

Screening of Antidepressant Activity of *Solanumnigrum*

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ABSTRACT

Solanumnigrum, black night shade contains alkaloid as its major component and its antidepressant like effect has been previously demonstrated in some earlier research works. The purpose of this study was to explore the possible contribution of *Solanumnigrum* in the antidepressant like effect of the serotonergic system in rats. Hydro-ethanolic extract of *Solanumnigrum* was quantified for phenolic content by Folin-Ciocalteu's method. Chronic unpredictable mild stress (CUMS) was employed to induce stress in rats. *Solanumnigrum* (50 and 100 mg/kg, body weight) and fluoxetine (20mg/kg, body weight) were administered during the 21 day stress exposure period. Antidepressant activities of *Solanumnigrum* were assessed in open field exploratory and behavioural despair paradigms, respectively. Our results indicate that *Solanumnigrum* possess anti-stress and moderate anxiolytic activity which may be due, in part, to its antioxidant effect.

Keywords: *Solanumnigrum*, antidepressant effect, serotonergic system, fluoxetine, anti-stress and anxiolytic activity.

INTRODUCTION

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being^[1]. Depressed people may feel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, irritable, hurt or restless. Many people, including lot of teen age people have suffered in this way. A depressed person has much less energy to do activities, feels like nothing matters and sees life in negative way, feel like it will never get better^[2]. Treatment for depression involves medication or taking spiritual as well as yoga therapy or usually a combination of the two^[3].

Serotonin is a neurotransmitter, synthesised from tryptophan and is responsible for emotional stability, mood anxiety, arousal, aggression, nutrient selection, sleep and sex^[4]. In the present study the

oxidative stress generated by the immobilization stress was measured in terms of free radical scavenging enzyme activities like superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GPx) and thiobarbituric acid reactive substance^[5].

MATERIALS AND METHODS

Procurement of Animals

Young male and female Albino rats of Westar strain (80+20g) procured from Trissur, Kerala, India were used for the study. The ethical clearance for handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals as per the guidance of the committee for the purpose of justice and empowerment, Government of India (CPCSEA/No:256/2014/IAEC).

Induction of Depression

Depression was induced by Chronic Unpredictable Mild Stress (CUMS) protocol in albino rats. The status of depression was diagnosed by the forced swimming test (FST) on the seventh day of induction, third and seventh day of treatment.

Preparation of Plant Extract

The hydro-ethanolic extract of *Solanumnigrum* whole plant was prepared [7, 8] at large scale (100g of leaf extract powder in 500ml of water and 500ml of ethanol). It was filtered and the filtrate was then concentrated to dryness under controlled temperature in a microwave oven. The hydro-ethanolic extract yield was weighed to be 14.46 g, brown in colour, hygroscopic and it was stored air tight in desiccators for use in various assays.

Experimental Setup

The animals were acclimatized for 3 days and maintained under standard laboratory conditions with controlled temperature ($29\pm 5^{\circ}\text{C}$), humidity ($55\pm 5\%$) and 12 hours light/dark cycles throughout the experimental period.

GROUP 1: The rats were provided with only standard pellet and water for 14 days and were considered as normal control.

GROUP 2: CUMS protocol induced depressed rats served as untreated depression control.

GROUP 3: Depressed rats were orally administered with the standard drug fluoxetine (20 mg/kg body weight) for 7 days.

GROUP 4: Depressed rats were orally administered with low dosage of hydro-ethanolic extract of *Solanumnigrum* (200mg/kg body weight) for 7 days.

GROUP 5: Depressed rats were orally administered with high dosage of hydro-ethanolic extract of *Solanumnigrum* (400mg/kg body weight) for 7 days.

Collection of Serum and Tissues

After the end of experimental treatment period (7 days), the animals were sacrificed by cervical dislocation under mild chloroform anaesthesia. Blood was collected by cardiac puncture and serum was separated by centrifugation at 2500 rpm.

Biochemical Analysis

The amount of serum protein, total cholesterol and enzymic & non-enzymic antioxidants (Superoxide Dismutase, Catalase, Glutathione peroxidase and Lipid peroxidation) were analysed in experimental rats.

Statistical Analysis

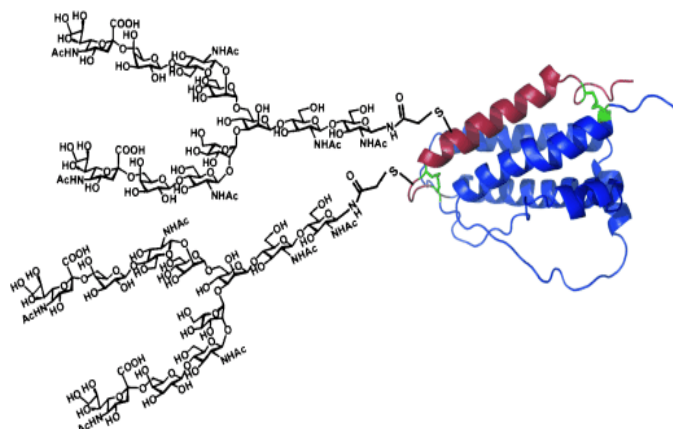
All the results were expressed as mean \pm SD and statistical evaluation was done using 'R' statistical package.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of CM was done and the results showed the presence of rich alkaloids, flavonoids, saponins, carbohydrates, phenols, glycosides, tannins and to lesser extent proteins.

Forced Swimming Test (FST): Depressed animals stay immobile for longer time during the final 4 minutes of observation. (Table1). In this study, serum protein and total cholesterol are low in depression and they are enhanced by plant extract and they also enhanced the antioxidant activity. The experimental animals were sacrificed and their serum and brain tissues are obtained for further studies. The protein concentration in the brain tissue was low in depression when compared to other groups which indicated reduced transcription and translational mechanisms. The serum cholesterol level also found to be reduced in depression and elevated levels are seen in normal and treated groups. The enzymic and non enzymic antioxidant were analysed as the oxidative stress has major influence on depression. The enzymic antioxidant

study revealed the decreased activity of SOD in depression but the levels was found to be elevated in treated groups as equal to that of normal levels. This may be due to the 150 kDa Glycoprotein fraction found in the *Solanumnigrum* (SNL-Glycoprotein) having cholesterol lowering and



antioxidant activities [9] (Fig. 1).

Table: 1 Forced Swimming Test (FST)

| Groups | Before treatment (min:sec:msec) | After treatment (min:sec:msec) | |
|------------------|------------------------------------|-----------------------------------|---------------------|
| | | 3 rd day | 7 th day |
| Normal control | 00:28:02 | 00:11:02 | 00:01:02 |
| Depression | 01:32:12 | 01:35:37 | 00:48:00 |
| Drug treated | 01:40:09 | 01:21:08 | 00:21:05 |
| Plant(low dose) | 01:44:77 | 01:23:86 | 00:39:49 |
| Plant(high dose) | 01:56:35 | 01:32:47 | 00:21:94 |

Table: 2 Estimation of Protein and Total Cholesterol

| Groups | Protein (µg) | Serum cholesterol (µg) | Triglyceride (mg/dl) |
|---------|--------------|------------------------|----------------------|
| GROUP 1 | 103±3.85 | 320±4.04 | 93.6±3.68 |
| GROUP 2 | 52±3.86 a* | 120±4.28a* | 125±4.07a* |
| GROUP 3 | 75±4.15b* | 150±4.28b* | 120±4.40b* |
| GROUP 4 | 92±4.02b* | 180±4.04b* | 126.3±3.96b* |
| GROUP 5 | 104±4.41b* | 180±4.48b* | 120.3±3.79b* |

Data are expressed as Mean *SEM*, n=6, using student t-test. * $P < 0.05$, "ns" denotes "no significant change". ^{a, b} denote comparison between group I and Group II and between

Table: 3 Estimation of Enzymic and Non-Enzymic Antioxidants

| Groups | SOD | CAT | GPx | LPO | Vit. C |
|---------|-------------|------------------------|------------------------|-------------|----------------------|
| Group 1 | 0.75±0.04 | 6.0±3.27 | 200±4.0 | 0.28±0.03 | 4.6±1.33 |
| Group 2 | 0.05±0.01a* | 6.0±3.50 ^{ns} | 500±4.17a* | 0.10±0.03a* | 5.4±1.37a* |
| Group 3 | 0.60±0.14b* | 7.4±3.54b* | 500±3.96 ^{ns} | 0.36±0.04b* | 5±2.05b* |
| Group 4 | 0.45±0.14b* | 6.4±4.3b* | 500±3.78 ^{ns} | 0.58±0.03b* | 5±1.45b* |
| Group 5 | 0.65±0.14b* | 9.2±4.0b* | 150±3.87b* | 0.34±0.03b* | 5±1.33 ^{ns} |

Data are expressed as Mean *SEM*, n=6, using student t-test. * $P < 0.05$, Group II (Depressed Controls), ^{ns} denotes "not significant change". ^{a, b} denote comparison between group I and Group II and between group

The analysis of vitamin C showed slightly increased levels in depression and returned to near normal on drug and plant extract treated groups (Table 2). The statistical analysis revealed that the proteins, serum cholesterol and Gpx in brain showed significant difference between normal and depressed groups which reveals that these parameters change during depression. LPO and triglyceride showed statistical significant difference between normal and control groups and they were found to return to near normal levels after the treatment. CAT showed no significant difference between normal and depressed groups and also the effect of plant extract of higher dosage had better efficacy in almost all parameters tested as a better antidepressant than the lower dose (Table 3).

CONCLUSION

Solanumnigrum fruit extract showed moderate anxiolytic and good antioxidant properties in rats. Hence it may be an important candidate herbal plant for treating depression and hence future research can be aimed at identifying and extracting

the active principles that may have antidepressant activity.

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