

Evaluation of safety profile and stress suppressant activity of *Rosa moschata* in mice

Muhammad Jamal¹, Mujeeb ur Rehman¹, Muhammad Nabi², Abdul Aleem Awan¹, Niaz Ali², Muhammad Azhar Sherkheli¹ and Rizwan ul Haq^{1*}

¹Department of Pharmaceutical Sciences, Abbottabad University of Science and Technology, Abbottabad, Pakistan

²Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University Peshawar, Pakistan

Abstract: Stress is a state that seriously disturbs psychological or physiological homeostasis of the body and subsequently affects the morphology and function of the hippocampus. Currently available anti-stress medications provide limited benefits with cost of severe adverse effects. In the present study, effect of *Rosa moschata* extract was evaluated using acute restraint model in mice. The stress suppressant activity of *Rosa moschata* was evaluated by using elevated plus maze test (EPM), dark light box test and open field test (OFT) following restraint stress protocol. Results showed that the *Rosa moschata* extract significantly enhanced the number of transitions and the time spent in the open arm in the EPM, increased the number of transitions and time spent in the light compartment of the dark light box, and also enhanced the locomotor activity in OFT, as compared to the stress group. In addition, LD₅₀ of the plant extract is greater than 5000mg/Kg. Thus the findings of our studies show that *Rosa moschata* significantly alleviates stress following the acute restraint stress in mice. Further studies dealing with underlying mechanism and characterization of active fraction/compound may provide an alternative therapy for stress and related neurological conditions.

Keywords: *Rosa moschata*, restraint stress, elevated plus maze, dark/light box, open field test.

INTRODUCTION

Stress is a pathological condition that seriously disturbs behavior and psychological or physiological homeostasis of the body. It disrupts cognition and memory processes by altering the morphology and function of the hippocampus (Kim and Diamond, 2002). Stress response initiates with an external or internal stimulus that ultimately causes the activation of the sympathetic nervous system and hypothalamic pituitary adrenal (HPA) axis. These stimuli extend to the many brain areas specifically limbic system, where the release of norepinephrine (NE), acetylcholine (Ach) and 5-Hydroxytryptamine (5-HT) occurs (Nguyen *et al.*, 1998). Brain has its innate capability to combat the stress but if the severity of stress is beyond the limits of the brain adaptive response against the stress then hazardous situations may occur that cause mental illness. Furthermore, brain has a distinct oxidative potential but an inadequate ability to neutralize oxidative stress. This oxidative stress is associated with mechanisms that lead to the neuronal cell injuries in numerous pathological conditions of the brain. In addition, brain is very susceptible to oxidative damage because it consumes more than 20% oxygen available via respiration even though it constitutes only 2% of the total body weight (Esch and Stefano, 2010). Similarly, nitric oxide synthase (NOS) has been involved in excitotoxicity phenomenon in various cellular systems and many neuropathological disorders such as Parkinson's disease, epilepsy, ischemia, Huntington chorea, Alzheimer's disease, due to the over

production of nitric oxide its subsequent activity (Moncada *et al.*, 1991, Gross and Wolin, 1995).

Common medications that are currently used against stress, depression and anxiety include tricyclic antidepressants, SSRIs, monoamine oxidase inhibitors. These medications have various side effects including jitteriness, memory lapses and sexual dysfunctions etc. Therefore there is an intense need of novel therapy with less side effects and more efficacy (Lanzenberger *et al.*, 2007).

Phytochemicals are very helpful in alleviating certain stress related disorders. Extracts of *Withania somnifera*, *Aralia mandshurica*, *Bryonia alba*, and *Panax ginseng*, containing terpenenes, phytosterol etc, exert their actions on the HPA axis. Moreover, these plants prevent or decrease levels of certain hormones that are characteristic of stress response (Panossian *et al.*, 1999, Kim *et al.*, 2003). Certain plants such as *Schisandra chinensis* and *Rhodiola rosea* has phenolic secondary metabolites such as phenyl and phenylethylpropanoids and their dimeric lignans (Saratikov and Krasnov, 2004). These compounds play a very important role in suppression of stress (Ataie *et al.*, 2016).

There are various animal models used to explore the basic mechanisms responsible for the induction of stress in physiological conditions of the body. Thus induction of inescapable stress and escapable stress has been widely used in different animals (rabbits, primates, mice, rats). Among these stress models, use of unavoidable physical stressors, such as restraint stress, exposure to extreme temperature or electroshock stress. Other models like

*Corresponding author: e-mail: rizwanhej@gmail.com

fasting or forced exercise is mostly used to study metabolic responses, while other models of stress like emotional or social stress such as social isolation or crowding have been used widely for various psychological disorders. Inescapable physical stress i.e. restraint stress or immobilization has been reported as the most validated and acceptable model in experimental studies relating to neurodegeneration (Jaggi *et al.*, 2011).

Experimentally it has been found that acute and chronic restraint stress induce the expression of inducible nitric oxide synthase (iNOS) in the brain, and also cause the production of peroxynitrite (ONOO⁻) in rats (Olivenza *et al.*, 2000, Madrigal *et al.*, 2001). In restraint stress, nitric oxide (NO) has been linked to the disruption of blood brain barrier (BBB) and reduced mitochondrial function (Madrigal *et al.*, 2001). Furthermore, acute and chronic stress evoke anxiety like behavior and depression in rats. Studies suggested that by inhibiting NOS acute and chronic stress induced behavioral changes may be prevented thus NO might be involved in the depression and anxiety like behavior (Sevgi *et al.*, 2006).

Antioxidant rich phytochemicals and/or extracts are gaining importance as anti-stress therapy. Various plant extracts are clinically and conventionally used for the management of different neurological disorders. From different studies, it has been evident that several medicinal plants and their extracts were used to protect neuronal damage (Kumar *et al.*, 2013), Alzheimer's disease and Parkinson's disease (Shim *et al.*, 2009, Hsieh *et al.*, 2010), to enhance neuronal differentiation (El Omri *et al.*, 2010) and to use against depression (Sasaki *et al.*, 2013), anxiety (Lo *et al.*, 2010) and epilepsy (Carro-Juárez *et al.*, 2012).

Rosa moschata belongs to the family rosaceae. More than 120 species of this genus are reported. Seven of them are reported to found in Malakand region of Pakistan (Barkatullah and Ibrar, 2013). There are various local names of this species such as "Kurach", "Zangley gulap", "Qorach" in Pakistan while in india the different local names of this plant are "Kojai", "Kuja", "Kunai" (Sharma and Devi, 2013, Ajmal *et al.*, 2012). Plant is perennial climbing shrub and the whole plant is used for medicinal purpose. Traditionally, this plant is used in many eyes disorders, wound healing, diarrhea and stomach disorders, and labour (Sharma and Devi, 2013). It is confirmed that the plant contain palmitic acid, margaric acid, and linolenic acid. Other compounds that are isolated from the species are Vitamin A, C, E, flavonoids and essential oil (Honarvar *et al.*, 2011).

MATERIALS AND METHODS

Animals

Experiments were performed on male Balb/c mice weighing 30 ± 3 g, with food and water available freely except during behavioral experiments where mice were deprived of food and water. Temperature was maintained

22 ± 2 C° under 12/12 hours dark-light cycle. Experiments were performed in the light cycle. Experimental procedures were performed according to the approved guidelines of the ethical committee of the institution.

Drug and testing material

The hydro-methanolic extract from fruits of *Rosa moschata* was obtained from Department of Pharmacology, Khyber Medical University (KMU), Peshawar, Pakistan. Briefly, plants were collected from Malakand, Pakistan and identified by a taxonomist at Department of Botany, University of Malakand, Pakistan. A voucher specimen (No. RM-2103) has been submitted in Pharmacology Department, KMU, Peshawar. Fluoxetine HCl was obtained as a gift from Ferozson laboratories, Nowshera Pakistan. The plant extract was dissolved in normal saline in different doses (50mg/kg, 100mg/kg and 150mg/kg). Fluoxetine HCl 10mg/kg solution was prepared by dissolving it in distilled water. Testing materials and drug were administered intraperitoneally (i.p.) in such way that dispensed volume didn't exceed 1 % of body weight of animal.

Acute toxicity test (LD₅₀)

The acute toxicity (LD₅₀) was performed as reported by Lorke in 1983 (Lorke, 1983). Different doses (10mg/kg to 5000mg/kg) of plant extract were administered intraperitoneally to different groups of mice and observed number of deaths after 24 hours (for detail see, Lorke, 1983, (Haq *et al.*, 2011)

Stress induction

Mice were transferred to the behavioral laboratory one to two hours prior to the start of experiments for acclimatization. Mice were weighed and labeled before commencement of the behavioral experiments. Drug, extract or normal saline were administered 30 min prior to the application of restraint stress procedure. In the restraint stress procedure, mice were kept immobilized for 90 minutes.

Animals were divided into six groups each having 8 mice. Group 1 served as negative control and administered 0.9% normal saline (NS); no stress was applied to these animals. Group 2 animals were taken as stressed control and 0.9% NS was administered to these mice followed by the restraint stress procedure. Group 3 mice were taken as positive control and were administered 10mg/kg Fluoxetine HCl; Groups 4 - 6 were administered extract at doses of 50mg/kg, 100mg/kg and 150mg/kg respectively.

For Stress induction, mice were restrained in a ventilated 50ml plastic syringe (Delaney *et al.*, 2012) and placed in an opaque surface for 90 minutes. After 90 minutes the animal was transferred to the cage for 5 minutes interval and thereafter mice were evaluated for behavior changes using elevated plus maze (EPM), open field test (OFT) and dark light box test.

Elevated plus maze (EPM)

EPM test has been extensively used for the evaluation of anxiety like behavior in rodents. EPM is a plus sign shape steel apparatus consists of two open arms (15cm x 5cm) and two closed arms (15cm x 5cm x 20cm). EPM was elevated 50cm above the ground. After stress induction procedure animal was put in the center of the EPM with head facing one of the open arms. Animal was permitted to explore for 5 minutes. During these five minutes the number of the entries in open and closed arms and time consumed in the open arms were noted.

Open field test (OFT)

OFT has been widely used to measure the locomotor activity of the rodents. The apparatus consisted of square chamber (72cm x 72cm x 15cm; length, width, and height respectively). The box was further divided into 4 x 4 small squares having area (18cm x 18cm). After stress protocol animal was kept in the center of the field and permitted to explore for 5 minutes. Numbers of squares crossed were recorded.

Dark light box

Dark light box has been used for the measurement of anxiety in rodents. It consist of a wooden box divided into two chambers, one was made dark and one light. Animal was placed in the light compartment facing its head towards the wall of the compartment. Number of transitions in the light compartment and percent time spent in the light and dark compartment were recorded during 5 minutes.

STATISTICAL ANALYSIS

Experimental data obtained from all groups were presented as mean \pm Standard error to the mean (SEM). The statistical assessment was performed using Cal ORIGIN software version 8.5. All the values were compared with stress control. The significance of difference was determined by One-way ANOVA by using Cal ORIGIN software. *p* value of < 0.05 was taken as significant.

RESULTS**Acute toxicity (LD₅₀)**

Hydro-methanolic extract from fruits of *Rosa moschata* showed a wide therapeutic index in our experiments as LD₅₀ of this extract was greater than 5000 mg/Kg when administered i.p. We didn't observe any death after 24 hours of administration of extract on any dose upto 5000 mg/Kg in mice.

Extract of *Rosa moschata* suppresses the stress parameters in EPM

Our data show that extract of *Rosa moschata* significantly suppresses the acute restraint stress in mice. Briefly, our

data indicate that extract of *Rosa moschata* significantly increases the number of transitions in open arm of EPM in dose dependent manner (fig. 1A; *n* = 8; *p*<0.05). As shown in fig. 1, acute restraint stress procedure markedly and significantly reduces the number of transitions to open arm in the maze as compared to unstressed group (negative control or saline control). Our data show that extract of *Rosa moschata* antagonizes the stress mediated alteration in parameters accessed in EPM. The highest stress suppressant effect represented by an increase in number of transitions in open arm of EPM is shown by administration of 150mg/kg dose of extract of *Rosa moschata*. This effect is well comparable with positive control as fluoxetine 10mg/kg significantly increased the number of entries in the open arm as compared to the saline control. Induction of stress, applying acute restraint stress procedures, significantly reduces the time spent in the open arm in EPM test (fig. 1B; *n* = 8, *p*<0.01). Similarly, administration of extract of *Rosa moschata* significantly and dose dependently increases time spent in the open arm in the elevated plus maze test (fig. 1B; *n* = 8 in each group, *p* < 0.01). Interestingly, administration of extract of *Rosa moschata* 150mg/kg increases the time spent in open arm of EPM in a similar manner to the administration of fluoxetine 10mg/Kg.

Extract of *Rosa moschata* increases the locomotor activity in open field test

Open field test (OFT) was applied to estimate the effects of extract of *Rosa moschata* on locomotor activity in stressed animals. As represented in fig. 2, application of acute restraint stress highly and significantly reduces locomotor activity of experimental animals (fig. 2; *n* = 8). The experimental results revealed that extracts of *Rosa moschata* at a dose of 50mg/kg, 100mg/kg and 150mg/kg significantly and dose dependently increase in the number of squares crossed in the OFT as compared to the negative control. The maximum increase in locomotor activity of stressed animals is shown by the extract at a dose of 150mg/kg (fig. 2; *n* = 8; *p*<0.01). This effect is well comparable to the positive control fluoxetine 10mg/Kg (fig. 2; *n* = 8).

***Rosa moschata* reverts stress symptoms in dark/light box test**

To validate our results representing that extract of *Rosa moschata* reverts the symptoms of stress in acute restraint stress model we conducted additional experiments using dark/light box test. As our data reconfirmed the finding that stressed mice are reluctant to enter light compartment frequently and usually spend more time in dark compartment (fig. 3).

Our results show that stress significantly decreases the transitions between two compartments (fig. 3A; *n* = 8) in dark/light box test. Administration of extract of *Rosa moschata* shows gradual and dose dependent increase in

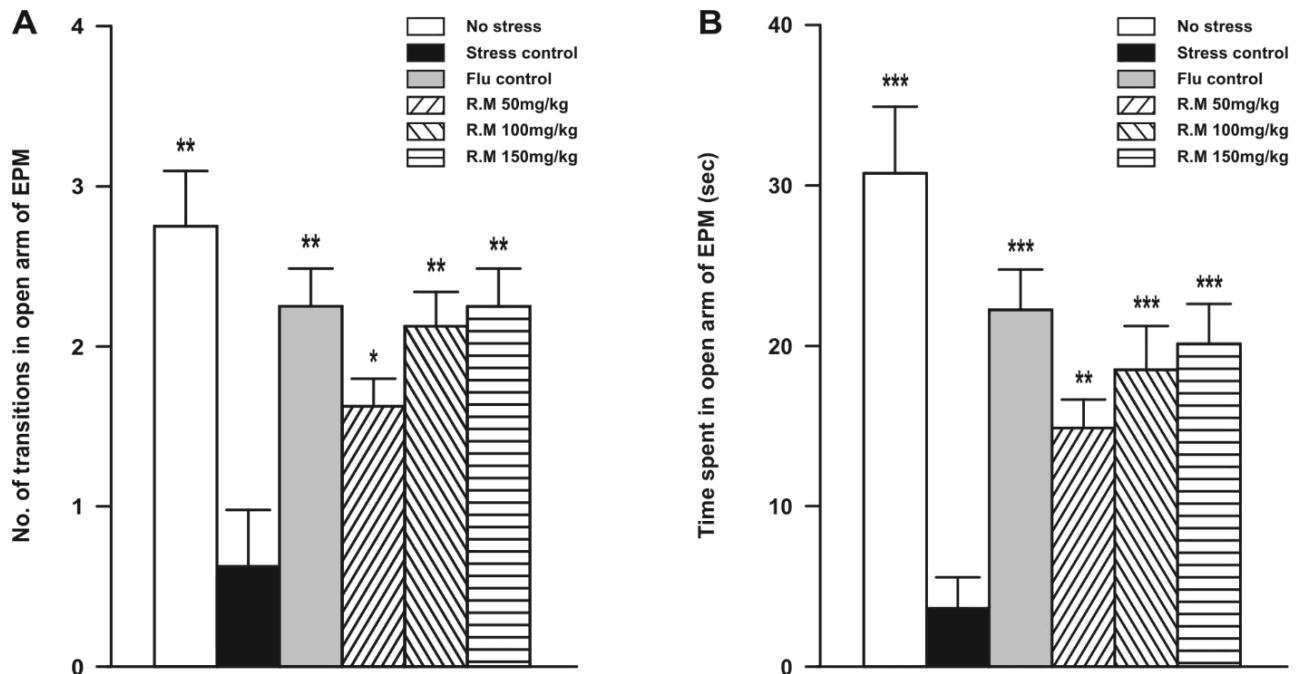


Fig. 1: Extract of *Rosa moschata* significantly and dose dependently suppresses the stress related parameters in elevated pulse maze (EPM) in mice. A; Bars diagram shows that administration of extract of *Rosa moschata* (R.M.) significantly increases in number of transitions in the EPM in dose dependent manner (n = 8 in each group). B; Bar graphs representing that application of extract of *Rosa moschata* significantly enhances the time spent in open arm of EPM (n = 8 in each group). Note that R.M. 150mg/Kg shows comparable effect with fluoxetine 10mg/Kg. *P<0.05, **P < 0.01 and ***P < 0.001 vs. stress control group by applying one-way ANOVA.

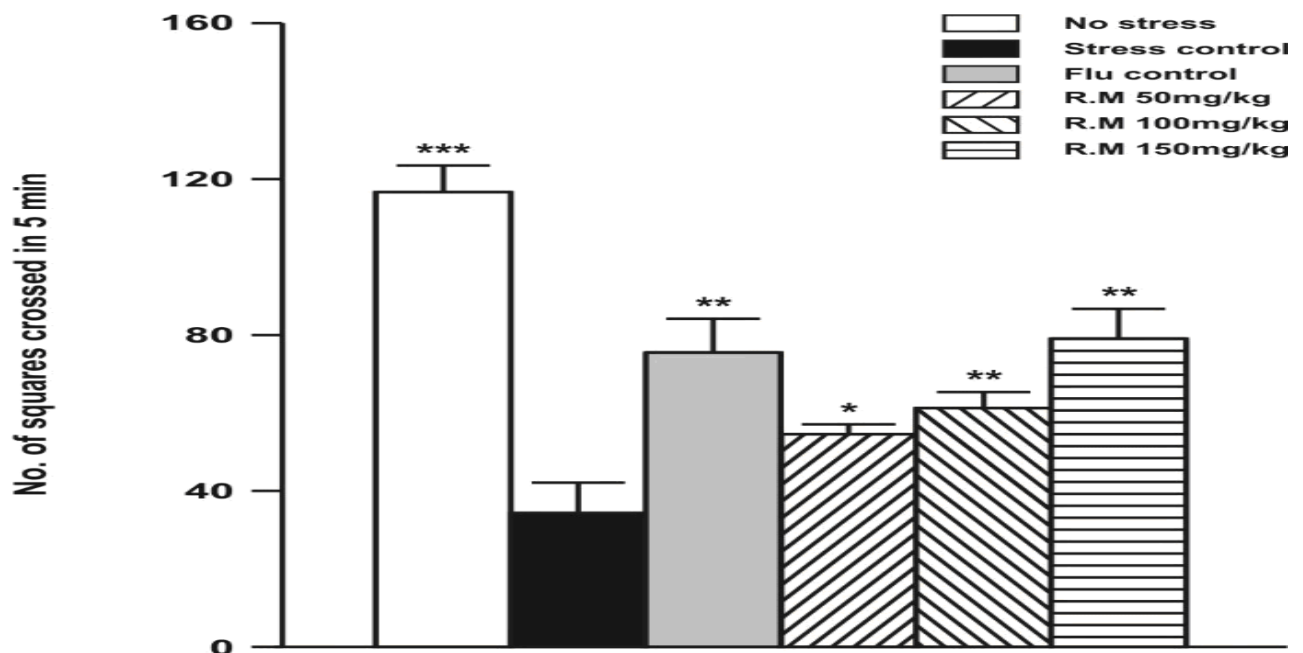


Fig. 2: Extract of *Rosa moschata* increases the locomotor activity in open field test. Graphical representation of effects of R.M. on locomotor activity in restraint stress mice. R.M. extract significantly augments locomotor activity in a dose dependent manner (n = 8 in each group) as indicated by an increase in number of square crossed in open field in all tested animals groups. *P<0.05 and **P<0.01 and ***P<0.001 vs. stress control group by applying one-way ANOVA.

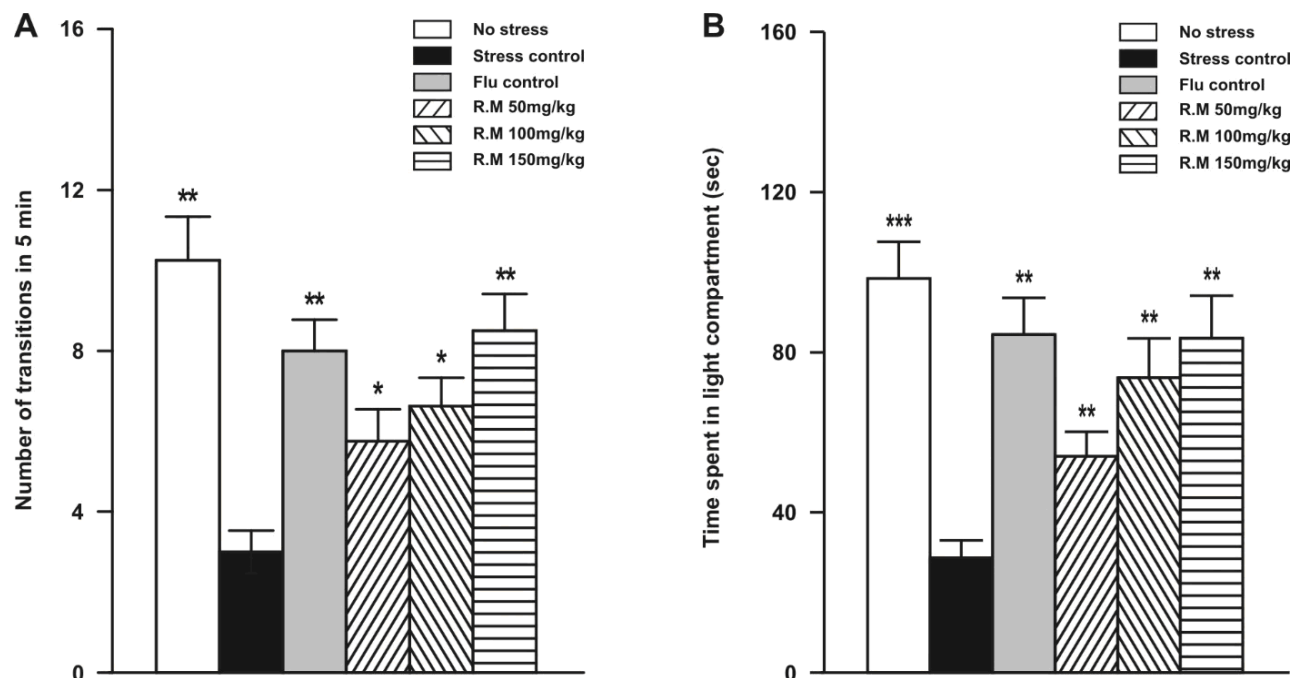


Fig. 3: Extract of *Rosa moschata* (R.M.) reverts stress symptoms in dark/light box test in mice. A; Bars diagram shows the extract of R.M. increases in number of transitions between two compartments of the light/dark box in mice (n=8 in each group). Note that plant extract shows significant increase in the number of transitions in dose dependent manner. B; Graphs representing that extract of *Rosa moschata* exerts a significant increase in the time spent in the light compartment of the light/dark box in mice (n = 8). Note that extract of *Rosa moschata* has similar effects to the fluoxetine 10 mg/Kg on both parameters measured in dark /light box test. *P < 0.05, **P < 0.01 and ***P < 0.001 vs. stress control group by applying one-way ANOVA.

transition between the two compartments (fig. 3A; n = 8 in each group; p < 0.05). The highest increase is shown by extract of *Rosa moschata* at a dose of 150mg/kg which is comparable to the fluoxetine 10mg/Kg (fig. 3A; n = 8). Similarly, our data reveal that application of extract of *Rosa moschata* significantly and dose dependently increases the time spent in the light compartment (fig. 3B; n = 8 in each group, p < 0.05). Extract of *Rosa moschata* at dose of 150mg/Kg exhibits maximum effects on time spent in light compartment and these effects are comparable with fluoxetine 10 mg/Kg (fig. 3B; n = 8).

DISCUSSION

In search of new therapeutic strategies for the cure of various neurological disorders and stressful conditions, plant products and phytochemicals are gaining more attention because of their potential in combating neurological disorders more effectively. Stress cause altered activities of various physiological systems of the body especially modulating hormonal factors and neurotransmitters those linked with the HPA axis and the generation of free radical (Zafir and Banu, 2009). In the present experiments the effect of *Rosa moschata* extract was evaluated in the restraint stress-induced behavioral changes in mice. Our results demonstrate that *Rosa*

moschata alleviates restraint-stress induced alteration in rodent behavior.

Phytochemicals containing antioxidants have the capability to reverse the cellular damage caused by the free radicals (Moosmann and Behl, 2002). The endogenous chemicals that prevent the oxidative damage include superoxide dismutase, glutathione peroxide and proteins like transferrin, metallothionein, and albumin. The exogenous dietary phytochemicals that help to prevent the oxidative damage of cells include quinones, catechins, polyphenols, terpenoids, flavonoids, coumarins and the smaller molecules like ascorbic acid (vitamin C), alphanatocopherol (vitamin E), beta-carotene etc. Although the mode of actions of these phytochemicals are not yet completely elucidated, these phytochemicals are very effective in controlling or reducing the progression of the certain neurodegenerative disorders like Huntington's disease, Parkinson's disease, Alzheimer's disease (Larson, 1988, Berger, 2005). It has been found that various stresses have been associated with increased production of free radicals that caused oxidative damage and also among all organs CNS is more prone to oxidative damage, due to high content of polyunsaturated fatty acid and low quantity of antioxidants in the brain. It has been reported that vitamin C, E, carotenoids, polyphenols and

flavonoids have antioxidant properties (Doyle and Pariza, 2002).

Studies shows that contents from *Rosa moschata* include vitamin A, C, E and flavonoids (Honarvar *et al.*, 2011). It has been also reported that vitamin C (Choi *et al.*, 2010) and vitamin E (Yargıçoglu *et al.*, 2003) play important role in anti-stress activities. The stress alleviating potential of vitamin C and Vitamin E both found to be effective but the their results are not additive (Kashif *et al.*, 2003). It was found that vitamin C is very effective in fearful stress conditions such as electroshock showing non-significant alteration in restraint stress-induced behavior changes (Choi *et al.*, 2010). Vitamin E play a major role among vitamins in combating stress and other neurodegenerative disorders (Zaidi and Banu, 2004, Kashif *et al.*, 2003). These result complies with the experimental study of *Rosa moschata* on alleviation of stress-induced behavior changes. It was found that vitamin E (Kayalvizhi *et al.*, 2012) but not vitamin C (Moretti *et al.*, 2013) significantly alter the locomotor activity of rodent in open field test and this result complies with our results: There is significant increase of locomotor activity by the rodent in open field at all tested doses of *Rosa moschata*. Similarly, *Rosa moschata* have flavonoids in their content and it was reported that flavonoids play an important role in reducing the stressful conditions. It was evaluated that flavonoids of *Ficus benghalensis* (Lotankar, 2016) and *Hypericum perforatum* (Butterweck *et al.*, 2000) having antistress activity on an elevated plus maze test, forced swimming test and tail suspension tests. Flavonoids of *Buckweat* also showed antistress activities and increased the open arm entries in elevated plus maze (Watanabe and Ayugase, 2008) and these results comply with the results of *Rosa moschata*.

CONCLUSION

From the results of Elevated plus maze, open field and dark light box test, it was concluded that the *Rosa moschata* have antistress effects. Moreover, it is assumed that this antistress activity of *Rosa moschata* may be due to antioxidant activity of flavonoids and vitamins.

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