Oral Administration of *Rauwolfia Serpentina* Plant Extract Mitigated Immobilization Stress-Induced Biochemical and Behavioral Deficits in Rats

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Summary: *Rauwolfia Serpentina* is a medicinal herb used for hypertension and psychotic disorders. In this study neuroprotective effects of *Rauwolfia serpentina* plant extract following the exposure to acute immobilization (2h) stress in rats were investigated. The extract of the plant administered orally at non-sedative dose 30mg/kg before immobilization (2h) to observe stress induced behavioral deficits. Neuroprotective efficacy of extract was assessed in terms of alteration in activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT). We also monitored leptin, corticosterone and glucose levels in plasma to obtain an imminent role of *Rauwolfia serpentina*. Animals were orally administered with *Rauwolfia serpentina* (30mg/kg) while controls receive saline (1ml/kg). Each group was subdivided into stressed and unstressed groups. Behavioral deficits were monitored in the open field and light dark activity box. Animals were decapitated; plasma samples were collected for CAT, SOD, corticosterone, leptin and glucose estimation. Orally administered *Rauwolfia serpentina* attenuates stress induced behavioral deficits and rise antioxidant enzymes levels. Plant extract also prevents the stress-induced increase in corticosterone but glucose levels do not manifest any significant change. Immobilization stress (2h) induced decrease of plasma leptin levels were reversed by *Rauwolfia serpentina*. Therefore, the present study suggests that *Rauwolfia serpentina* has potentiality to antagonize undesirable effects of immobilization stress (2h) by reducing stress perception and inhibitory effects of stress on the activity of hypothalamic pituitary adrenal (HPA) axis and animal behaviors. Despite an apparent role of *Rauwolfia serpentina* the mechanism of action at molecular level causing the acute anxiolytic effects of oral administration of plant extract remains to be determined.

Keywords: Acute stress; *Rauwolfia serpentina*; SOD; CAT; Behavioral deficits; Psychotic disorders.

Introduction

Disorder in the balance between antioxidant defenses and the production of reactive oxygen species is defined as an oxidative stress (OS) [1]. Universal condition in neurodegeneration seems to be the imbalance between OS and antioxidant defense systems [2]. In order to counter balance the free radical induced damage of biological molecules, antioxidant mechanisms and enzymes alike, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are activated. These enzymes catalyze their alteration into less reactive species and are considered as body's primary line of protection against ROS [3].

Now a day's global population is moving towards the herbal remedies which contain bioactive compounds to cure the diseases [4]. *Rauwolfia serpentina (*family: Apocynaceae), due to the occurrence of its enormous therapeutic properties, is considered as an important herbal plant in the pharmaceutical industries [5]. *Rauwolfia serpentina* is effectual in the treatment of hypertension as well as in psychotic diseases like anxiety, schizophrenia, insanity, insomnia etc [5-6]. Roots of *Rauwolfia serpentina* plant have various biological activities due to isolation of different indole alkaloids and associated constituents from it [7]. Extracts of *Rauwolfia serpentina* have antioxidant [5, 8] and antimicrobial activities [8-9], which were reported in earlier studies. The principle alkaloid of *Rauwolfia serpentina* is reserpine [10]. It holds at least 0.03% to 0.14% of the reserpine, available in stem, leaves and roots of the plant [6]

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Previously, numerous studies have been reported from our laboratory that establishes the capability of phytochemicals present in Rice bran Oil [11], olive oil [12] and aqueous fruit extract of Sea buckthorn [13] to attenuated/or reversed anxiety in rats. Oral administration of Red rice bran oil averted haloperidol-induced anxiety syndrome in rats [14]. Conversely, oral administration of Olea europaea (OE) oil (common name: olive oil) and Nigella sativa (NS) (common name: Black cumin) oil did not show anxiolytic effects in rats [15]. In perpetuation of our research on the plant, the current study was focused to examine the neuroprotective effects *Rauwolfia serpentina* in rats following acute exposure to the immobilization (2h) stress. The herb extract was orally administered at a non-sedative dose 30mg/kg [16] before immobilization for 2h to monitor any behavioral changes in the activities. The neuroprotective efficacy of *Rauwolfia serpentina* present in the roots assessed in terms of its potency to attenuate oxidative stress induced alterations of antioxidant enzymes activities a like SOD and CAT and locomotor deficits. In order to get insight in the role of *Rauwolfia serpentina* in HPA axis, we also monitored plasma leptin, corticosterone and glucose levels. The aim of the present study is to establish the beneficial significant effect of root extract of *Rauwolfia serpentina* plant for the control of stress and interrelated disorders.

Experimental

Animals

Twenty-four male albino wistar rats, having weight 180-200g purchase from PCSIR were housed separately with free access to *ad lithium* water and standard rodent-diets, for at least one week before experimentation. Every procedure conducted was approved by the Local Animal Care Committee.

Preparation of Plant Extract

Ground powder of roots of *Rauwolfia serpentina,* weight thirty grams were extracted with methanol (1 L; 95%) overnight and filtered twice by using Whatman No.1 filter paper. This filtrate was then concentrated at 40°C till dryness under vacuum in rotary evaporator (Eyela-NE) to attain a brown residue which was mentioned as methanolic root extract (MREt) [16]. The procedure yielded 3 - 4% (w/w) of the dry root. The MREt was stored below 10°C in an airtight container in refrigerator until used.

Immobilization Procedure (Restraining Procedure)

During immobilization procedure wire grid of 10" × 9" fitted with a Per-spex plate of 9" × 6.5" were used. The method was similar as described before [12, 17–19]. Briefly, the animals were immobilized by pressing their legs through the gap in the metal grid and tape them together by zinc-oxide plaster. Hind limbs were also taped and the head of animal rested on the Per-spex plate[12].

Behavioral Analysis

Activity in Novel Environment (Open Field)

Open field test was used to assay locomotion in rats. This method followed in the current study was same as described previously [18, 20]. The parameter noted to observe exploratory activity were in terms of number of square crossed by rats as described in earlier studies [21].

Light- Dark Transition Test

Anxiety level in animal is observed by using light and dark activity box. Time expended and entries in light box were noted. Increased number of entries and time of stay in light compartment will be served as an index of decrease anxiety state in rats. The method was same as performed previously in our laboratory [22].

Blood Sample Collection

Blood was collected from rats in heparinized centrifuge tubes. Centrifugation was done for 10 minutes. Plasma collection was stored at -70 °C until biochemical estimation of the plasma glucose, corticosterone, leptin, CAT and SOD.

Biochemical Estimation of Glucose, Catalase and Superoxidedismutase in Plasma

Determination of Catalase (ec1.11.1.6)

Activity of CAT was measured by Patterson's method [23]. In this method, H_2O_2 decomposition was determined at 240 nm taking De at 240 nm as 43.6 $mMcm^{-1}$. The assay mixture (3.0mL) contained 10.5 mM H_2O_2 in potassium phosphate buffer (0.05 M,pH 7.0). The addition of 0.1 mL enzyme extract at 25 °C started there action. Catalase activity was calculated by the decrease in absorbance at 240 nm. The amount of enzyme dismuting 1mM of H_2O_2 min^{-1} is define as one unit of CAT activity [24].

Determination of Superoxide Dismutase (ec.1.15.1.1):

Activity of SOD was carried out according to the method of Beyer and Fridovich [25]. For the assay mixture 1.5 mL of L-methionine, 27.0 mL of 0.05M potassium phosphate buffer (pH 7.8), 1.0 mL of nitroblue tetrazolium salt, and 0.75 mL of Triton X-100 were mixed. Aliquots (1.0 mL) of this mixture with the addition of 10 mL of riboflavin (4.4 mg per 100 mL) and 20 ml enzyme extract were transferred into small glass tubes. The cocktail was mixed and then illuminated in an aluminum foil-lined box, having 25W fluores- cent tubes for 15 minutes. The sample was replaced by 20 mL of buffer in a control tube, and measured the absorbance at 560 nm. The reaction was stopped up by placing the tubes in the dark and switching off the light. Formation of formazan causing the increase in absorbance was calculated at 560 nm. According to the describe state, increase in absorbance in the control was accepted as 100% and the enzyme activities were measured by determining the percentage inhibition per minute. Amount of enzyme that cause a 50% inhibition under the conditions of the assay is taken as one unit of SOD [24].

Estimation of Glucose in Plasma by God Pap Method

The glucose concentration in plasma was measured by using glucose-oxidase method (GOD-PAP, Solo per USO diagnostic in vitro)

Estimation of Leptin and Corticosterone in Plasma by Elisa Kit

Animals were decapitated followed by the collection of blood in heparinized centrifuge tube. Centrifugation processed for 20 minutes at 2000g and 4 °C to obtain plasma. All samples were stored at-70°C until the assay of plasma corticosterone and leptin by using respective ELIZA kit (Cat # EZRL-83K).

Experimental Protocol

Twenty four animals randomly assigned into two equal groups, each were divided into unstressed and stressed groups. Animals were subdivided into groups of four (i) saline unstressed; (ii) *Rauwolfia serpentina* unstressed; (iii) saline stressed; (iv) *Rauwolfia serpentina* stressed, which were orally administered with saline (1 ml/kg) or *Rauwolfia serpentina* (30mg/kg). Animals of the stressed group were immobilized for 2h. Meanwhile animals of the unstressed group were remained in their home cages. Behavioral activities were monitored in light dark transition box and open field activity after the termination of 2h immobilization period. Plasma samples were collected for CAT, SOD, corticosterone, glucose and leptin estimation. The experiment was completed in such a balanced way to avoid the order effects between control and test treated rats.

Statistical Analysis

Results are presented as mean $\pm$ SD. Analysis of the data was performed by two way ANOVA using Newman-keuls post hoc test. Results with the value of $p<0.01$ were considered significant.

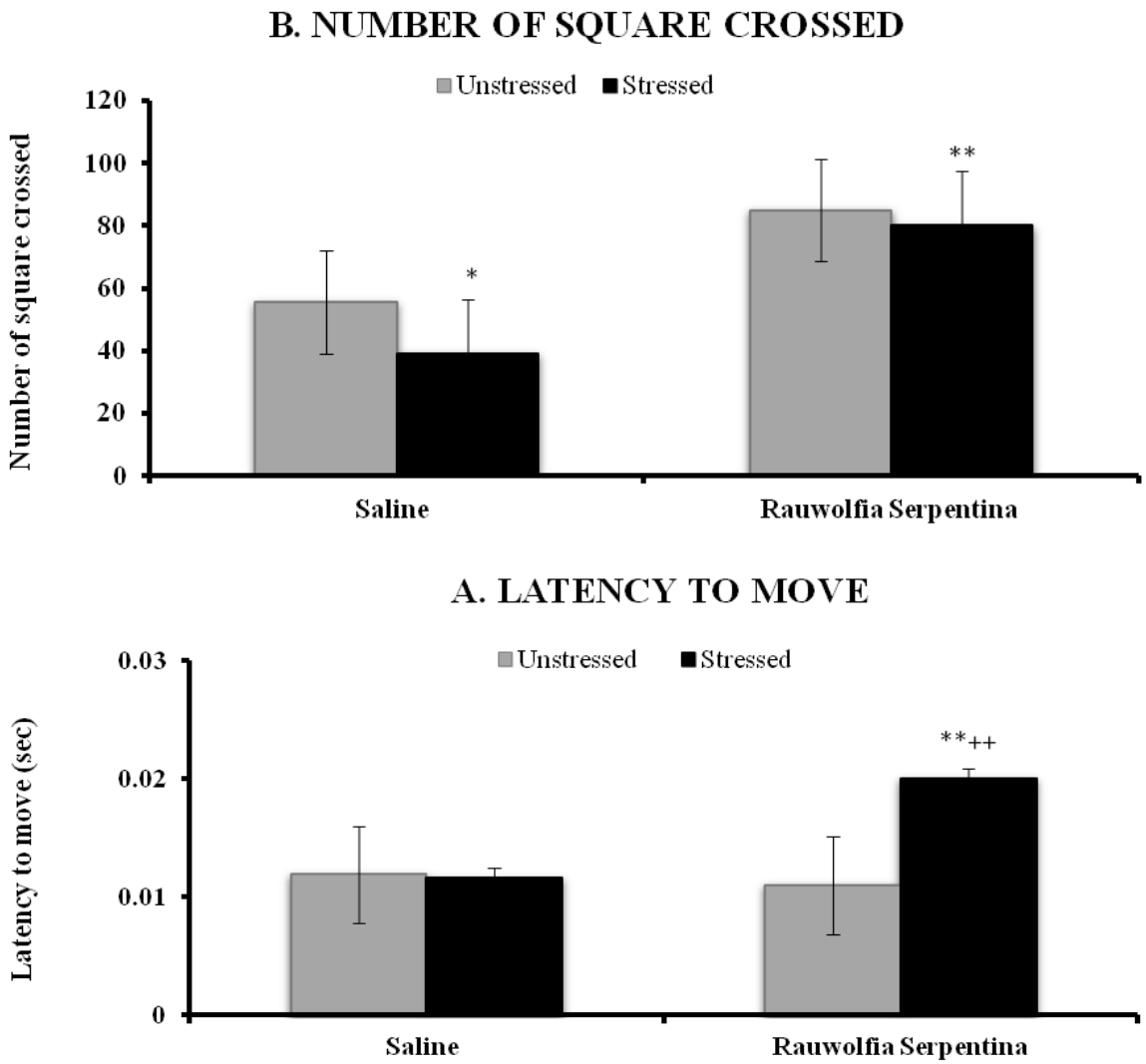
Result and Discussion

Fig. 1(A) Shows changes of motor activity in a novel environment in animals orally administered with *Rauwolfia serpentina* 2h before exposing animal to acute immobilization stress for 2h. Analysis of the data on latency to move (Fig 1 A) showed significant effects of stress ($F=7.737$ $p<0.01$ df 1,20) and *Rauwolfia serpentina* ($F=7.737$ $p<0.01$ df 1,20) as well as the interaction between two factors ($F=8.796$ $p< 0.01$ df 2,20).

Newman keuls post hoc analysis revealed that administration of *Rauwolfia serpentina* at dose of (30mg/kg) to stress rats showed increase in latency to move as compare to unstressed rats. On the other hand saline + stressed rats did not show any significant difference in latency to move as compare to unstressed rats. In comparison with *Rauwolfia serpentina* + stressed rats showed increase in latency to move with saline + stressed rats.

Fig. 1(B) Shows changes of motor activity in a novel environment in animals orally administered with *Rauwolfia serpentina* 2h before exposing animal to acute immobilization stress for 2h. Analysis of the data on number of square crossed (Fig 1 B) showed significant effects of stress ($F=4.017$ $p<0.05$ df 1,20) and *Rauwolfia serpentina* ($F=43.136$ $p<0.01$ df 1,20). The interaction between the two factors was not significant ($F=1.143$ N.S).

Post hoc analysis showed decrease number of square crossed by saline + stressed rats but not in *Rauwolfia serpentina* + stressed rats. Alone *Rauwolfia serpentina* rats showed increased locomotor activity in open field. On the other hand, stress induced decreases of locomotor activity was reversed in *Rauwolfia serpentina* administered stressed rats.



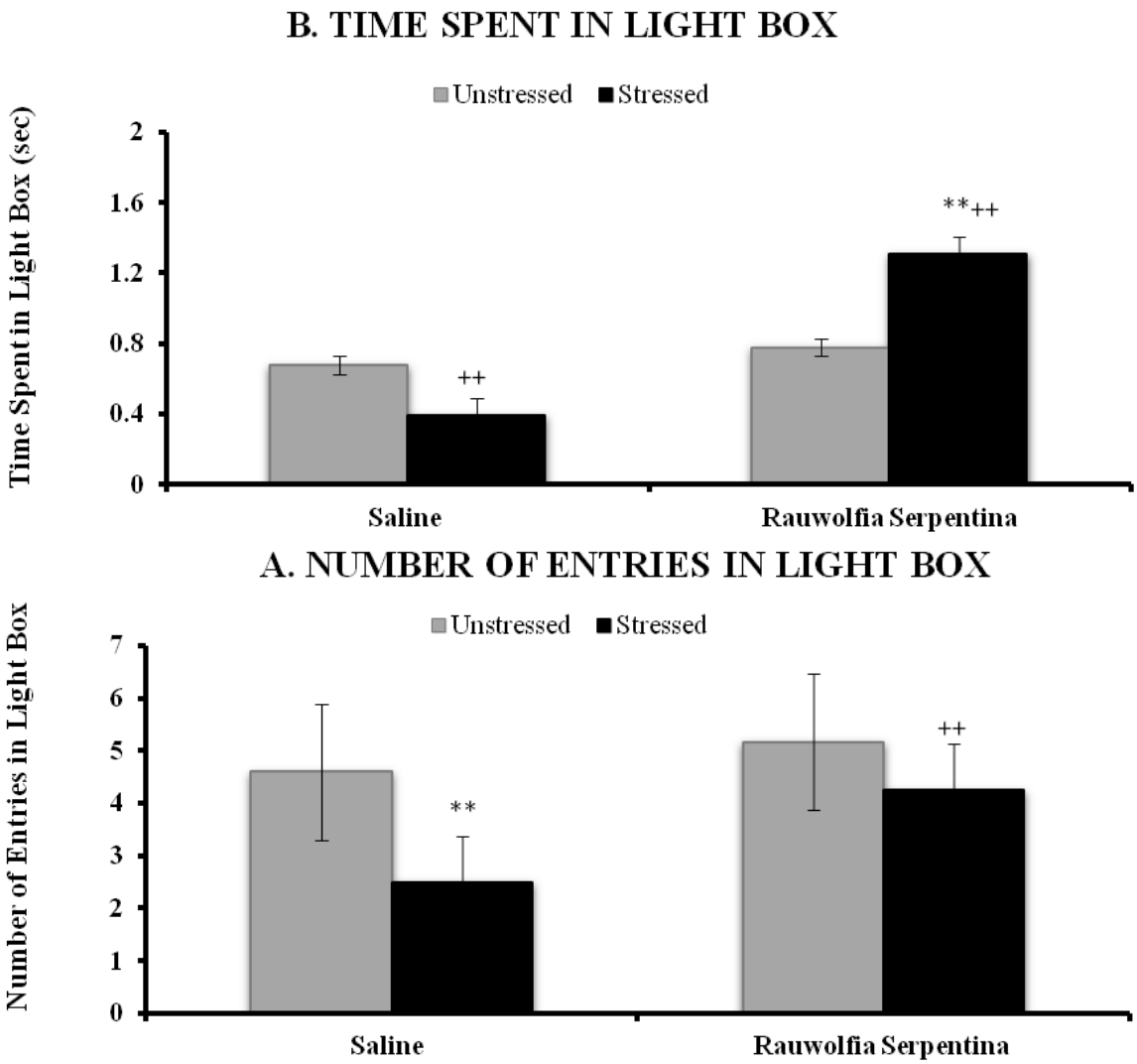
Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 nd +p<0.05 versus their respective (unstressed or stressed) animals.

Fig. 1: Changes of Locomotor Activity in a Novel Environment in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

Fig. 2(A) Shows changes of behavior in light dark transition test in animals orally administered with *Rauwolfia serpentina* 2h before exposing animals to acute immobilization stress for 2h. Analysis of the data on entries in light box (Fig 2 A) showed significant effects of stress ($F=16.298$ $p<0.01$ df 1,20) and interaction between the two factors ($F=5.391$ $p<0.01$ df 1,20). Effects of *Rauwolfia serpentina* was not significant ($F=1.589$ N.S).

Post hoc analysis by Newman keuls showed decrease number of entries in light dark transition box in *Rauwolfia serpentina* + stressed and saline + stressed animals as compare to their respective controls. Alone *Rauwolfia serpentina* rats showed increase number of entries in light dark transition box. On the other hand, stress induced decreases of number of entries in the light dark box was reversed in *Rauwolfia serpentina* administered rats.

Fig. 2(B) Shows changes of behavior in light dark transition test in animals orally administered with *Rauwolfia serpentina* 2h before exposing animals to acute immobilization stress for 2h. Analysis of the data on time spend in light box (Fig 2 B) showed non significant effects of stress ($F=1.146$ N.S) and significant effects of *Rauwolfia serpentina* ($F=20.861$ $p<0.01$ df 1,20) and interaction between the two factors ($F=7.740$ $p<0.01$ df 2,20).



Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 and +p<0.05 versus their respective (unstressed or stressed) animals.

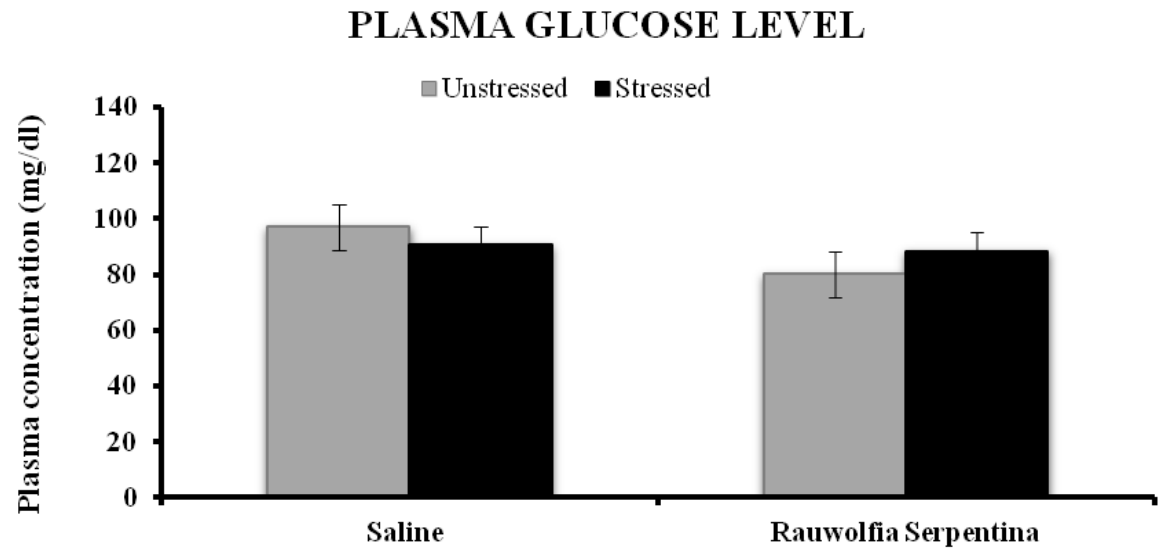
Fig. 2: Changes of Behaviour in Light Dark Transtion Test in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

Newman keuls post hoc test showed decrease time spent in light dark transition box (sec) in saline + stressed rats but significant increased in *Rauwolfia serpentina* + stressed rats. *Rauwolfia serpentina* alone did not increased locomotor activity in a light dark transition box. On the other hand, stress induced decrease of locomotor activity was reversed in *Rauwolfia serpentina* administered stressed rats.

Fig. 3 Shows effects of stress on oral administration of *Rauwolfia serpentina* on plasma glucose level. Analysis of the data on glucose level (Fig 3) showed non significant effects of stress (F=0.566 N.S), *Rauwolfia serpentina* (F=2.144 N.S) as well as the interaction between the two (F=3.142 p<0.005 df 2,20).

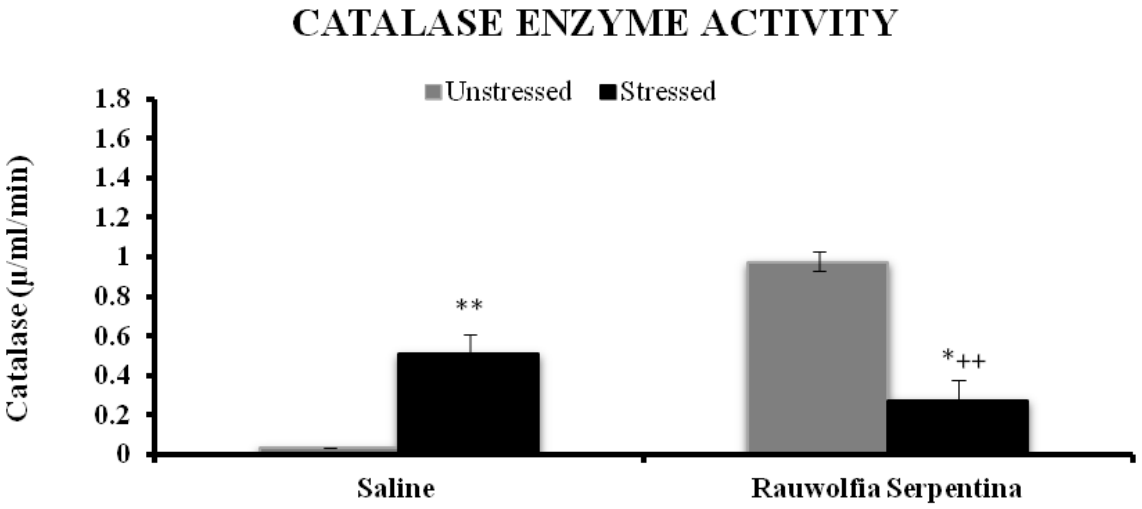
Post hoc analysis revealed that concentration of plasma glucose was not significant in all groups.

Fig. 4 Shows effects of stress on oral administration of *Rauwolfia serpentina* on plasma CAT activity. Analysis of the data on CAT activity (Fig 4) showed non significant effects of stress (F=0.508 N.S) and interaction between the two factors (F=2.802 N.S). Effects of *Rauwolfia serpentina* was significant (F=4.858 p<0.05 df 2,20).



Values are means ± S.D. (n=24).

Fig. 3: Changes in the Levels of Glucose in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

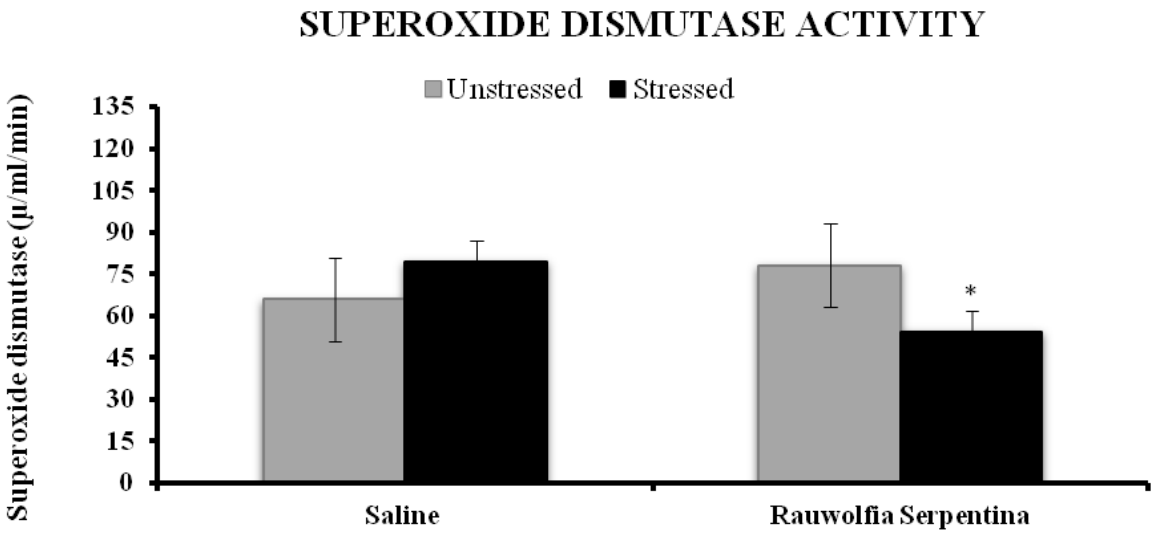


Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 and +p<0.05 versus their respective (unstressed or stressed) animals.

Fig. 4: Changes in the Levels of Catalase Activity in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

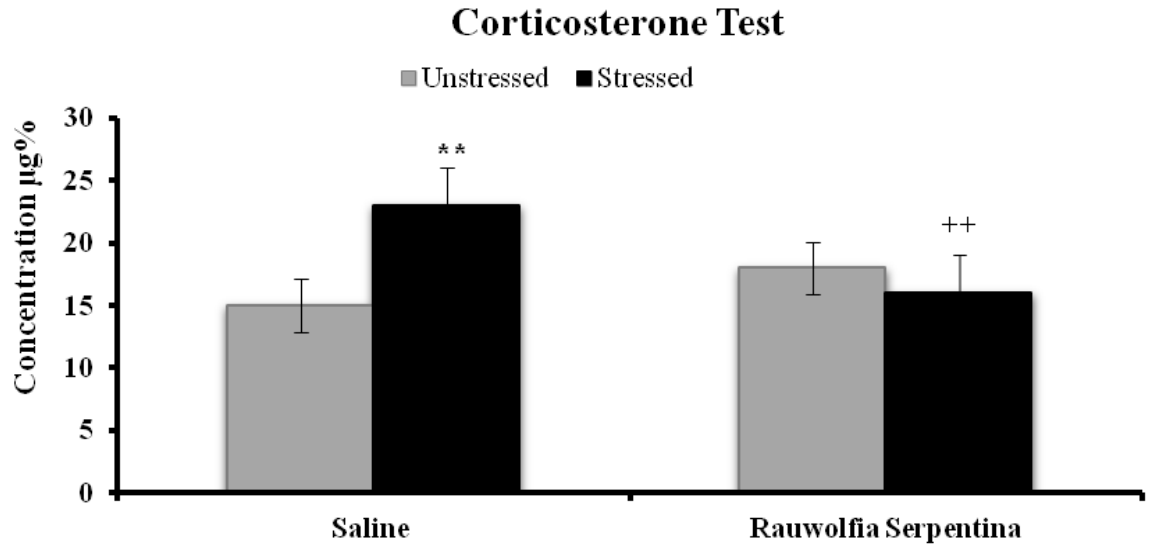
Newman keuls post hoc analysis revealed that activity of CAT was significantly increased in saline + stressed rats but significant decreased in *Rauwolfia serpentina* + stressed rats. *Rauwolfia serpentina* alone administration increased CAT activity. On the other hand, stress induced increase of CAT activity was attenuated in *Rauwolfia serpentina* administered stressed rats.

Fig. 5 Shows effects of stress on oral administration of *Rauwolfia serpentina* on plasma SOD activity. Analysis of the data on SOD activity (Fig 5) showed significant effects of stress ($F=3.282$ $p<0.05$ df 1, 20). Effects of *Rauwolfia serpentina* ($F=2.256$ N.S) and the interaction between two were not significant ($F=1.121$ N.S).



Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 and +p<0.05 versus their respective (unstressed or stressed) animals.

Fig. 5: Changes in the Levels of Superoxide Dismutase Activity in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

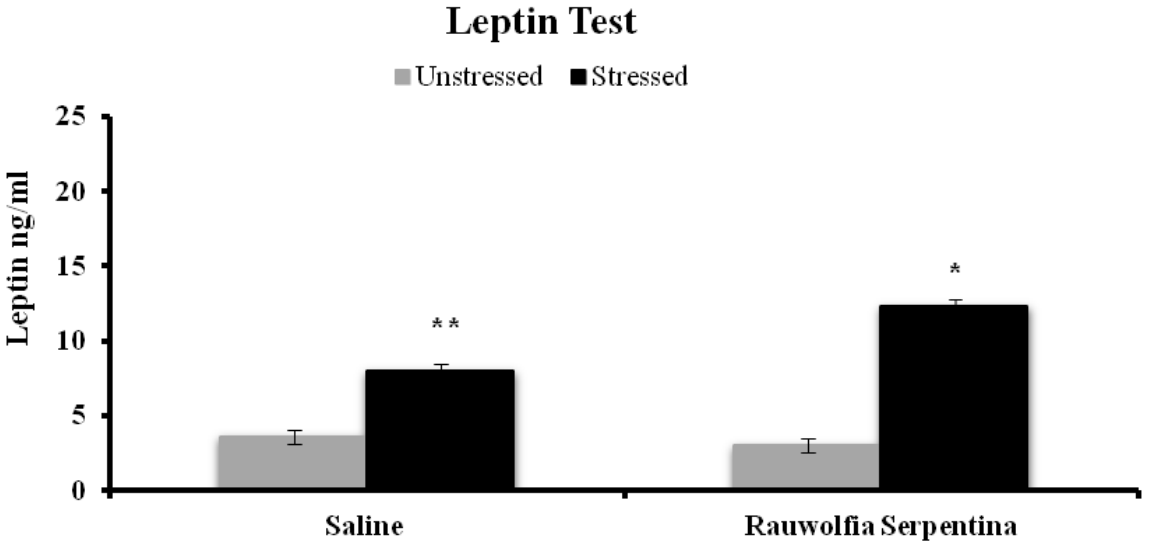


Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 and +p<0.05 versus their respective (unstressed or stressed) animals.

Fig. 6: Changes in the Levels of Corticosterone in Animals Orally Administered with *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

Newman keuls post hoc test showed that activity of SOD was significantly decreased in *Rauwolfia serpentina*+ stressed rats. But *Rauwolfia serpentina* alone did not alter the activity of SOD. On the other hand, the activity of SOD was not significant in other group.

Fig. 6 Shows effects of stress on oral administration of *Rauwolfia serpentina* on plasma corticosterone level. Analysis of the data on corticosterone level (Fig 6) showed significant effects of stress ($F=9.0$ df 1,20 $p<0.01$), *Rauwolfia serpentina* ($F=7.92$ df 2,20 $p<0.01$) as well as the interaction between the two ($F=26.01$ df 1,20 $p<0.01$).



Values are means ± S.D. (n=24). Data was analyzed by Newman-keuls test. **p<0.01 and *p<0.05 versus their control animals. ++p<0.01 and +p<0.05 versus their respective (unstressed or stressed) animals.

Fig. 7: Changes in the Levels of Leptin in Animals Orally Administered With *Rauwolfia Serpentina* Exposed to Acute 2h Immobilization Stress.

Post hoc analysis showed significant increased level of corticosterone in saline + stressed animals but decreased in *Rauwolfia serpentina* + stressed animals. On the other hand immobilization stress induced increase of corticosterone did not occur in single *Rauwolfia serpentina* administered animals.

Fig. 7 shows effects of stress on oral administration of *Rauwolfia serpentina* on plasma leptin level. Analysis of the data on leptin level (Fig 7) showed significant effects of stress ($F=9.0$ df 1,20 $p<0.01$) and *Rauwolfia serpentina* ($F=7.92$ df 2,20 $p<0.05$). A non significant interaction between the two factors ($F=26.01$ N.S).

Post hoc analysis showed significant increased in both saline + stressed and *Rauwolfia serpentina* + stressed animals as compare to their unstressed control rats respectively. In comparison with *Rauwolfia serpentina* + stressed rats showed increase level of leptin with saline + stressed rats.

Previously, it was reported that acute exposure to the immobilization stress can impair motor control, affect memory performance, cause pain perception [26], anxiety [27] and depression-like behaviors [28] in the animals. The purpose of this current study was to observe the neuroprotective effects of *Rauwolfia serpentina* on the behavioral activity of animals in the novel environment and light dark transition box activity following acute exposure to 2h immobilization stress in rats. Alterations in the levels of corticosterone, glucose and leptin were also measured to establish a link between oxidative stress and HPA axis following administration of plant extract. We also probed concentration of antioxidant enzymes like SOD and CAT to delineate the relationship of oxidative stress with behavioral deficits in rats. A consistent result of the current study is that an oral administration of *Rauwolfia serpentina* plant extracts attenuated immobilization stress-induced behavioral deficits and alteration in antioxidant enzymes levels in rats. Moreover, plasma leptin and corticosterone were also mitigated in these rats proposing the role of antioxidant components of plant extract which may elicit neuroprotective effects.

In the current study, we investigate the effects of *Rauwolfia serpentina* on the modulation of immobilization stress-induced behavioral deficits by two extensively used behavioral models of anxiety like behaviors including light dark transition test and open field. The present results showed that 2h immobilization exhibits a significant decrease in the number of square crossed but not latency to move in the open filed as compare to the unstressed animals (Fig. 1). Our results are in support with the previous findings [29-30] that acute exposure to (2h) immobilization stress produces anxiety like symptoms in rats. Therefore, immobilized stress animals avoid exploring new environment in light dark box as well as in the open field exploration test. Conversely, oral *Rauwolfia serpentina* extract alone

administration increased number of square crossed in the open field in rats. On the other hand, oral administration of *Rauwolfia serpentina* extract attenuated 2h immobilization stress-induced decreases in the locomotor activity in the open field. Similarly, a significant increase in the numbers of entries in light box and time spent in light compartment of the light dark transition box activity were also observed in these animals suggesting a reduction in novel environment-induced anxiogenic effects (Fig 2). Therefore, this anxiolytic effect of *Rauwolfia serpentina* plant extract could be explainable in terms of presence of numerous phytochemical compounds or secondary metabolites like alkaloids, flavonoids, phenols, resins, terpenes and etc in the plant extract [10, 31-32]**.** The present results are therefore in accordance with the earlier data that phenolic antioxidants present in the plant extracts could produce anxiolytic effects [33].

Oxidative stress has been associated with responses to stress [34] and linked to various neurological and psychiatric disorder [35]. Endogenous antioxidants play a vital role in conserving optimal cellular functions. While, on the other hand endogenous antioxidants could not be adequate under certain situation that could support oxidative stress [36-37] as observed in the current results (fig 4 & 5). Thus, elevated SOD and CAT activities were found in rats immobilized for 2h than control animals signifying that acute exposure to stress can encourage the formation of ROS and exhibits oxidative stress. In such instance, maintenance of optimal cellular functions could be provided by giving dietary antioxidants [38]. It has been suggested that consuming a antioxidants rich diet can reap many health benefits [39]. In recent years, many studies evidenced that most of the antioxidant properties of the plants were due to the presence of compounds such as phenolic acids, flavonoids and ascorbic acids that can provide protection against ROS [40–43]. In this perspective, plant extract containing flavonoids and ascorbic acid content of *Rauwolfia serpentina* exhibit antioxidant capacity which expand its nutraceutical values [44]. In present study, oral administration of *Rauwolfia serpentina* (Fig 4 & 5) attenuated immobilization induced increase in antioxidant enzymes CAT and SOD activities suggesting antioxidant capacity of plant extract component particularly flavonoids and ascorbic acid. Conversely, we also observed that oral administration of *Rauwolfia serpentina* alone increases CAT but not SOD antioxidant enzymes activity. It has been indicated that under physiological conditions pro-oxidants were comparatively favors during the balance between pro-oxidant and antioxidant compounds. Consequently, it causing a minor state of oxidative stress and requiring the involvement of endogenous antioxidant network of the organism [45]. It seems possible that alkaloids and flavonoids components of *Rauwolfia serpentina* plant extract could be contributed along with endogenous antioxidant system to counteract oxidative stress under basal conditions.

It is well recognized that exposure to acute stress causes the formation of free radicals that may leads to oxidative damages [46]. The HPA axis is the neuroendocrine system which controls the response to stress [47]. Activation of a stress response system is related to the production of elevated level of free radicals into the glands that involves the HPA axis [48–50]. Concerning, the HPA axis activity it is now eminent that neurons in the paraventricular nucleus (PVN) release corticotropin-releasing factor (CRF). CRF from the anterior pituitary stimulate the synthesis and release of adrenocorticotropin (ACTH). ACTH after that travels to the adrenal gland and induces the rapid [51] release of corticosteroids that later on activate a variety of physiological actions to deal with the stressful condition and assist an organism to reinstate homeostasis under a potential threatening condition [18, 52-53]. The present investigation demonstrates that animals subjected to immobilization stress exhibits an increased corticosterone levels (Fig 6). This is not unexpected since it has been previously reported that acute restraint stress [54] and immobilization stress [55] increases corticosterone levels and it is considered to be an important indicator of stress [56–58]. However, oral administration of *Rauwolfia serpentina* alone did not alter corticosterone levels as compare to saline plus unstressed animals. Conversely, the elevated corticosterone levels induced by immobilization stress were attenuated in *Rauwolfia Serpentina* treated animals (Fig 6). Previously, it was reported that chronically immobilized [46] and restraint [59]stress-induced attenuation of corticosterone levels explainable in terms of anti-stress activity. It is therefore interesting to relate the *Rauwolfia Serpentina* induced modulation of corticosterone levels in terms of suppressing HPA mobilization in responses to stress by normalizing elevated plasma corticosterone levels back to baseline. Thus, oral administration of *Rauwolfia Serpentina* reduced the adverse effects of acute exposure to (2h) immobilization stress and thought to be beneficial for the body to prevent from stress-induced damages.

As per clinical evidences elevated level of corticosterone in response to stress also increases the

plasma glucose concentration [60-61]. From the earlier studies, it was reported that stress causes increase in plasma glucocorticoid levels [62–64] which stimulate liver gluconeogenesis that leads to the elevated blood glucose [65]. Several authors [66-67]state that the usage of plasma glucose as the only indicator of stress, should be handle with care. It is because of many inconsistencies during the glucose measurements. It could be an auxiliary test of stress rather than a main indicator [68]. In the present result acute (2h) exposure to immobilization stress unable to alter the plasma glucose concentration. Previously, pre clinical studies on antidiabetic potential of methanolic root extract of *Rauwolfia serpentina* have been reported. It was found to be effective in lowering the blood glucose levels [16]. However, in our findings oral administration of *Rauwolfia serpentina* did not show any significant decrease in the levels of glucose as compare to the saline plus unstressed rats (Fig 3). Similarly, treatment with *Rauwolfia serpentina* also did not alter stress induced changes in glucose concentration in rats (Fig 3). It seems that 30mg/kg of *Rauwolfia serpentina* was not sufficient to produce significant hypoglycemic effects in our present study paradigm. The cause of variation between our observation and that in the mentioned study is uncertain but it may possibly due to discrepancy in nature of stress or ambient environment.

We are here reporting for the first time about the potential therapeutic role of *Rauwolfia serpentina* on endogenous leptin and corticosterone levels. Influence of leptin on HPA axis is one of the mechanisms by which leptin can improve stress controllability to produce antidepressant and anxiolytic-like effects. Previously, preclinical studies reported that exposure to 1h immobilization [69], 10 min forced swimming [70], showed an increase in the levels of leptin. These studies are consistent with our present data that exposure to acute (2h) immobilization stress resulted in significant increase in the leptin levels (Fig 7). As several components of the HPA axis carry receptors of leptin, it appear promising that stress response can be alter by the systemically circulating leptin [71]. In contrast, stress-induced releases of corticosterone have an opposite influence on leptin expression in adipocytes and its secretion into the blood circulation. It has been reported that stress-mediated stimulation of plasma corticosterone and ACTH in mice were inhibited by pretreatment with recombinant mouse leptin [72] and this inhibitory effects could be produced by receptors in the hypothalamus. The present results showed that oral administration of *Rauwolfia serpentina* significantly augment immobilization stress-induced increase of plasma levels of leptin (Fig 7) but inhibit corticosterone levels (Fig 6). It is therefore suggested that leptin could elicit a feedback effect on the HPA axis activity. Thus, the role of leptin in HPA axis functioning suggest that their relationship is bidirectional [71]. However, a role of leptin in alleviating stress perception is also apparent from studies reporting anxiolytic effects of pharmacological doses of exogenous leptin in rodent models of anxiety and an inhibition of stress-induced anxiety in these models [72]**.** It has also been reported that responsiveness of corticosterone to an acute stressor is inhibited by the conventional anxiolytic compounds [73-74] and reversed stress-induced behavioral deficits [75-76]. Similarly, we found that oral administration of *Rauwolfia serpentina* reversed acute (2h) immobilization stress induced behavioral deficits (Fig 1 & 2). It is therefore recommended that oral administration of plant extract could possibly elicits an anxiolytic like effect (Fig 1 & 2) by modulating endogenous leptin levels and thus inhibiting stress induced activation of the HPA axis.

Conclusion

We recommend that *Rauwolfia serpentina* has capability to antagonize the unpleasant effects of acute (2h) immobilization stress by reducing stress perception. Despite an apparently promising role in reducing the stress perception, the mechanism of action at molecular level causing the acute anxiolytic affects of oral administration of *Rauwolfia serpentina* plant extract remains to be determined. Furthermore, studies are also required to figure out the effects of oral administration of *Rauwolfia serpentina* plant extract on before and after exposure to unpredictable stress perception to further evaluate its potential as an anxiolytic compound.

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Conflict of interest

There is no conflict of interest.

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