5	110.9 ± 3.1*	90.9 ± 2.3***	85.3 ± 1.9***	83.2 ± 2.6***
G 6	114.6 ± 2.1**	105.3 ± 2.4***	93.2 ± 2.5***	90.9 ± 3.1***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as matched to G 2.

Table 2: METSF Effect on Brain LPO and Nitrites.

	Brain	
	LPO (nM/mg protein)	**Nitrates (nM/mg protein)**
G 1	1.10 ± 0.2	2.68 ± 0.4
G 2	3.21 ± 0.1$^{@}$	6.50 ± 0.7$^{@}$
G 3	1.35 ± 0.1***	2.93 ± 0.2***
G 4	2.91 ± 0.2***	5.53 ± 0.3*
G 5	2.50 ± 0.3***	4.23 ± 0.5**
G 6	1.92 ± 0.1***	3.39 ± 0.7**

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
as matched to G 2.

Table 3: METSF Effect on brain GSH and Total protein.

Groups	**Brain**	
	GSH (nM/mg protein)	**Total protein (nM/mg protein)**
G 1	6.01 ± 0.21	10.68 ± 0.41
G 2	1.56 ± 0.35$^{@}$	2.50 ± 0.72$^{@}$
G 3	5.83 ± 0.53***	8.93 ± 0.21***
G 4	2.88 ± 0.33*	3.53 ± 0.39*
G 5	3.61 ± 0.41**	5.69 ± 0.53**
G 6	4.01 ± 0.19***	7.39 ± 0.72***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
as matched to G 2.

Table 4: METSF Effect on memory performance in elevated plus maze.

	Inflation ratio
G 1	0.96 ± 0.01
G 2	0.26 ± 0.03$^{@}$
G 3	0.85 ± 0.02***
G 4	0.47 ± 0.01***
G 5	0.51 ± 0.06***
G 6	0.72 ± 0.03***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
as matched to G 2.

Table 5: METSF Effect on brain Acetylcholinesterase.

	AChE activity (U/mg protein)
G 1	12.65 ± 1.12
G 2	26.35 ± 1.35$^{@}$
G 3	14.85 ± 1.95***
G 4	21.37 ± 1.18^{ns}
G 5	18.51 ± 1.61**
G 6	15.72 ± 1.03***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
as matched to G 2.

Table 6: METSF Effect on Escape Latency in MWM test.

	Escape Latency Time (ELT) in seconds
G 1	36.65 ± 2.56
G 2	59.35 ± 2.35$^{@}$
G 3	38.85 ± 1.55***
G 4	52.37 ± 2.11^{ns}
G 5	46.51 ± 2.21**
G 6	40.72 ± 2.36***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
as matched to G 2.

Table 7: METSF Effect on oxidative stress level and AChE of mice brain in MWM test.

	MDA (nmoles/mg of protein)	**AChE activity (U/mg protein)**
G 1	1.10 ± 0.2	14.65 ± 1.12
G 2	4.21 ± 0.1$^{@}$	31.35 ± 1.35$^{@}$
G 3	1.85 ± 0.2***	18.69 ± 2.06***
G 4	3.10 ± 0.3**	28.41 ± 1.96^{ns}
G 5	2.60 ± 0.1***	24.25 ± 1.10*
G 6	1.92 ± 0.1***	19.13 ± 1.56***

Mean ± SEM, (n=6), where $^{@}$ $p < 0.001$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as matched to G 2.

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