Pharma Science Monitor 6(3), Jul-Sep 2015

PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
Journal home page: http://www.pharmasm.com

POSSIBLE BENEFICIAL EFFECT OF GINSENG AGAINST HALOPERIDOL-INDUCED COGNITIVE IMPAIRMENT, NEUROINFLAMMATION AND BIOCHEMICAL ALTERATIONS IN RATS

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ABSTRACT

The aim of the study is to investigate the possible beneficial effect of ginseng against haloperidol induced cognitive impairment, neuroinflammation and biochemical alterations in rats. Haloperidol (1mg/kg) administration intraperitoneally to rats for 21 days produced TD like symptoms as observed from behavior and biochemical alterations in rat brain homogenates. Behavior parameters like orofacial dyskinesia, rotarod, elevated plus maze, open field and beam crossing task were assessed weekly. On 22nd day, rats were sacrificed and biochemical parameters like reduced glutathione, nitrite level, lipid peroxidation and catalase activity were measured in brain homogenates. Haloperidol produced oxidative stress as depicted from increased levels of lipid peroxidation, nitrite levels and reduced antioxidant defense (glutathione and catalase levels), neuroinflammation as it increased TNF-α and IL-6 levels and altered cognition as suggested from elevated plus maze (EPM) studies along with behavioral disturbances like orofacial dyskinesia (VCMs), decreased muscle grip, locomotor activity and gait abnormalities. Ginseng was co-administered orally with haloperidol for 21 days at 100 mg/kg (low dose) and 200 mg/kg (high dose). Ginseng administration attenuated behavioral, neuroinflammation & biochemical alterations in rats' dose dependently. Therefore, it may be concluded that ginseng may have neuroprotective effect against haloperidol induced cognitive impairment, neuroinflammation and behavioral alterations in rats.

KEYWORDS: Haloperidol, Ginseng, Cognition, Neuroinflammation.

INTRODUCTION

TD is a hyperkinetic disorder and characterized by involuntary movements of the face, tongue and lips. Vacuous chewing movements, tongue protrusions and facial jerking are main symptoms of tardive dyskinesia. Mainly orofacial region get affected, but sometimes body's other parts also get affected[1]. Haloperidol is neuroleptic used in psychotic disorders. It induces cognitive impairment, behavioral and biochemical alterations and neuroinflammation to produce tardive dyskinesia (TD) like symptoms Haloperidol and other antipsychotic drugs are used in schizophrenia and other psychiatric disorders. Oxidative stress and lipid peroxidation are the

main culprits involved in the pathophysiology of various neurodegenerative diseases. Long-term treatment with neuroleptics causes oxidative stress and increase in free radical production[2]. It was suggested that oxidative stress plays a key role in pathology of neuroleptic induced TD[3,4]. Vacuous chewing movements observed in tardive dyskinesia are due to neuronal loss, mainly present in striatum region[5].

Various models like haloperidol, reserpine, chlorpromazine induced, isoniazid induced and primate models are used for development of TD. Haloperidol is commonly used model for tardive dyskinesia. Haloperidol is administered continuously for 21 days. Antipsychotics are mainly dopamine receptor antagonist[6]. It is suggested that mechanism of action involved in haloperidol is blockade of D_2 dopamine receptor in brain. Dopamine turnover is increased which lead to excessive production of free radicals and causes damage of neuronal cells[7]. Studies suggest that long use of neuroleptics causes dopamine supersenstivity and a selective loss of GABAergic neurons along with reduced nigral GAD activity resulting in development of dyskinesia[8]. Haloperidol also increases glutamate release in the striatum region and morphological changes are related to increased glutamatergic activity[9,10]. Calcium influx also plays role in pathophysiology of TD. Calcium influx occurs through NMDA receptor and it increases reactive oxygen species which increases neuronal damage[11]. Haloperidol administration triggers the different damaging pathways, like dopamine turnover increases the hydrogen peroxide in the basal ganglia[12].

Panax ginseng is a perennial herb of the family *Araliaceous*. From many years it has been used as traditional medicine[13]. *P. ginseng* inhibit the over production of reactive oxygen species (ROS) and other apoptotic mediators those causes cell death[14]. It also act as anti-inflammatory agent by inhibiting the activation of NF – κB/p65, Akt and the ERK1/2 in H_2O_2 model[15]. In some studies it was suggested that ginseng can inhibit the neurological impairment and striatal cell death in Huntington's disease (HD). It was also reported that it inhibits the activation of proinflammatory cytokines like TNF-α and IL-6[16]. It was also reported that ginseng attenuates neurodegeneration in Parkinson's disease (PD) and Alzheimer's disease (AD)[17]. It was found out that ginseng also inhibits microglial activation in rat model of traumatic head injury[18]. Therefore, the present study was designed to check the possible beneficial effect of ginseng in haloperidol induced TD attenuation of cognitive impairment, neuroinflammation and biochemical alterations.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 180-220 g were obtained from Central Animal House facility of I.S.F. college of Pharmacy, Moga, Punjab, India. Animals were housed in group of six in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. All the behavioral assessments were carried between 9:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of ISF college of Pharmacy, Moga and work was carried out in accordance with the CPCSEA guideline for the use and care of the experimental animals. All the experiments for a given treatment were performed using age matched animals in an attempted to avoid variability between experimental groups.

Drugs and treatment schedule

Haloperidol was dissolved in distilled water. Ginseng was obtained as ex-gratia sample from Vagus Remedies Pvt. Ltd. Gujarat, India. Ginseng was suspended in 0.5% Carboxy methyl cellulose (CMC), administered at doses of 100 mg/kg and 200 mg/kg orally. These doses were selected on the basis of previous studies[18]. Group I: vehicle treated (CMC *p.o.*), Group II: Ginseng per se, Group III: Haloperidol treated (1 mg/kg *i.p.*), Group IV: Haloperidol (1 mg/kg) + Ginseng low dose (100 mg/kg *p.o.*) treated, Group V: Haloperidol (1 mg/kg *i.p.*) + Ginseng high dose (200 mg/kg *p.o.*) treated. The duration of study was 21 days.

Haloperidol was administered *i.p.* in a volume of 1 ml per 200g of body weight. Ginseng was administered orally in a constant volume/body weight of rat once daily for 21 days. Ginseng was administered 30 min before the haloperidol treatment.

All the behavioral parameters were observed on day 1st, 7th, 14th and 21st of haloperidol treatment. Behavioral parameters were observed in sequence on each day starting with locomotor activity then Rota rod followed by VCMs, tongue protrusion, facial jerking in each animal. Treatments were given in all the groups in the morning before behavioral observation on respective day.

Measurement of Body weight

Animal body weight was recorded on the first and last day of experimentation. Percent change in body weight was calculated as:

$$\frac{\text{Body weight (day 1)} - \text{Body weight (day 21)}}{\text{Body weight (day 1)}} \times 100$$

Assessment of behavioral parameters

Assessment of orofacial movements:-

On the test day the rats were placed individually in plexiglass (30 x 20 x 30 cm) cage for assessment of oral dyskinesia. Animals were allowed 10 min to get used to observation cage before behavioral assessments. To quantify the occurrence of oral dyskinesia, hand-operated counter were employed to score vacuous chewing, tongue protrusion, facial jerking frequencies. In the present study, VCMs are referred to as single mouth opening in the vertical plane not directed towards physical material. If tongue protrusion or VCMs occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for the period of 10 minutes in all the experiments the score was unaware of the treatment given to the animals[1,19,20,21].

Motor coordination:-

Motor coordination was assessed for all rats on a rotarod. Rats were placed individually on a rotating rod with a rod of 7 cm (speed 25 rpm). Prior to any treatment, rats were trained in a single session until they attained 300s on rotarod. The fall of time was recorded.

Spatial memory:-

The elevated plus maze was used to evaluate spatial long term memory and was performed on 20th and 21st day. Briefly the apparatus consisted of two open arms and two close arms. The arms extended from a central platform and the maze was elevated to a height of 50 cm from the floor. On the first day each animal was placed at the end of an open arm. Transfer latency (TL), that is the time taken by the rat to move into one of the closed arm, was recorded on the first day. If the animal did not enter into a closed arm within 90s it was gently pushed into one of the closed arms and the TL was assigned as 90s. The rat was allowed to explore the maze for 20s and then was returned to home cage. The rat was again placed in the maze next day (24 h later) and TL was recorded[1]. Percentage retention was calculated by the formula:

$$\frac{\text{Transfer Latency (Day 1)} - \text{Transfer Latency (Day 2)}}{\text{Transfer Latency (Day 2)}} \times 100$$

Locomotor activity:-

To monitor activity in a novel environment, an open field apparatus was used. That used in the present investigation consisted of a square area 76 x 76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity a rat was placed in the

center square of the open field. The numbers of squares crossed with all four paws were scored for 5 min[22].

Gait abnormalities:-

Narrow beam walk apparatus used for measuring the gait abnormalities. The apparatus consist of 50 cm wooden strip supported by two wooden stick at each end, with height of 100cm above ground. The rats were supposed to walk on narrow beam, suspended between straight platform and their home cage. Straight platform was at a height of at least 100 cm above the ground, to make sure that the animal fears the height and really attempt to reach the home cage. The rats must be trained 5 days before actual task. Time taken to reach home cage was measured (cut off time 30 sec) along with their number of slipping errors on 1st, 7th, 14th and 21st day[23,24].

Dissection and biochemical studies

On day 22, after behavioral quantification the animals were sacrificed by decapitation. The brains were removed, forebrain was dissected out and cerebellum was discarded. Brains were put on ice and the cortex and striatum were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). For enzyme assays, brains were homogenized with the help of homogenizer and centrifuged at 12,000 x g for 60 min at 4°C. The supernatants from the above samples were taken and used for further oxidative stress enzymatic assays.

Lipid peroxidation:-

The quantitative measurement of lipid peroxidation in forebrain was performed according to method of Wills (1965)[25]. The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nmol of malondialdehyde /mg protein using the molar extinction coefficient of the chromophore (1.56×10^5 M^{-1} cm^{-1}).

Estimation of reduced glutathione:-

Reduced glutathione in the brain was estimated according to the method of Ellman (1959)[26]. A sample (0.75 ml) of homogenate was precipitated with 0.75 ml of 4% sulfosalicyclic acid. The samples were centrifuged at 1200 x g for 15 min at 4 °C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M (in 0.1 M phosphate buffer, pH 8.0) DTNB (5-5' – Dithio Bis – (2 – Nitrobenzoic acid)). The yellow color developed was read immediately at 412 nm spectrophotometrically. The results are expressed as nmol of GSH per mg protein.

Catalase activity:-

Catalase activity was assayed by the method of Luck (1971)[27], by which the breakdown of H_2O_2 is measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 – phosphate buffer (1.25 x 10^{-2} H_2O_2 M) and 0.05 ml of supernatant of brain homogenate (10%) and the change in absorbance was recorded at 240 nm spectrophotometrically. The results were expressed in µmol H_2O_2 decomposed/min/mg protein.

Protein estimation:-

The protein was estimated by Biuret method using bovine serum albumin as standard[28].

Estimation of Nitrite:-

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by calorimetric assay using Greiss reagent (0.1% N-(1-naphthylethylenediaminedihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid)[29]. Equal volume of supernatant and Greiss reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and absorbance determined at 546 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as microgram per ml.

Estimation of TNF-α and IL-6 levels:-

The quantifications of tumor necrosis factor (TNF-α) and interleukin (IL-6) were done by rat TNF-α and IL-6 immunoassay kit. The quntikine rat TNF-α and IL-6 immunoassay is a 4.5 h solid phase ELISA designed to measure rat TNF-α and IL-6 levels. The assay employs the sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat TNF-α and IL-6 has been pre-coated in the micro-plate. Standards control and samples was pipette out into the wells and any rat TNF-α or IL-6 present was bound by the immobilized antibody. After washing away any unbound substance, an enzyme-linked polyclonal antibody specific for rat TNF-α or IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution was added. The intensity of the color measured was in proportion to the amount of rat TNF-α or IL-6 bound in the initial steps. The sample values were then read off the standard curve.

Statistical analysis

One specific group of rats were assigned to one specific drug treatment condition and each group comprised six rats (n=6). All the values are expressed as mean $\pm$ S.E.M. The data were analyzed by using one way analysis of variance (ANOVA) followed by Tukey's test and two way analysis of variance (ANOVA) followed by Bonferroni's post hoc test.

RESULTS

Effect of ginseng on vacuous chewing movement, tongue protrusions and facial jerking in haloperidol treated rats

Haloperidol (1mg/kg *i.p.*) treatment resulted in time dependent increase in vacuous chewing movements, tongue protrusions and facial jerking. Administration of ginseng low dose (100 mg/kg *p.o.*) and high dose (200 mg/kg *p.o.*) significantly and dose dependently decrease the haloperidol induced VCMs, tongue protrusions and facial jerking [Fig 1,2,3].

Effect of ginseng on body weight in haloperidol treated rats

Body weight significantly decreased in haloperidol (1mg/kg, *i.p.*) treated group as compared to vehicle treated group. There was a dose dependent significant improvement in the body weight on 22nd day in low dose (100 mg/kg *p.o.*) and high dose (200 mg/kg *p.o.*) of ginseng treated groups [Fig 4].

Effect of ginseng on rotarod performance in haloperidol treated rats

Haloperidol (1mg/kg *i.p.*) treated rats significantly decreased the muscle grip strength on day 7th, 14th and 21st as compared to normal group. Ginseng per se had no effect on its own and results were similar to the normal group. Ginseng low dose (100 mg/kg *p.o.*) for 21 days improved the grip strength in haloperidol treated group. Ginseng (100 mg/kg *p.o.*) for 21 days significantly improved the muscle grip strength as compared to haloperidol and ginseng low dose group respectively [Fig 5].

Effect of ginseng on locomotor activity in haloperidol treated rats

Haloperidol treated group significantly reduced locomotor activity and number of rearing in open field as compared to the normal group. Ginseng per se shows results similar to normal group. Ginseng low dose (100 mg/kg *p.o.*) for 21 days improved the locomotor activity as compared to haloperidol treated group. Ginseng high dose (200 mg/kg *p.o.*) significantly improved the number of ambulation and rearing activity as compared to both haloperidol and ginseng low dose group respectively [Fig 6,7].

Effect of ginseng on spatial memory in haloperidol treated rats

Haloperidol treatment resulted in a significant decrease in % retention of memory as compared to control group on day 22nd. Ginseng low dose (100 mg/kg *p.o.*) for 22 days improved the transfer latency as compared to haloperidol treated group. Ginseng high dose (200 mg/kg *p.o.*) for 22 days significantly improved the transfer latency as compared to both haloperidol and ginseng low dose [Fig 8,9,10].

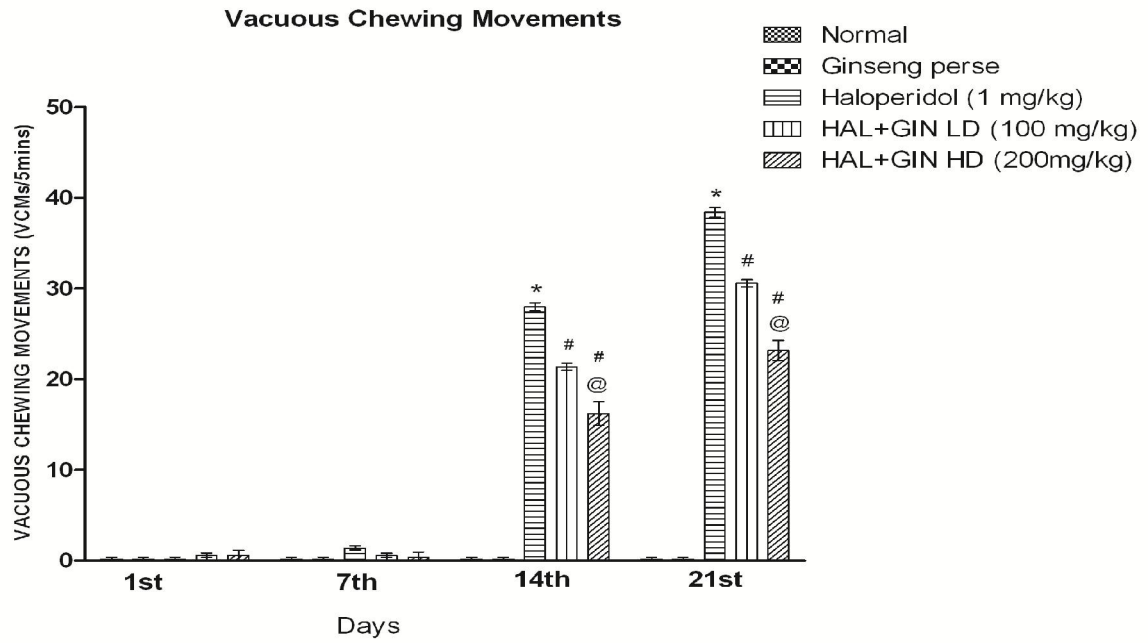

Fig.1. Effect of Ginseng on vacuous chewing movements in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

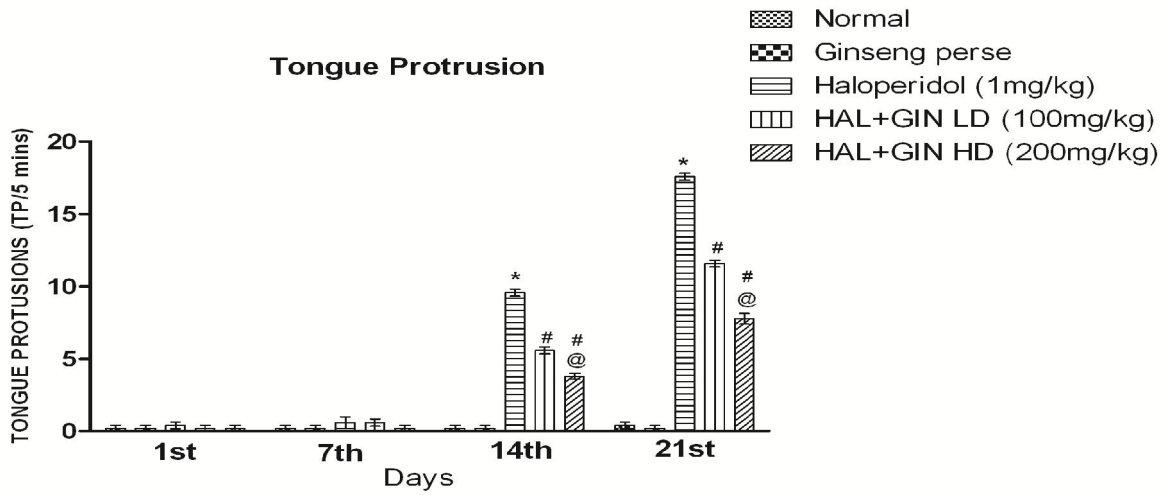

Fig.2. Effect of Ginseng on tongue protrusion in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

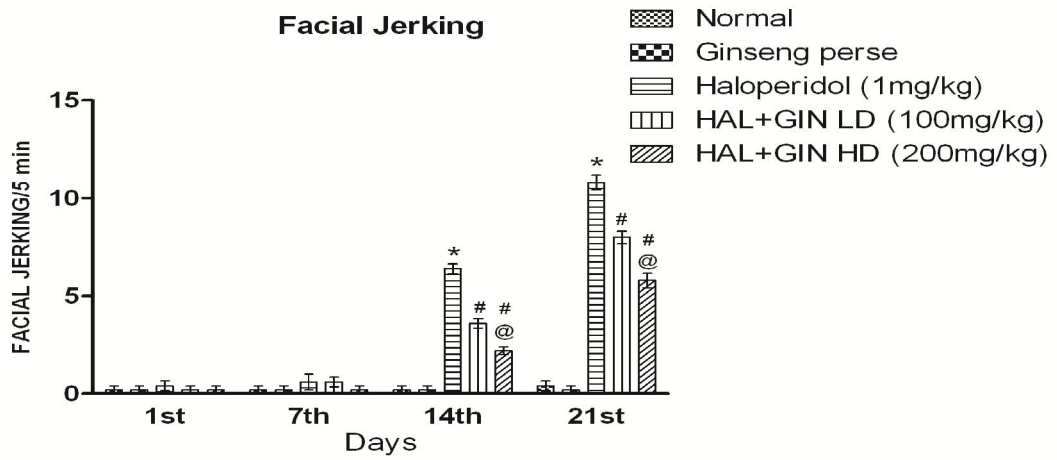

Fig.3 Effect of Ginseng on facial jerking in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN - Ginseng

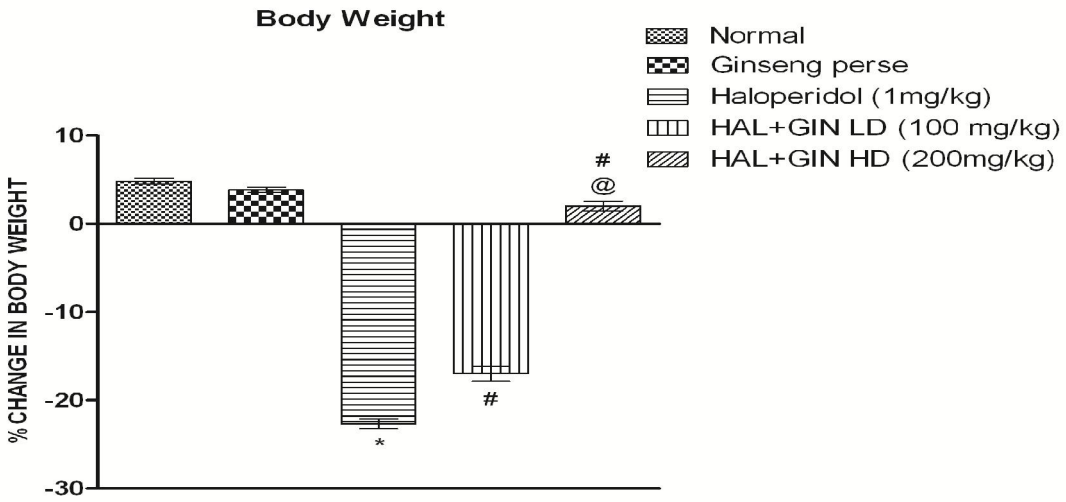

Fig.4. Effect of Ginseng on body weight in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN - Ginseng

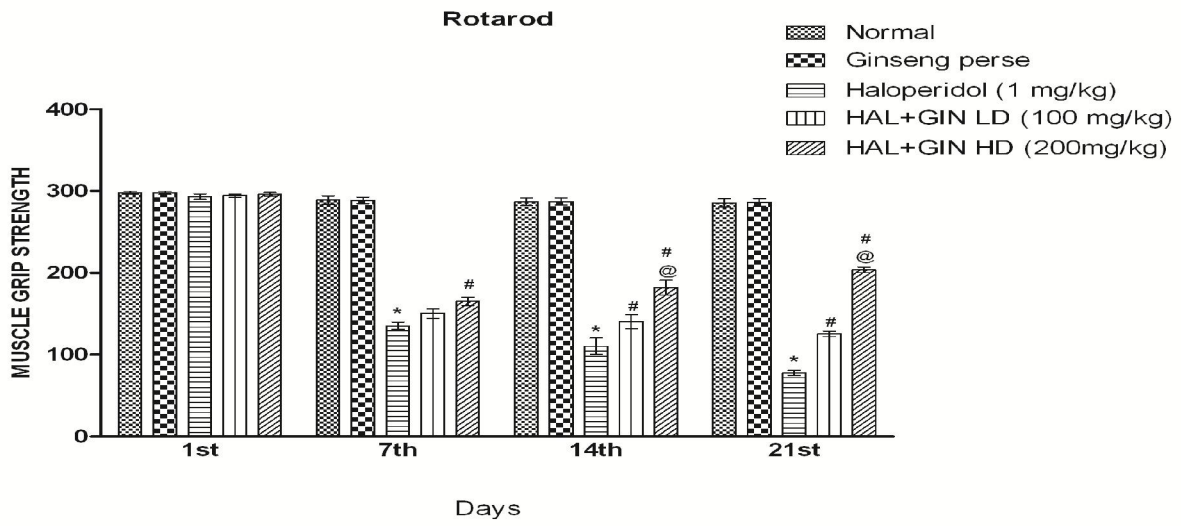

Fig.5. Effect of Ginseng on rotarod activity in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

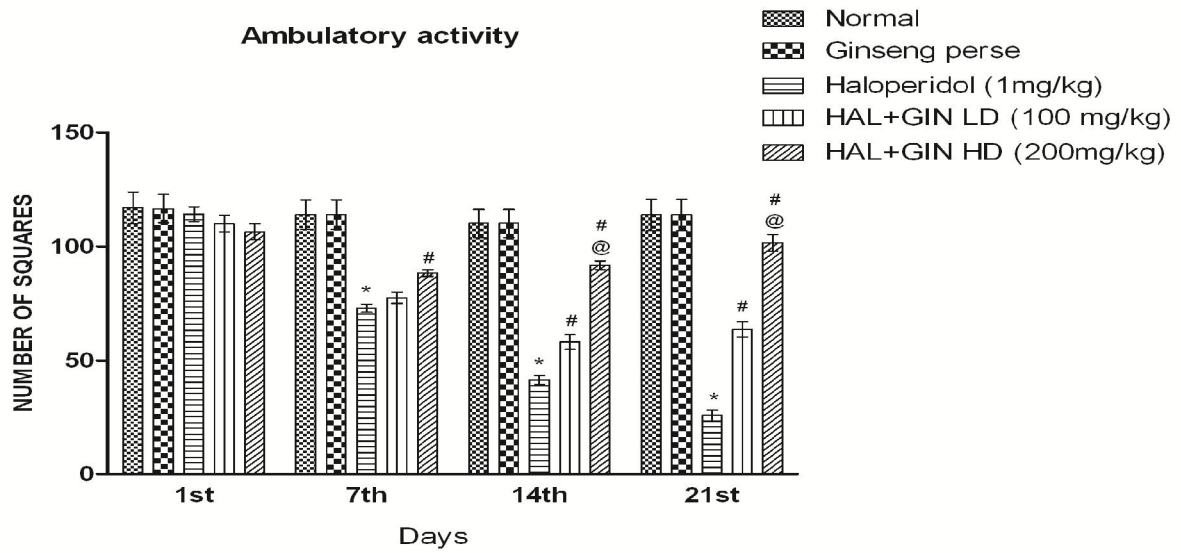

Fig.6. Effect of Ginseng on ambulation activity in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

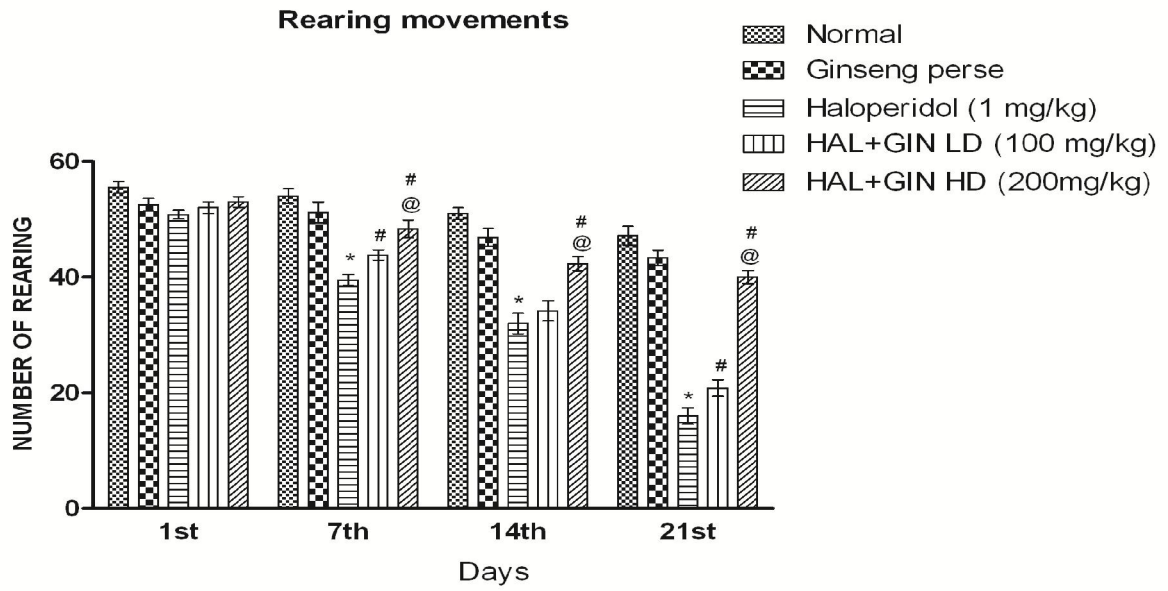

Fig.7. Effect of Ginseng on rearing activity in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). $^{*}p<0.05$ vs. normal; $^{\#}p<0.05$ vs. haloperidol; $^{@}p<0.05$ vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

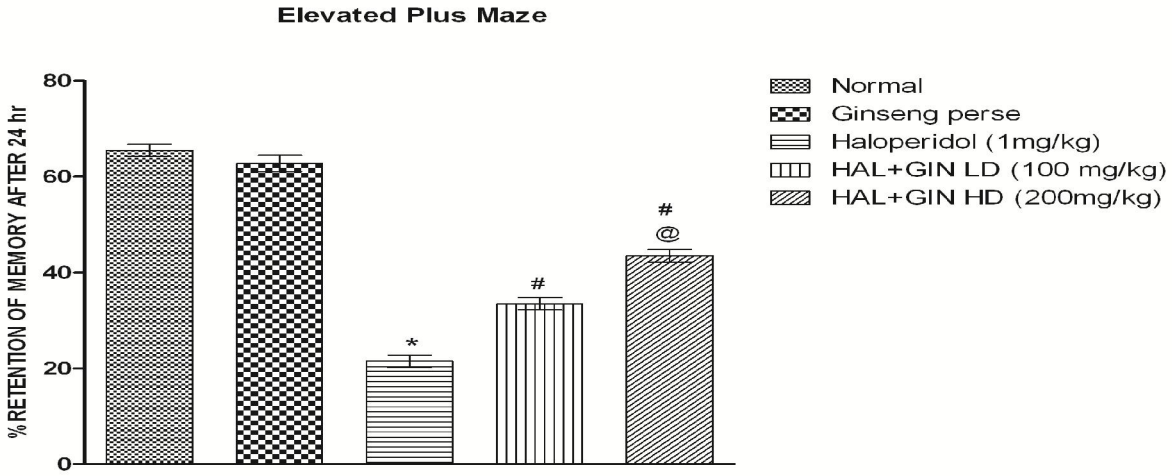

Fig.8. Effect of Ginseng on spatial memory (elevated plus maze) in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). $^{*}p<0.05$ vs. normal; $^{\#}p<0.05$ vs. haloperidol; $^{@}p<0.05$ vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

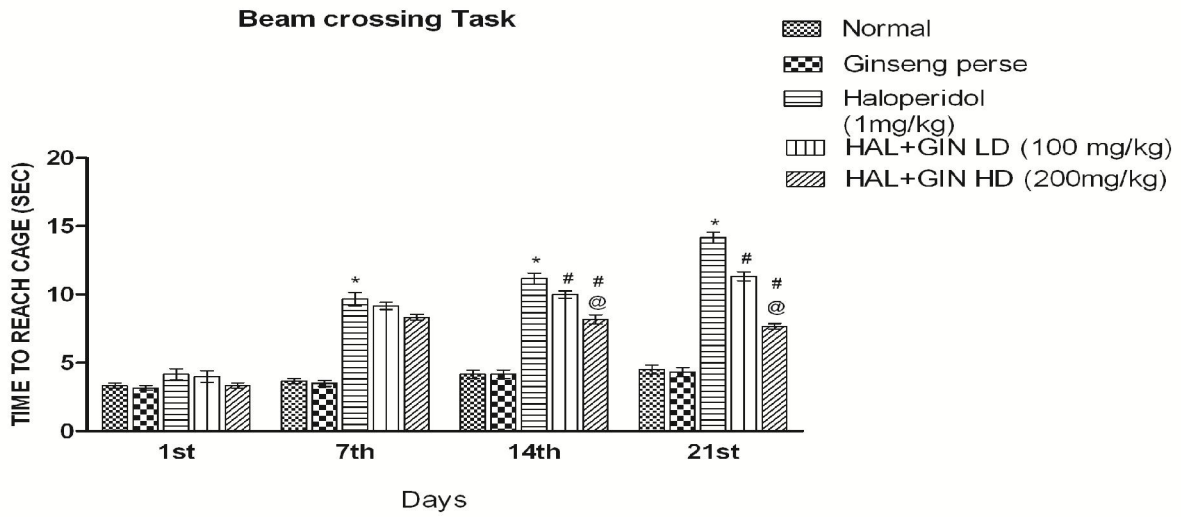

Fig.9. Effect of Ginseng on time taken to reach cage in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

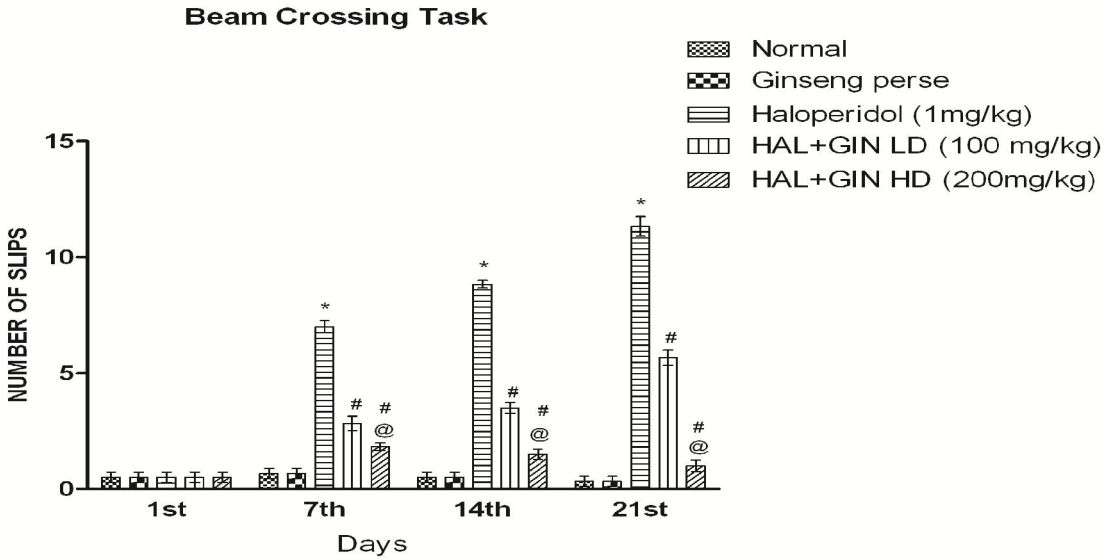

Fig.10. Effect of Ginseng on number of slips in beam crossing task in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

Effect of ginseng on lipid peroxidation level (LPO) in haloperidol treated rats

Haloperidol significantly increased the MDA level in striatum and cortex on 22nd day as compared to the normal group. Ginseng per se group had no effect of its own and result similar

to that of normal group. Ginseng low dose (100 mg/kg *p.o.*) had decreased the MDA level as compared to haloperidol group. Ginseng high dose (200 mg/kg *p.o.*) significantly decreases the MDA level as compared to haloperidol and ginseng high dose respectively [Fig 11].

Effect of ginseng on nitrite level in haloperidol treated rats

Haloperidol significantly increased the nitrite level in striatum and cortex on 22nd day as compared to the normal group. Ginseng per se group had no effect of its own and result similar to that of normal group. Ginseng low dose (100 mg/kg *p.o.*) had decreased the nitrite level as compared to haloperidol group. Ginseng high dose (200 mg/kg *p.o.*) significantly decreases the nitrite level as compared to haloperidol and ginseng high dose respectively [Fig 12].

Effect of ginseng on reduced glutathione (GSH) level in haloperidol treated rats

Haloperidol significantly decreased the GSH level in striatum and cortex on 22nd day as compared to the normal group. Ginseng per se group had no effect of its own and result similar to that of normal group. Ginseng low dose (100 mg/kg *p.o.*) had increased the GSH level as compared to haloperidol group. Ginseng high dose (200 mg/kg *p.o.*) significantly increases the GSH level as compared to haloperidol and ginseng high dose respectively [Fig 13].

Effect of ginseng on catalase activity in haloperidol treated rats

Haloperidol significantly decreased the catalase level in striatum and cortex on 22nd day as compared to the normal group. Ginseng per se group had no effect of its own and result similar to that of normal group. Ginseng low dose (100 mg/kg *p.o.*) had increased the catalase level as compared to haloperidol group. Ginseng high dose (200 mg/kg *p.o.*) significantly increases the catalase level as compared to haloperidol and ginseng low dose respectively [Fig 14].

Effect of ginseng on TNF-α and IL-6 levels in haloperidol treated rats

Haloperidol significantly increased the levels of TNF-α and IL-6 as they were assessed on 22nd day, as compared to normal group. Ginseng per se group had no effect of its own and result similar to that of normal group. Ginseng low dose (100 mg/kg *p.o.*) had decreased the TNF-α and IL-6 levels as compared to haloperidol group. But ginseng high dose (200 mg/kg *p.o.*) significantly decreased the TNF-α and IL-6 levels as compared to haloperidol and ginseng low dose respectively [Fig 15,16].

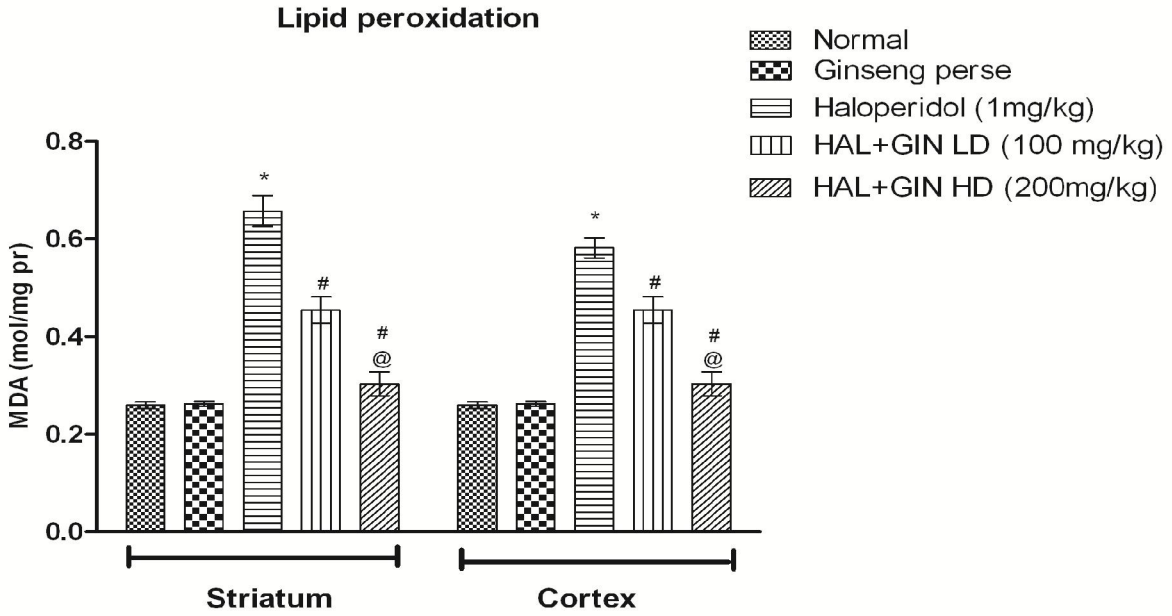

Fig.11. Effect of Ginseng on lipid peroxidation in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

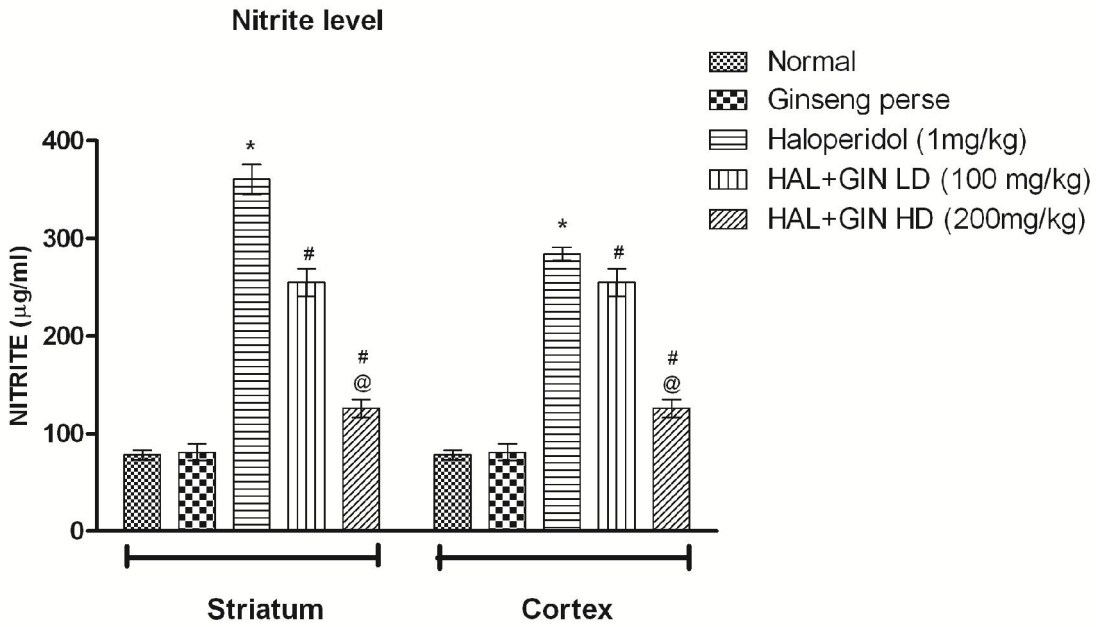

Fig.12. Effect of Ginseng on nitrite levels in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

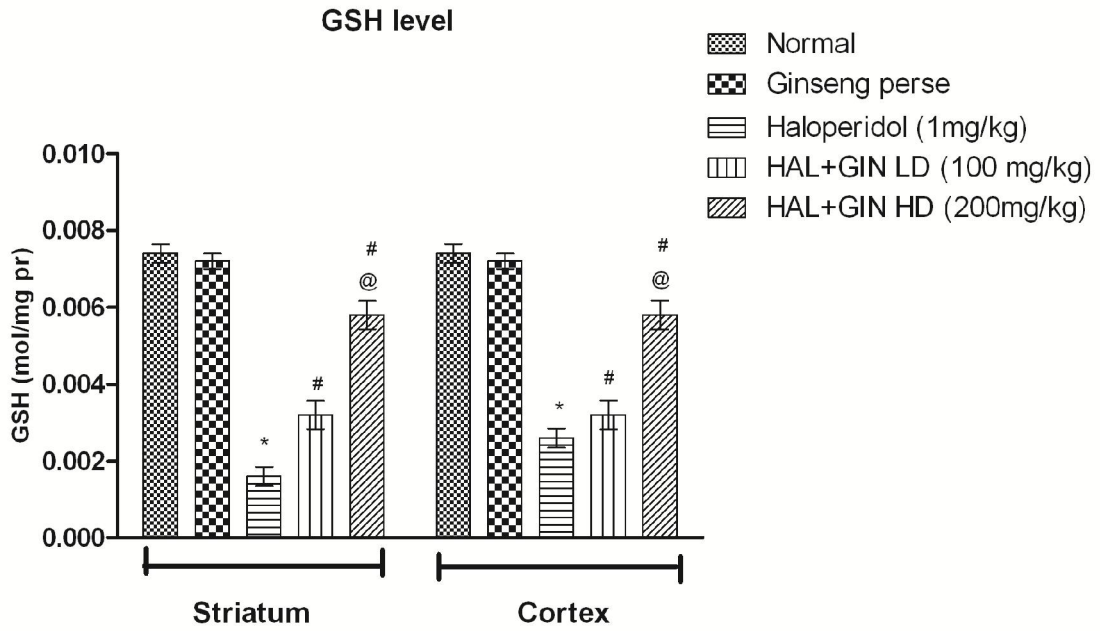

Fig.13. Effect of Ginseng on GSH levels in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

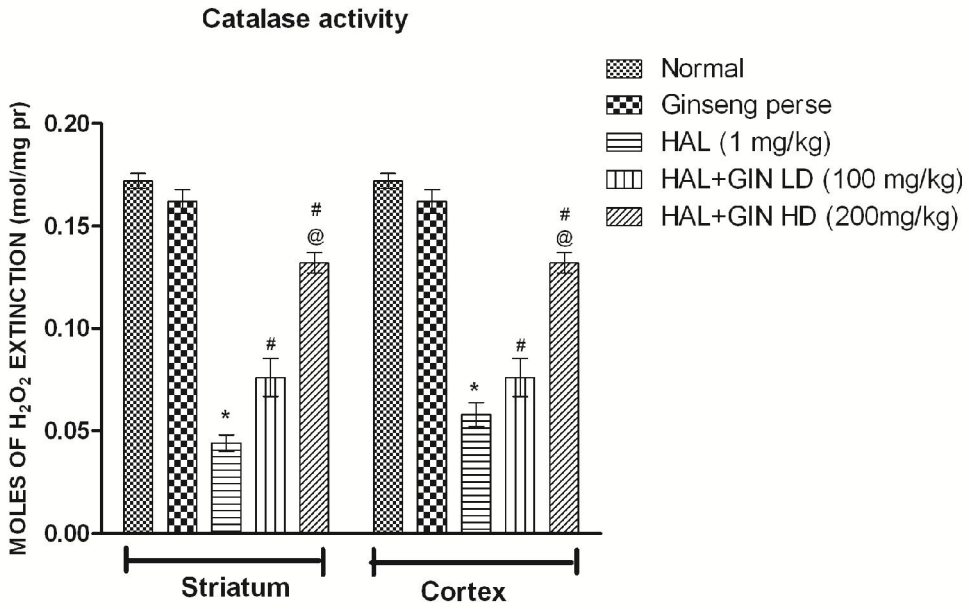

Fig.14. Effect of Ginseng on catalase activity in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL-Haloperidol; GIN – Ginseng

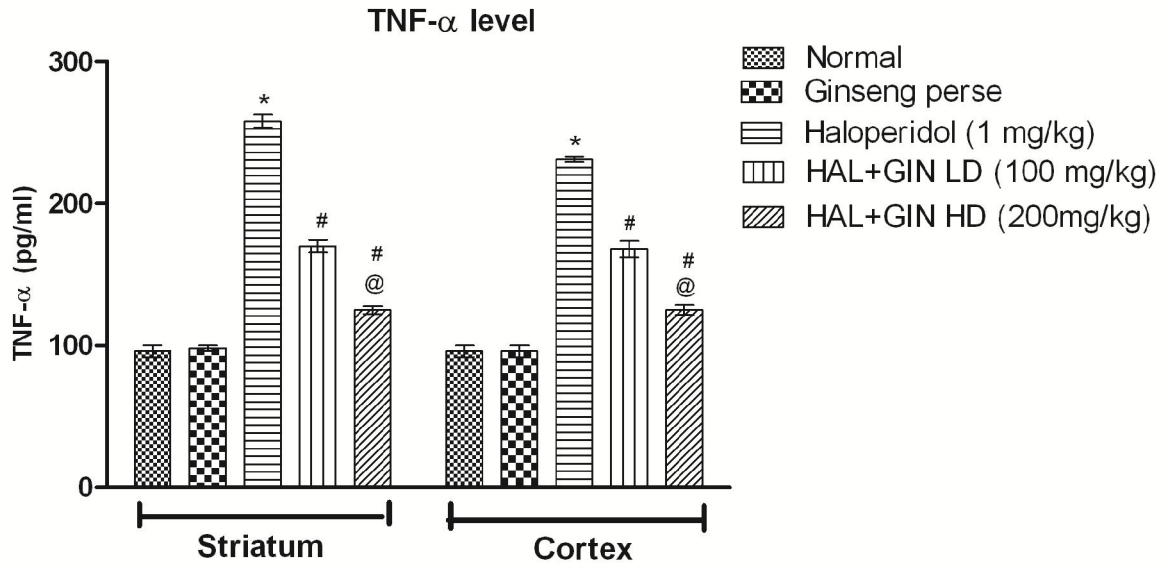

Fig.15. Effect of Ginseng on TNF-α level in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @p<0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

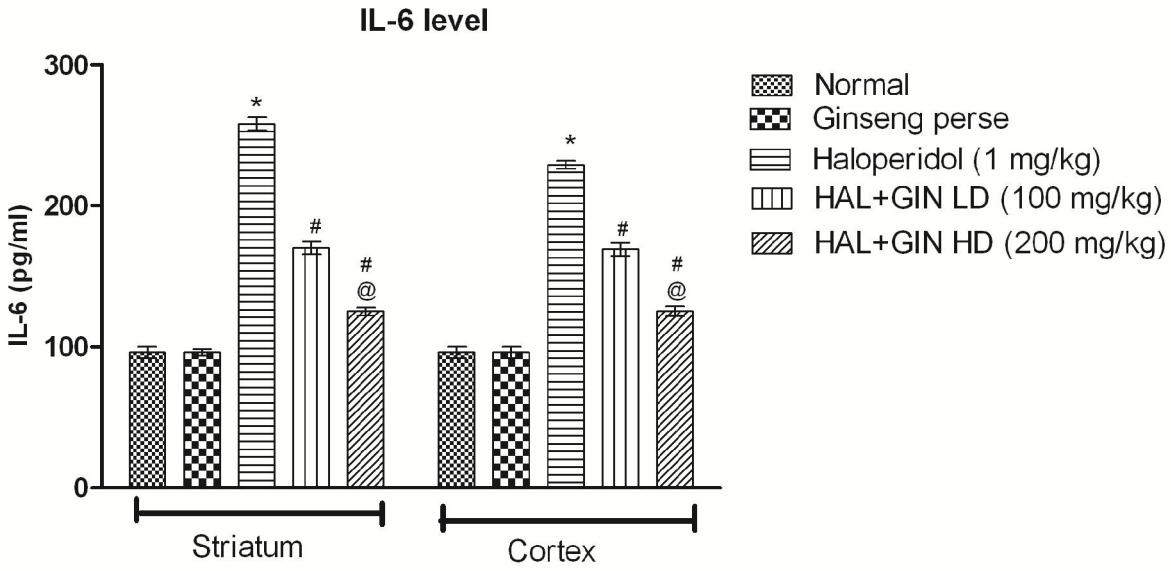

Fig.16. Effect of Ginseng on IL-6 level in haloperidol treated rats. Values are expressed as mean ± S.E.M. (n=6). *p<0.05 vs. normal; #p<0.05 vs. haloperidol; @ p< 0.05 vs. haloperidol+ginseng low dose (100 mg/kg) were considered as statistically significant. HAL- Haloperidol; GIN – Ginseng

DISCUSSION

Tardive dyskinesia is a movement disorder caused by long term use of neuroleptics. However the pathology of TD is not well known till yet. The present study represents that treatment with ginseng could reverse haloperidol induced disturbances in cognition, behavior and biochemical. Haloperidol was administered at 1mg/kg dose for 21 days. Animals produced tardive dyskinesia like symptoms which were determined by vacuous chewing movements, tongue protrusions and facial jerking. The behavior parameters were measured from and biochemical alterations were checked on brain homogenates. Haloperidol also affects the body weight, locomotor activity and muscle grip strength. Haloperidol significantly increased VCMs, tongue protrusions and facial jerking's in rats on 14th day (Fig. 1, 2 & 3). The previous studies also report that haloperidol induce VCMs, tongue protrusions and facial jerking's [30,31]. A significant decrease in body weight was also observed. The decrease in locomotor activity was measured by open field apparatus. There was also a significant decrease in the ambulatory and rearing movements (Fig. 6 & 7) of rats. There was significant decrease in muscle grip strength. Haloperidol also produces cognitive impairment and gait abnormalities as previous studies hypothesized that haloperidol developed learning deficits in animals[32]. Cognitive defects were checked on elevated plus maze (Fig. 8) on last two days. Gait abnormalities were checked on narrow beam walk. In this test number of slips and time taken to reach the cage was noted. Haloperidol significantly increased the time taken to reach the cage and number of slips as compared to normal rats.

It is suggested in previous studies that haloperidol increases the oxidative stress. Due to which VCMs, cognitive impairment and other biochemical alterations occur. So, oxidative stress may be the main culprit for symptoms like TD[33,34]. *Panax ginseng* is the drug used in the present study to attenuate haloperidol induced effects. It was suggested in previous studies that ginseng showed neuroprotective effects. It produced beneficial effects in PD, AD and HD. It blocks the neurodegeneration in these diseases[17]. So, from these studies we have chosen our drug. It was administered at two different doses 100 mg/kg and 200 mg/kg for 21 days. Some studies shows that ginseng do not show any effect on 50 mg dose. So, we preferred 100 mg/kg and 200 mg/kg doses[18].

Treatment with ginseng (100 mg & 200 mg) shows beneficial effects. VCMs, tongue protrusion and facial jerking were significantly decreased by ginseng. It significantly increased the body weight of animals (Fig. 4). Ginseng improved muscle grip strength in animals. It also significantly increased ambulatory and rearing movements. Previous studies reported that ginseng improved the cognitive deficits in traumatic head injury[18]. This is the first report to

check ginseng for its neuroprotective effect against haloperidol induced cognitive deficits, neuroinflammation and biochemical alterations in rats. In our study, ginseng decreased cognitive impairment which was assessed on elevated plus maze. Gait abnormalities also significantly improved as time taken to reach the cage and number of slips (Fig. 9 & 10) were decreased which indicate improvement in motor co-ordination. Ginseng per se does not show any significant change in behavior studies. Therefore, ginseng improved the body weight, cognitive impairment, VCMs, gait abnormalities, locomotor activity and muscle grip strength significantly suggesting its neuroprotective potential by altering behavioral parameters.

Oxidative stress is the main culprit responsible for neurotoxicity. Neuroleptics block dopamine D_2 receptors[35] which results in increased dopamine turnover on basal ganglia and thereby production of free radicals. Haloperidol increased lipid peroxidation in brain and decreased the antioxidant defense mechanism by decreasing reduced glutathione and catalase levels[36,37]. In our biochemical studies, which were performed on striatal and cortex brain homogenates, we checked that there were increased levels of lipid peroxidation and nitrite in haloperidol treated rats. But the levels were high in striatal regions than cortex region suggesting the impact of disease in striatum due to oxidative stress there was decreased reduced glutathione levels and catalase activity. Neuroinflammation is also responsible for neurodegeneration[38]. Some cellular studies showed that marked increase in neuroinflammatory markers like TNF-α and IL-6 were observed in striatal and cortex regions in haloperidol treated rats[39]. In our study haloperidol moreover, increased levels of inflammatory markers like TNF-α and IL-6. Ginseng co-administration with haloperidol showed decreased levels of lipid peroxidation and nitrite. It may be suggested that ginseng decreased the oxidative stress in animals. Moreover the elevated levels of reduced glutathione and catalase activity were also observed (Fig 13 & 14). Literature review suggested that ginseng decreased the proinflammatory cytokines like TNF-α and IL-6 and reduced striatal toxicity in 3-NP treated rats[40]. Our study also supported that ginseng decreased the TNF-α and IL-6 levels (Fig. 15 & 16) in both striatal and cortex regions dose dependently. So, ginseng showed beneficial effects by improving antioxidant & reducing ROS to lower oxidative stress and it decreased TNF-α & IL-6 levels to reduce neuroinflammation.

Ginseng per se group does not show any significant change in behavioral and biochemical studies as compared to normal group.

The above discussion reveals that ginseng improved behavioral parameters, cognitive deficits, reduced oxidative stress and neuroinflammation in haloperidol induced neurodegeneration in rats. So, ginseng has produced neuroprotective effect by attenuating the cognitive impairment,

neuroinflammation and biochemical alteration in rats. Further studies are needed to explore this potential plant for other neurodegenerative disorders.

ACKNOWLEDGMENT

Authors are thankful to Mr. Parveen Garg, Chairman, ISF College of Pharmacy, Moga, and Punjab, India for providing entire facilities for conduction of experimental work.

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