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INVESTIGATION OF ANTIPARKINSONIAN POTENTIAL OF CALOTROPIS GIGANTEA (FLOWER)

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ABSTRACT

Calotropis gigantean (flower) belongs to family Asclepiadaceae. The total phenolic content equivalent respectively. Irwin schedule was used to evaluate C.N.S depressant or stimulant activity of extract. Tacrine induced vacous jaw movement, bursting and tongue protrusion was used to evaluate. Hence the protective effect of extract can be attributed to effect on dopamine receptor and also due to protection against oxidative stress in brain. Thus effect of extract can be considered due to presence of quercetine in crude extract.

KEYWORDS: Calotropis gigantean, quercetine, tacrine, Jaw movement, oxidative stress.

INTRODUCTION

Calotropic gigantean commonly known as **Madar** belongs to a family **Asclepidaceae** or **Milkweed**. It is widely naturalized in the East and West Indies and Ceylon. The plant contains flavonoids, triterpinoids, alkaloids, steroids glycosides, saponins, terpenes, alcohol, resin, fatty acid flower of Calotropis gigantean contain cardiac glycosides calotropin, uscharin, gigantin. Traditionally, the plant Calotropis gigantean is used in the treatment of paralysis, asthama, epilepsia, fever, eczema, dyspepsia.[1] The flower of Calotrapis gigantea has analgesic activity.[2,3] The antioxidant property of ethanolic, methanolic, chloroform and aqueous leaf, bud and flower extracts of Calotropis gigantea by DPPH (2, 2 Diphenyl-1-picrylhydrazyl) and hydrogen peroxide free radical scavenging methods with an additional reducing power test.[4,5]

Extraction: (Maceration)- Extraction was performed using maceration technique. Briefly 250gm of plant material was packed in airtight container. 70% methanol was added, sufficiently to make a layer above plant material. Container was kept at room temperature for 5 days with vigorous shaking intermittently. After 5 days plant material was filtered and filterate was concentrated in water bath at 60^{o}C till no further variation in amount of extract was kept in air tight container at 4^{o}C till any further use.

METHOD

Animal studies

Effect on gross behaviour using Irwin schedule- Body weight of animal was recorded and were randomly divided into 4 group having 6 rats in a group on the experimental day. The test sample was administered orally to the rats 60 min. Before the experimental and observed the behavioural activities under the Irwin schedule.[6] The behaviour activity viewed under this method were rearing, grooming, searching, exploratory behaviour activity, calm, sleep, paw, licking, writhing, salivation, lacrimation, piloerection, motor activities.

Group A: The vehicle was administered orally to rats 60min. Before the experimental and all the behaviour parameters were observed.

Group B: The methanolic extract of flower of Calotropis gigantean (200 mg/kg) was administered orally to rats 60 min. Before the experiment and all the behaviour parameter and all the behaviour parameters were observed.

Group C: The quercetin (200mg/kg) was administered orally to rats 60 min. Before the experiment and all the behaviour parameters were observed.

Group D: The methanolic extract of flower of Calotropis gigantean (400mg/kg) was administered orally to rats 60 min. Before the experiment and all the behaviour parameters were observed.

Table 1: Effect of vehicle on gross behaviour.

Rat no.	Wt (g)	Behavioral profile									
		Grooming	Rearing	Licking	Paw licking	Writhing	Teeth chattering	Head twitches	Searching	Calm (min.)	Sleep (min.
1	120	3	0	0	1	1	1	10	5	10	8
2	130	11`	0	0	3	1	2	2	7	6	10
3	140	5	0	0	0	0	0	10	15	9	16
4	150	13	0	0	1	0	1	4	7	5	12
5	130	7	0	0	1	1	1	6	6	7	8
6	120	0	0	0	0	1	0	4	7	5	9

Table 2: Effect of methanolic extract of flower of Calotropis gigantean (200mg/kg, orally) on gross behaviour.

Rat no.	Wt(g)	Behavioral profile									
		Grooming	Rearing	Licking	Paw licking	Writhing	Teeth chattering	Head twitches	Searching	Calm (min.)	Sleep (min.
1	120	6	3	14	9	0	10	35	13	6	0
2	125	8`	4	10	17	3	4	35	12	8	0
3	95	5	0	5	9	1	0	17	20	13	0
4	110	8	9	13	8	0	0	28	21	9	0
5	115	9	9	16	9	0	0	29	26	10	0
6	120	8	8	17	8	0	0	24	29	9	0

Table 3: Effect of methanolic extract of flower of Calotropis gigantean (400mg/kg, orally) on gross behaviour.

Rat no.	Wt(g)	Behavioral profile									
		Grooming	Rearing	Licking	Paw licking	Writhing	Teeth chattering	Head twitches	Searching	Calm (min.)	Sleep (min.
1	95	9	3	9	3	0	3	8	8	8	0
2	95	4`	3	8	2	0	4	11	11	4	0
3	95	4	3	8	3	0	3	10	7	3	0
4	75	3	3	10	3	0	3	13	5	5	0
5	80	4	3	8	3	0	3	12	6	4	0
6	80	3	4	9	2	0	2	11	7	4	0

Table 4: Effect of Quercetin (200mg/kg, orally) on gross behaviour.

Rat no.	Wt(g)	Behavioral profile									
		Grooming	Rearing	Licking	Paw licking	Writhing	Teeth chattering	Head twitches	Searching	Calm (min.)	Sleep (min.
1	95	8	2	36	4	0	5	32	18	9	0
2	95	4`	2	23	17	0	3	35	29	17	0
3	90	3	2	13	9	0	14	48	14	15	0
4	85	4	2	7	14	0	12	40	19	16	0
5	80	4	2	20	13	0	11	33	17	14	0
6	80	3	2	19	10	0	5	38	14	16	0

Tacrine induced vacous jaw movement (Sanjay Kasture, 2009) (Salamone J.D., 1998)- Rats divided into 4 groups and treated with vehicle, methanolic extract flower of Calotropis gigantean (200mg/kg,orally) and quercetin (200mg/kg, orally) and methanolic extract of flower Calotropis gigantean(400mh/kg,orally) Tacrine (2.5mg/kg,i.p) was administered 20 min. After vehicle methanolic extract of flower of Calotropis gigantean and quercetin the number of tremenulous jaw movements and bursts were measured for 60 min. Administration of tacrine induces perioral tremor, which mostly occurs in bursts of jaw movements. Therefore, the number of bursts was recorded. Tremulous jaw movements were defined as vertical deflection of the lower jaw not directed at a particular stimulus. Tongue protrusion and bursting also included in the evaluation.

Group A: The vehicle (5ml/kg) was given to rat 30 minutes before the practical and the number of bursts, jaw movements and tongue protrusion was counted for one hour.

Group B: The methanolic extract of flower of Calotropis gigantean (200mg/kg, orall) was given to rats 30 minutes before the practical and the number of bursts, jaw movements and tongue protrusion was counted for one hour.

Group C: Quercetine (200mg/Kg) was given to rat 30 minutes before the practical and the number of bursts, jaw movements and tongue protrusion was counted for one hour.

Group D: The methanolic extract of flower of Calotropis gigantean (400mg/kg, oral) was given to rats 30 minutes before the practical and the number of bursts, jaw movements and tongue protrusion was counted for one hour.

Table 5: Tacrine induced Vacuous Jaw Movement (After 1HOUR, 5 min duration).

S NO.	Treatment	Jaw movement
1	Vehicle+ Halo	178.2+_19.23
2	Extract+Halo	119+_14.07*
3	Extract+Halo	67.83+_10.36**
4	Quercetin+Halo	63.83+_8.58**

`All the value expressed as Mean +_ SEM (n=6), **P<0.050 VS `Vehicle+ Haloperidol

Table 6: Tacrine induced orofacial bursting (After 1HOUR, 5 min duration).

S NO.	Treatment	Bursting
1	Vehicle+ Halo	48.33+_4.835
2	Extract+Halo	17.830+_1.720**
3	Extract+Halo	4.830+_0.750**
4	Quercetin+Halo	0+_0**

`All the value expressed as Mean +_ SEM (n=6),**P<0.050 vs `Vehicle+ Haloperidol

Table 7: Tacrine induced torgue protrusion (After 1HOUR, 5 min duration).

S NO.	Treatment	Tongue protrusion
1	Vehicle+ Halo	6.66+_0.61
2	Extract+Halo	4.5+_0.76**
3	Extract+Halo	4.0+_0.854**
4	Quercetin+Halo	1.0+_0.25**

`All the value expressed as Mean +_ SEM (n=6),**P<0.050 vs `Vehicle+ Haloperidol

Haloperidol induced catatonia (Kasture Snajay B, 2009)(Kulkarni S.k,2003)- Twenty four albino wistar rats were divided into 4 groups, each group contained 6 animals. The animals treated with vehicle, Calotropis gigantean (200mg/kg and 400 mg/kg, orally) and quercetin (200mg/Kg, orally) was given to rats 30 minutes before the practical and cut off time was observed at 0,30,60,90, and 120 minutes.

Table 8: Showing the effect of catalepsy in different treatment groups.

S no.	Treatment	Duration of catalepsy in sec.				
		0 min.	30 min	60 min	90 min	120 min
1	Vehicle+ Halo	5.33+_0.42	177.5+_16.53	152.3+_1`2.64	155.7+_14.79	154.5+_13.11
2	Extract(200mg/kg)+ Halo	3.83+_0.30**	119.3+_7.0**	124.0+_8.81**	129.5+_8.3**	109.7+_8.71**
3	Extract(400mg/kg)+Halo	3.33+_0.4216**	119.0+_21.4**	87.17+_4.19**	88.17+_3.6	86.0+_5.6**
4	Quercetin(200mg/kg)+Halo	1.5+_0.6**	57.33+_8.25	51.83+_4.9**	51.83+_5.9	48.0+_8.4**

All the values expressed as mean +_SEM,**p<0.050vs Vehicle + Haloperidol

Action against oxidative stress in brain

Dissection and homogenization: On the 21st day of haloperidol treatment, the animal were sacrificed by decapitation immediately after behavioural assessment. The brains were removed, forebrain was dissected out and rinse with isotonicsaline and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1M phosphate buffer (p^H 7.4) and biochemical estimation of enzymes viz, superoxide dismutase (SOD), Glutathione and lipid per oxidation (LPO) were carried out.

Assay of reduced Glutathione

Principle: 5,5-dithiobis-2- nitro benzoic acid (DTNB) is reduced in presence of GSH to produced a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm

Procedure: Brain homogenate was prepared (10% 0.1 M) phosphate buffer, p^H -7.4) 0.2ml of homogenate was taken20% TCA and 1Mm EDTA was added to it. The solution was kept aside for 5 minutes and then centrifuged at 2000 rpm for 10 minutes.200ul supernatant was taken in a separate tube. 1.8ml Ellman's reagent (EDTA) (0.1Mm) prepared in 0.3M phosphate buffer, p^H 7 with 1% sodium citrate solution was added to it. The volume was made upto 2 ml with distilled water and the absorbance was measured immediately at 412 nm (water as blank)

Calculation

In blood

= A sample X 66.66 mg/dl

= A sample X 2.22 m mol/dl

In tissue
= (A sample X 66.66)/g tissue used mg/g tissue
=Mg/g tissue
= (A sample X 2.22)/g tissue used m mol/g tissue.

Table 9: Showing Glutathion (GSH) in different treatment groups.

S. No.	Treatment group	GSH(N/mol/mg)
1	Vehicle	2.940+_0.120**
2	Vehicle + Halo	0.640+_0.0900
3	Extract (200mg/kg)+Halo	0.830+_0.0300
4	Extract (400 mg/kg)+Halo	1.910+_0.0500**
5	Quercetin(200mg/kg)+Halo	2.36+_0.500**

All the values expressed as Mean+_ SEM (n=6),** P<0.050 vs VEHICLE+ HALOPERIDOL

Lipid peroxidise (LPO)

Procedure- 10% w/v tissue homogenate in 0.15M tris HCl buffer (p^H 7.4) was prepared 0.2ml tissue homogenate was taken and added 0.2 ml 8.1% sodium dodecyl sulphate (SDS) + 1.5 ML 20% Acetic acid +1.5ml 8%TBA to it (adjusted volume upto 4 ml with distilled water).The solution was heated on water bath (95^{o}C) for 60 minute using glass ball as condenser and then cooled. The volume was adjusted upto 5 ml of butanol: pyridine (15:1) was added to the solution and then vortexed for two minutes. The resulting solution was centrifuged at 3000 rpm for 10 minutes. Upper organic layer was taken and its OD WAS NOTED AT 532 NM (blank- butanol: pyridine 15:1). This absorbance was that of total MDA formed. Interpretation was based on standard curve of MDA.

Table 10: Showing Lipid peroxidise (LPO) level found in different treatment groups.

S NO.	Treatment group	LPO (Nm/mg wet tissue)
1	Vehicle	1.25+_0.138**
2	Vehicle + Halo	3.65+_0.098
3	Extract (200mg/kg)+Halo	2.83+_0.122
4	Extract (400 mg/kg)+Halo	2.45+_0.125
5	Quercetin(200mg/kg)+Halo)	2.42+_0.24**

CONCLUSION

Parkinson's disease is caused by degradation of dopaminergic neurons of substantia nigra, pars compacta. Oxidative stress contributes to the cascade leading to dopamine cell degeneration in Parkinson's disease. In present investigation extract of Calotropis gigantean flower was investigated for its effect on behaviour analysis (Irwin schedule), haloperidol induced catatonia, tacrine indiuced vacuous jaw movement, orofacial burst and tongue protrusion, oxidative stress in brain due to chronic administration of haloperidol. As it is the good source of quercetine,it was expected that flowers extract would provide significant protection against oxidative stress due to haloperidol administration.

Intraperitoneal chronic injection of haloperidol significantly increased malondialdehyde and nitrite levels, while it significantly attenuated the activity of reduced glutathione, catalase and superoxide dismutase (Adeyemi OO.et al., 2013). Superoxide dismutase (SOD) `is an enzyme system that is implicated in the oxidant stress model of Parkinson's disease (PD) `pathogenesis. GSH levels were reduced in substantia nigra in Parkinson's disease patients (40% compared to control subjects).Hence in present study the protective effect of extract can be attributed to effect on dopamine receptor and alsodue to protective against oxidative stress in brain. Thus effect of extract can be considered due to presence of quercetin in crude extract.

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