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EFFECT OF *OXALIS CORNICULATA* ON CORTECOSTERONE INDUCED MEMORY IMPAIREMENT IN MALE ALBINO MICE

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ABSTRACT

In the traditional system of medicine, the whole plant *oxalis corniculata* (oxalidaceae) have been employed for treating anaemia, wounds, cancer, piles, abortifacient. The present study was undertaken to investigate the effects of *oxalis corniculata* on corticosterone induced memory impairment in male albino mice. Morris water maze test and passive shock avoidance paradigm were employed to test learning and memory. Two doses of methanolic extract of *oxalis corniculata* MEOC (200 and 400mg/kg P.O) were administered for 21 days in separate group of animals. The dose of MEOC (400mg/kg P.O) significantly improved learning and memory than the MEOC (200 mg/kg P.O). The chronic administration of corticosterone (5mg/kg) injection subcutaneously for inducing memory impairment in mice by damaging the hippocampus subregion CA_3. The MEOC significantly reduced memory deficits in the mice by the facilitation of cholinergic transmission in mouse brain. However further studies are required to identify exact mechanism of action. In the present study, *oxalis corniculata* has showed potential memory enhancing agent in the laboratory models employed.

Key words: *Oxalis corniculata*, Memory, Learning, Hippocampus.

INTRODUCTION

Dementia is syndrome or set of symptoms and signs occur at the same time due to disease in brain. It associate's (Berrios, 1987) the impairment of memory thinking, orientation, learning capacity, language and judgments. Changes is cognition occurs determination in the person's emotional control, social behavior or motivation and other cognitive changes often include apraxia, agnosia, aphasia, depression, anxiety, agitation, restlessness, apathy (Jewart *et al.*, 2005) and suspicion. Mainly dementia occurs in Alzheimer's disease (AD), (Dinesh Dhingra *et al.*, 2004), cerebrovascular disease, lewy body weight disease (LBD),

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Dementia occur due to the cerebral ischemia, energy failure, calcium overload, glutamate mediated cytotoxicity, oxidative stress and structural and functional changes (Juan Wang *et al.*, 2009). The changes in behaviour associated with the level or stage of severity of the dementia is a clinical dementia rating scale (CDRS) developed by Berg and published in 1988. Dementia occurs three stages characterized by severe memory loss, disorientation time and place and inability to make judgments. Dementia is a syndrome of failing memory and other intellectual functions with little in consciousness (Adam *et al.*, 2001). The central cholinergic pathways play a prominent role in learning and memory process (Nabeshima, 1993), centrally acting antimuscarnic drugs (e.g. scopolamine) impair learning and memory both in animals (Higashida and Ogawa, 1987).

Structural integrity of the hippocampus is necessary for certain types of learning and memory as declarative and spatial memories. The hippocampus has

glucocorticoid (GK) receptors in brain and participitates in the GK-mediated negative feedback of the hypothalamus-pituitary adrenal (HPA) axis (Sapolsky *et al.,* 1986). In the animal hippocampus corticosterone regulate neuronal metabolism, physiological functions, and after the cell morphology, chronically elevated levels corticosterone can produce neuronal atrophy and cell death in the hippocampus (Magarinos *et al.,* 1997). It also changes in neurotransmitters such as catecholamines, serotonin and γ-aminobutyric acid (GABA) in several brain structures. In the hippocampus corticosterone impairs GABA-mediated inhibitory neurotransmission and causes neurodegeneration via diminished expression of GABA receptors, (Weizman *et al.,* 1997). High amount of corticosterone enhance action of NE (Norepinphrine) via β-adrenoreceptors and increased dopamine (DA) turnover in prefrontal cortex is accompanied by the decreased spatial memory performance (Dziedzicka-Wasylewska *et al.,* 1997).

The plant *oxalis corniculata* (Creeping wood sorrel) also called procumbent yellow sorrel belongs to family oxalidaceae. It is very popular perennial herb that is distributed in world wide. The leaves of wood sorrel are quite edible with a tangy taste (Lee Allen Peterson, 1977).

The entire plant is rich in vitamin-C the plant *oxalis corniculata* leaves having three major c-glycosylflavones are reported. These are isoorientin, isovitexin and swertisin etc (Hiroki Mizokami *et al.,* 2008) *oxalis corniculata* used in wound healing (Taranalli *et al.,* 2004) antibacterial activity (Satish *et al*., 2008), Abortifacient and anti implantation (Sharangouda and Patil, 2007), anti fungal activity (Iqbal *et al.,* 2002) Relaxant activity (Achola *et al*., 1995) and other traditionally used in anaemia, dyspepsia, cancer, piles, dementia, convulsions (Madhavachetty *et al.,* 2008). The vitamin C supplementation effects on brain acetyl cholinesterase and neurotransmitter levels and treated in dementia induced by scopolamine in animals (Lee *et al.,* 2001). May be this is one of the claim to support this plant *oxalis corniculata* having dementia activity

Hence the present work we evaluated the effect of two different doses of methanolic extracts of *oxalis corniculata* on male mice with memory impairment induced by corticosterone using the morris water maze (Morris, 1989) and passive stock avoidance test (Leo *et al.,* 2003a).The *oxalis corniculata* showed significantly in two different doses 200mg/kg, 400mg/kg in corticosterone induced memory impairment in the mice.

MATERIALS AND METHODS

Plant material

The whole plant of *oxalis corniculata* was collected from Talakona forest, Chittoor district of Andhra Pradesh, India, in the month of September 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, W. Tambaram, Chennai. The voucher specimen (PARC/2009/343) of the plant was deposited at the college, for further reference.

Preparation of extracts

The whole plant of *oxalis corniculata* was dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through the 40 mesh sieve. A weighed quantity (200gm) of powder was subjected to continuous hot extraction with methanol in soxhlet apparatus for 48 hours. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage yield of methanolic extract of *oxalis corniculata* was found to be 24.94% w/w.

Animals used

Male albino mice (30-40mg) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet fed (Hindustan Level Limited, Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No./AEC/XIII/05/SVCP/2008-09).

Acute toxicity study

The acute toxicity of MEOC was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study (OECD, 2002).

Treatments

Animals were divided into four groups, each consisting of six male albino mice. The methanolic extract of *oxalis corniculata* was blackish oily extract divided into two doses MEOC-200mg/kg, MEOC-400mg/kg given orally daily for 21 days, 30 min before cortecosterone injection.

Corticosterone (VHB life sciences, 5mg/kg) was dissolved in absolute ethanol and subsequently diluted in water to the final concentration of 10% ethanol and injected subcutaneously in a volume 1ml/kg (De Quervain *et al.,* 1998).

Group - I: Normal control mice administered normal saline (0.9% w/v), Group - II: Disease control administered cortecosterone inj subcutaneously (5mg/kg). Group – III: cortecosterone + MEOC 200mg/kg, Group – IV: Cortecosterone + MEOC 400mg/kg given orally for 21 days, 30min before cortecosterone administration.

Morris Water Maze Test

The modified procedure from morris (Morris, 1989). The Morris water maze is a circular pool (90cm in diameter and 45cm in height) with featureless inner surface. The circular pool was filled to a height of 30cm with water

(18 ± 1°C), in which 500ml of milk was mixed. A white platform (6cm in diameter and 29cm I height) was centered in one of four quadrants of the pool (Southest area) and submerged 1cm below the water surface so that it was invisible at water level. In the water maze experiments the first week of the experiment was dedicated to swimming training for 60s. All animals were four groups we investigated the 3 weeks for treatment. In these days the mice were given one session of two trials each day for 21 days. During each trial, the mouse's escape latency, measured with a stop watch, were recorded. The parameter was averaged for each session of trials and for each mouse. One the mouse located the platform, it was permitted to remain on it for 10s. If the mouse did not locate the platform within 120s, it was placed on the platform for 10s. During this period, the platform was located in a fixed position. In the last day of training, mice were given a probe trial which considered of removing the plat form from the pool and allowing the mice to swim for 60's in search of it. A record was kept of the swimming time in the pool quadrant where the platform had previously been placed. Solutions of MEOC were given orally 30min prior to the consecutive training.

Passive shock avoidance paradign

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. The apparatus consisted of a box (27x27x27cm) having three walls of wood and one wall of plexiglass featuring a grid floor (3mm stainless steel rods set 8mm apart), with a wooden platform (10x7x1.7cm) in the center of a grid floor.

The box was illuminated with a 15W bulb during the experimental period; electric shock (20VAC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed in the wooden platform set in the center of the grid floor. When the mouse stepped down and placed on the wooden platform set in the center of the grid floor. When the mouse stopped down and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step down latency (SDL) was recorded. SDL was define as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor, animals showing SDL in the range (2-15 sec) during the first test were used for the second session and the retention test. The second-session was carried out 90min after the first test. When the animals stepped down before 60sec, electric shocks were delivered for 15sec. During the second test, animals were removed from the shock free zone if they did not step down for a period of 60sec. Retention was tested after 24h in similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300sec (Parle Milind and Dhingra, 2003).

In this passive avoidance shock test we divided four groups of animals. Group-I: Normal control group for mice (n=6), normal saline (0.9% w/v) was administered P.O. for 8 days. After 90 min of administration on 8th day, SDL was recorded retention was examined after 24h, Group-II and Group-III (n=6 each); MEOC (200 and 400mg/kg) respectively orally for 8 days. SDL was recorded after 90 min of administration on 8th day and after 24h. In this 30 min before corticosterone injection administration subcutaneously. Group-IV: corticosterone (5mg/kg) was administered subcutaneously in the negative control mice for upto 8 days continuously and 8th day SDL was recorded, retention were examined after 24h.

Statistical analysis

The statistical significance of the results of Morris water maze as well as passive shock avoidance tasks were analysed using ANOVA, followed by Tukey-Krammer multiple comparison test, the P values <0.05 were considered as significance.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the MEOC at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioural changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 21 days of observation.

Morris water maze test

In the Morris water maze ANOVA followed by Tukey-Krammer multiple comparison tests. All the latencies to reach the location of the platform significantly (P<0.05) Table: 1. The methanolic extract of *oxalis corniculata* (MEOC) showed the significant activity in 21 days study. Saline treated control mice rapidly learned the location of the submerged platform at 21st day compared 0th day, corticosterone inj (5mg/kg) group memory impairment was rapidly increased significantly (P<0.001) from 0 to 21st day compare to saline treated control. MEOC treated mice (200 and 400mg/kg) found the platform significantly (P<0.001) earlier then corticosterone injected mice and also significantly (P<0.001) than saline treated control from 6th day to 21st day.

Passive shock avoidance paradigm

In this passive avoidance model the higher dose of MEOC-400mg/kg pretreatment for 8 days successively protected mice (P<0.001) against cortecosterone induced memory impairment. The step down latency (SDL) of cortecosterone injected mice was significantly (P<0.001) poor when compared to that of saline treated mice (Table-2). MEOC (400mg/kg P.O) profoundly increased step-down latency (SDL) significantly (P<0.001) compared to saline treated mice, indicating improvement in memory. MEOC (200mg/kg P.O) increased SDL significantly (P<0.001) compared to saline treated mice and lesser than MEOC (400mg/kg P.O) treated group.

TABLE 1: Effect of *Oxalis corniculata* transfer latencies of mice on Morris water maze.

Groups	Treatment	Transfer Latency							
		0	3	6	9	12	15	18	21
I	Normal saline control	10.7± 0.9	10.2± 0.95	10.8±0.95***	9.83± 0.83***	7.8± 0.70***	7.7± 0.71***	8.8± 1.2***	8.2± 0.8***
II	MEOC 200mg/kg	7.5± 0.43	8.8± 0.7	10.3± 1.0***	14.3± 0.84***	11.3± 1.02***	9.4± 1.04***	7.3± 0.6***	7.2± 0.4***
III	MEOC 400mg/kg	6.7± 0.7	9.8± 0.95	11.5± 0.05***	10.5± 0.95***	9.83±0.8***	8± 0.58***	7.67±0.42***	7.3± 0.61***
IV	Corticosterone	7.3± 0.42	10.5± 0.62	22± 1.7	35± 1.6	49.7± 1.38	58.3± 1.67	67.3± 1.86	70.5± 2.1

Values are expressed as mean±SEM, ANOVA followed by Tukey-Krammer multiple comparison test, 6 male albino mice in each group. *** P<0.001, as compared to corticosterone injected group.

TABLE 2 : Effect of *oxalis corniculata* on step down latency (SDL) using passive avoidance apparatus.

Group	Mice	Treatment	Dose (mg/kg)	SDL after 24h (score/sec±SEM)
I	Normal control	Normal saline	---	107.5±4.64
II	Test drug	MEOC	200	199±4.43***
III	Test drug	MEOC	400	279±3.61***
IV	Cortecosterone	Cortecosterone	5	19.5±1.77***

Values are expressed as Mean ± SEM, ANOVA followed by Tukey-Krammer multiple, 6 male albino mice is comparison test each group. ***P<0.001, as compared to control.

DISCUSSION

In this present study, memory was assessed by using water Morris maze and the step-down avoidance test. The effect of *oxalis corniculata* on memory impairment induced by cortecosterone in male albino mice was performed by using methanolic extract, cortecosterone significantly impaired other forms of hippocampus-dependent memory such as object recognition and retrieval of the passive avoidance behavior. Corticosterone, the predominant glucocorticoid in rodents, chronic administration of cortecosterone it damages hippocampal subregion CA_3 that leads to impair spatial memory (Coburn-Litvak *et al.*, 2003). Also chronically elevated levels of cortecosterone inj administration is mice for 21 days can produce neuronal atrophy and cell death in the hippocampus while leaving other brain regions (Magarinos *et al.*, 1997), the elevated levels of cortecosterone changes in various neurotransmitters such as catecholamines, serotonin and γ-aminobutyric acid (GABA) in several brain structures. In the hippocampus corticosterone impairs GABA-mediated inhibitory neurotransmission and causes neurodegeneration via diminished expression of $GABA_A$ receptors (Weizman *et al.*, 1997). High amounts of corticosterone enhance action of norepinephrine (NE) via β-adreno receptors and increased dopamine (DA) turnover in prefrontal cortex is accompanied by the decreased spatial memory performance (Dziedzicka-Wasylewska *et al.*, 1997).

In this study we investigated the two memory assessment behavioural model, Morris water maze test and passive avoidance paradigm in both models the methanolic extract of *oxalis corniculata* showed significant activity on memory impairment induced by chronic administration of cortecosterone 5mg/kg subcutaneously into the male albino mice. In this morris maze test chronically elevated levels of cortecosterone administered for 21 days that leads to memory impairment occurs in the hippocampal subregion. This can be overcome by the MEOC treated (200 and 400mg/kg) two groups showed significant action compared to saline treated mice and only cortecosterone treated mice, cortecosterone treated mice showed increasing latency period due to memory impairment. In the treatment 0^{th} day to 1^{st} day there is no action on the mice.

In the passive avoidance paradigm cortecosterone injected mice showed decreased step down latency compared to saline treated mice after 24 hours later. In this continuously 8 days cortecosterone occurs. The MEOC treated (200 and 400mg/kg) showed significant activity and increasing the SDL after 24 hours compared to normal as well as cortecosterone treated mice the higher dose of MEOC (400mg/kg P.O) pretreatment for 8 days successively protected mice against cortecosterone induced memory impairment. The higher dose of MEOC (400mg/kg P.O) increased the SDL after 24 hours then the MEOC treated (200mg/kg P.O) group.

The plant *oxalis corniculata* (Oxalidaceae) contain three C-glycosylflavones, rich in vitamin-C, the vitamins C, and E can effects the neurotransmitters and acetyl cholinesterase activity in the brains of rodents treated with scopolamine inducing dementia (Lee *et al.,* 2001). This is supported to our research work may be rich in the vitamin-C supplementation increases the Acetylcholinestrase activity in brain. Oxygen-free radicals and other products of oxidative metabolism have been shown to be neurotoxic (Sayre *et al.,* 1997). The protective effect of *oxalis corniculata* extract may be attributed to antioxidant property due to rich in vitamin-C by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function there by enhancing the memory. There conditions were showed the neuroprotective role of methanolic extract of *oxalis corniculata* induced memory deficits.

CONCLUSION

In the present investigation methanolic extract of *oxalis corniculata* significantly shown memory enhancing agent in cortecosterone induced dementia. These natural memory enhancing agents will help to develop new drug candidates for dementia therapy.

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