International Journal of Neuroscience and Behavioral Science 5(3): 45-54, 2017 http://www.hrpub.org
DOI: 10.13189/ijnbs.2017.050301

Effects of Ginsomin® on Selected Behaviours in Mice

Onaolapo O J[1], Onaolapo A Y[2,*]

[1]Department of Pharmacology, Ladoke Akintola University of Technology, Nigeria
[2]Department of Anatomy, Ladoke Akintola University of Technology, Nigeria

Abstract Objective: To study the effects of ginsomin® on behaviours of healthy mice using the open-field, Y-maze, eight-arm radial maze and the elevated-plus maze. Method: Groups of mice were administered vehicle (distilled water), scopolamine (2 mg/kg), diazepam (2 mg/kg), or one of two doses of ginsomin (0.5 and 1.0 mg/kg) for 21 days. Behavioural assessments were carried out after the first and final dose of treatment. Result: Repeated administration of ginsomin was associated with a decrease in weight gain, in comparison to vehicle. In the elevated plus maze (EPM), administration of ginsomin resulted in an increase in time spent in the open arms and number of open arm visits in comparison to vehicle, but not diazepam control. A dose-related increase in open-field horizontal locomotion and rearing behaviours; and improvements in Y-maze and radial arm maze spatial working-memory performances were also observed. However, self-grooming behaviours were significantly reduced after repeated administration. Conclusion: Ginsomin administration in healthy mice is associated with significant behavioural changes; hence, our findings support its perception as a ginseng-containing mixture with profound central nervous system effects.

Keywords Ginsomin, Open-field Behaviours, Memory, Anxiety-related Behaviours, Supplements

1. Introduction

Ginsomin® is a nutritional supplement which contains Korean Panax ginseng (KPG) extract enriched with multivitamins and minerals. The vitamins and minerals in ginsomin include the B complex vitamins, protective anti-oxidants like vitamins C and E, natural beta carotene, copper, selenium and zinc. Ginsomin is promoted for its perceived ability to improve general well-being and mental health, and/or help in the management of several diseases [1].

Ginseng is a very popular herbal plant (belonging to the genus *Panax*), widely used in both traditional and contemporary medicine for its varied health benefits. Over the years, ginseng has been reported to be useful for its antineoplastic, antioxidant [2], aphrodisiac [3], adaptogenic [4], weight lowering properties [5]; it is also used in the management of erectile dysfunction [6,7] among many other uses. The medicinal potentials of Panax ginseng has been recognized for centuries, especially amongst the Asians [8, 9]. It is generally recommended for boosting energy levels, protecting the body against stress, and improving physical and mental endurance. The root extract of Panax ginseng contains biologically-active saponins called ginsenosides, which are known to play a major in the physiological and pharmacological activities of ginseng [10, 11].

A number of studies have examined the effects of ginseng extracts on human and animal behaviours; reporting that ginseng can directly modulate behavioural deviations in animal models of autism [7] and modulate cerebral electrical activity by significantly reducing the latency of the P300 component of cortical evoked potential [12]. Certain ginsenosides belonging to the panaxadiol group e.g. Rb_1, and panaxatriol group e.g. Rg_1 are known to play major roles in the stimulation and/or inhibition of the central nervous system and modulation of neurotransmission. In certain studies, ginsenosides in these groups have been documented to improve cognition [13]; although a few studies have reported contrary opinions. For instance, in a study using the Morris water maze test, Lee et al [14] did not find evidence of any pharmacological mechanism through which ginseng therapy could improve cognition. Other ginsenosides like Rg_3 and Rh_2 have also been shown to reduce anxiety levels in rodents [15]. Certain minerals such as zinc [16] have recognized beneficial central nervous system effects; hence, fortifying KPG with multivitamins and minerals is generally assumed to help retain and boost the core central nervous system effects of ginsenosides, ensuring that the effects ascribable to 'pure' ginseng are also derivable from products like ginsomin.

Panax ginseng, the main constituent of ginsomin has been studied extensively [17-21]. In an earlier study [1], we reported the protective potential of ginsomin supplementation in acetaminophen overdose in rat; however, there appears to be a dearth of scientific literature examining the behavioural effects of ginsomin supplement in health;

hence, this study. Considering the increase in the use of ginsomin for its immune and mental-health boosting properties, as well as its perceived ability to increase the body's resistance to stress and fatigue, there is a need to examine the behavioural changes that may be associated with ginsomin use in health. A number of studies have reported the effects of Panax ginseng extracts on learning and memory [22], in Alzheimer's disease [23], ischemic brain injury [24] and brain superoxide dismutase activity [25]. However, in this study, we tested the hypothesis that oral ginsomin supplementation can significantly alter behaviours of healthy mice in the open field, Y-maze, elevated plus maze and radial arm-maze; and compared the effects observed to those of a standard anxiolytic (diazepam) in the elevated plus maze, and a standard amnesiac (scopolamine) in the spatial working memory tests.

2. Materials and Methods.

2.1. Drugs

Ginsomin® (Mega Life Sciences, Australia), Scopolamine (Locin® as Hyoscine N-butylbromide, 10 mg/mL, Greenfield Pharmaceuticals Ltd), Diazepam at 2 mg/kg/day (Valium® 5mg of diazepam/ tablet, Roche Pharmaceuticals, Lagos, Nigeria)

2.2. Animal Care

Male, Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing 18-20 g each were used for this study. Mice were housed in groups of five, in cages located in a temperature-controlled quarters (22-25 degree Celsius) with 12 hours of light daily (lights on at 7.00 a.m). All animals were fed commercial standard chow (Calories: 29% protein, 13% fat, 58% carbohydrate) from weaning. Animals had access to food and water *ad-libitum,* except during the behavioural tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.3. Experimental Method

Eighty mice were randomly assigned into two main groups (group 1 and group 2) of 40 animals each, based on the behavioural tests they were exposed to [(group 1- anxiety and open field tests), (group 2 -Y maze and radial arm maze)]. Animals were then further divided into four groups of ten (n=10) mice each. Animals received vehicle (oral distilled water at 10 ml/kg and i.p injection of distilled water at 2 ml/kg); or one of two oral doses of ginsomin (0.5 and 1.0 mg/kg) and i.p injection of distilled water at 2 ml/kg. A fourth group of animals received i.p. injection of a standard amnesiac (scopolamine at 2 mg/kg) for the memory tests or i.p diazepam (2 mg/kg) for the anxiety tests, plus oral distilled water at 10 ml/kg. Treatments were given daily for 21 days. Doses of ginsomin were calculated by dissolving weighed quantities in distilled water; and given at a volume of 10 ml/kg. Behavioural tests were conducted after the first and last dose of the treatments.

2.3.1. Behavioural Testing

Open field novelty-induced behaviours such as locomotion, rearing and grooming were assessed after the initial and final dose of treatments. Behavioural tests were conducted in a quiet room between 10 a.m. and 3 p.m. On each of the test days, mice were transported in their home-cages to the behavioural testing laboratory, and allowed to acclimatise for 30 minutes before administration of treatments. Behavioural tests were conducted in a room lit by white fluorescent light (130 Lux). One hour after administration of vehicle or ginsomin, and 30 minutes after administration of diazepam or scopolamine, behavioural tests were conducted; timing was based on the knowledge of the differences in the pharmacokinetic characteristics of the drugs. Animals in group 1 were allowed to explore the elevated plus maze first (5 minutes) and then the open-field (for 10 minutes), while animals in group 2 explored the Y-maze first and were then immediately placed in the radial arm maze (5 minutes each). At the beginning of the behavioural tests, each animal was placed in the apparatus and the behaviour videotaped {by a ceiling-mounted digital video camera (SMX-F543B), placed 1.5 meters above the arena} for subsequent analysis. After testing, each mouse was removed from the maze and returned to its home cage, and all interior surfaces cleaned thoroughly with 70 % ethanol, and then wiped dry. At least, 5 minutes was allowed between the testing of individual animals to ensure that the maze was completely dry, and that dispersal of the residual odour of alcohol had occurred. The behavioural parameters were later scored by two independent observers who were blind to the groupings.

2.3.1.1. Anxiety Model: Elevated plus-maze (EPM)

The EPM is validated in mice for assessment of anxiety-related behaviours. It relies upon rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights and open spaces (avoidance). It is plus-shaped, with two open arms measuring 25 x 5 x 0.5 cm lying across from each other and perpendicular to two closed arms measuring 25 x 5 x 16 cm with a central platform (5 x 5 x 0.5 cm). The closed arms are enclosed by 2 high walls (16 cm), while the open arms have no side wall. Each mouse was placed in the central platform, facing a closed arm, and behaviours recorded for 5 minutes. The criterion for arm visit is considered only when the animal decisively moved all its four limbs into an arm [26, 27]. The number of arm entries and the amount of time spent in the open and closed arms are recorded. Anxiety behaviour is measured by

time spent (duration) in the open arm and number of open arm visits; while general motor activity is measured by time spent (duration) in the closed arm and number of closed arm visits [28]. Percentage open arm or closed arm time is calculated by dividing time spent in the open or closed arms by test duration, multiplied by 100.

2.3.1.2. Open Field Novelty-Induced Behaviours

Ten minutes of horizontal locomotion, rearing and grooming were observed in the open-field, and scored. The open field apparatus is a rectangular arena made of white-painted wood, measuring 36 x 36x 26 cm. The floor is made of hard wood and divided by permanent red markings into 16 equal-sized squares. The mice were placed in the centre of the field and covered by a small dome (for 5 seconds) which was removed at the beginning of the 10 minutes countdown. Spontaneous locomotor activity was monitored in the open-field [29, 30]. Thirty minutes after administration of diazepam, and hour after vehicle or ginsomin; each mouse was introduced into the arena and the total horizontal locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind legs either with its fore-arms against the walls of the observation cage, or free in the air) and grooming episodes (number of body-cleaning with paws, picking of the body and pubis with the mouth, and face-washing actions; indicative of a stereotypic behaviour) within the 10 minute period was recorded.

2.3.1.3. Memory (Y and Radial-maze)

The Y-maze and the radial-arm maze are used to measure general activity and spatial working-memory. Spontaneous alternation behaviour (SAB) was used to measure spatial working-memory. SAB measures the tendency for rodents to alternate their (conventionally) non-reinforced choices of Y-maze or radial maze arms on successive opportunities. The Y-maze spontaneous alternation is validated in rodents as a measure of working-memory, general locomotor activity and stereotypic behaviour. Spontaneous alternation was assessed using a Y-maze made of white painted wood with three equally-spaced arms (120°, 41cm long, 15 cm high and 5 cm wide). The floor was also made of hard wood and painted white. Each mouse was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The sequence of arm entries was recorded. An alternation was defined as entry into all three arms consecutively. The number of actual alternations is number of sequential arm entries into the three arms, designated A, B and C. The percentage alternation is calculated as {(Actual alternations/Total arm entry minus two) x 100} within a 5 minute period [28, 31].

Spatial working-memory in the radial-arm maze was measured as sequential arm entries before committing an error. The apparatus is made of white painted wood with eight equidistantly spaced arms, each about 33 cm long, 15 cm high and 5 cm wide, all radiating from a small central circular platform. Working-memory was assessed when the mouse enters each arm a single time. Re-entry into the arms would result in a working-memory error [28].

2.4. Statistical Analysis

Data was analysed using Chris Rorden's ezANOVA for windows, version 0.98. Hypothesis testing was performed using analysis of variance (ANOVA). We tested the hypothesis that a single or repeated oral administration of ginsomin could significantly alter anxiety related behaviours, open-field novelty-induced behaviours and spatial working-memory in healthy mice. Two-factor ANOVA (one between subject, one within subject) was used to test effects of 2 main factors: treatment (four levels: vehicle, ginsomin at 0.5 and 1.0 mg/kg, scopolamine or diazepam) and repeated administration (2 levels: initial and final administration) on behaviours in the elevated plus maze, open-field, Y-maze and radial-arm maze. Tukey's honest significant difference (HSD) test was used for within and between-group comparisons. Results were expressed as mean ± S.E.M and p values less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of Ginsomin on Body Weight

The effect of ginsomin on percentage change in body weight (measured as the difference between final and initial weight divided by initial weight and multiplied by 100) is shown in figure 1. There was significant [$F_{(4, 45)} = 33.3$, $p<0.001$)] decrease in weight with ginsomin at 0.5 and 1.0 mg/kg compared to vehicle.

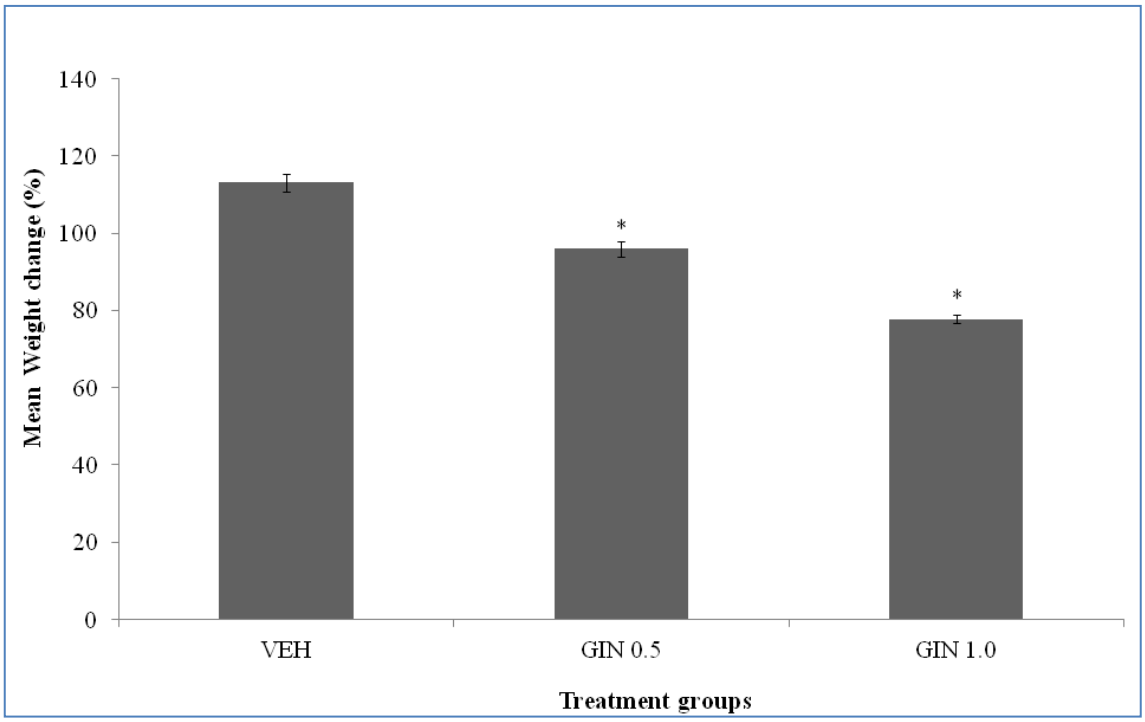

Figure 1. Effect of ginsomin on % weight gain Results presented as mean ± S.E.M, *p<0.05 significantly different from VEH. VEH: Vehicle, GIN: ginsomin, number of mice per treatment group = 10.

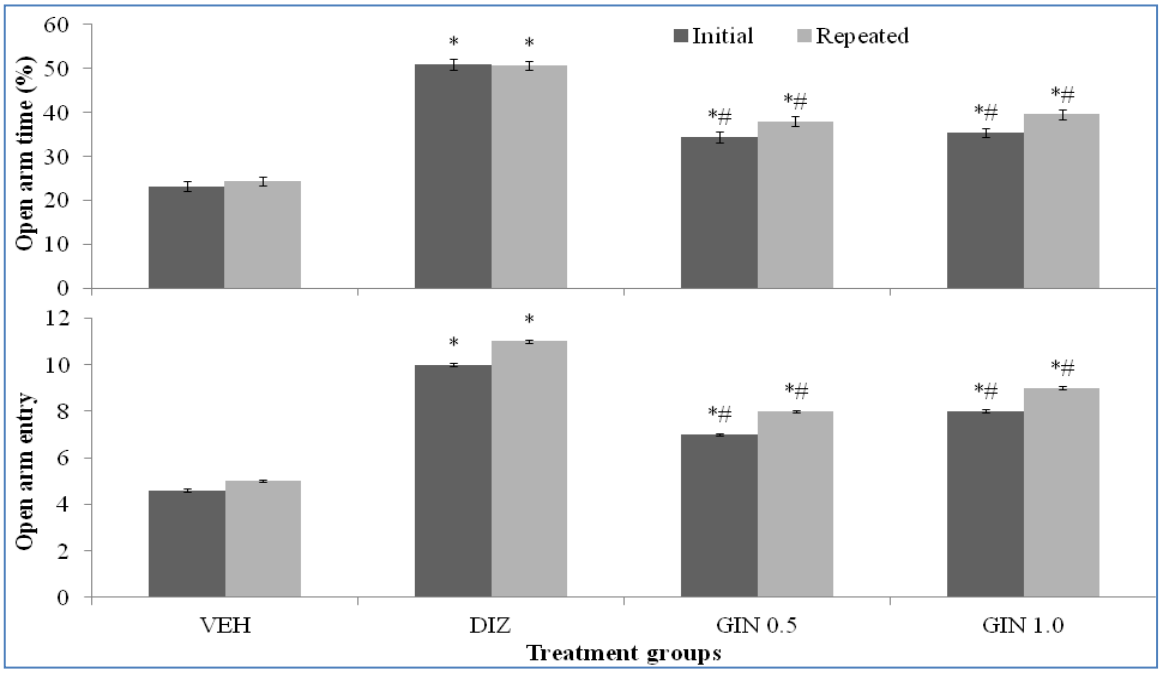

Figure 2. Effect of initial and repeated administration of ginsomin on % time spent in the open arm (upper panel), and number of open arm visits (lower panel) in the elevated plus maze. Values are means ± S.E.M. (*p<0.05 significantly different from VEH, $^{\#}$p<0.05 significantly different from DIZ, VEH: vehicle, DIZ: diazepam, GIN: ginsomin, number of animals per group=10.

3.2. Effect of Ginsomin on Anxiety-Related Behaviours

Anxiety-related behaviour measured as % time spent in the open arm (upper panel) and number of open arm visits (lower panel of for all groups are summarised in figure 2. Two-factor ANOVA revealed a significant effect of treatment {$F_{(3,\ 36)}$ = 10.22, p<0.001}, duration of administration {($F_{(1,\ 36)}$= 7.24, p<0.002)}, and interactions between treatment and duration of administration{($F_{(3,36)}$= 4.12, p<0.032)}. Tukey HSD tests revealed a significant increase in open arm time with diazepam (p<0.001, p<0.001), ginsomin at 0.5 mg/kg (p<0.003, p<0.002) and 1.0 mg/kg (p<0.002, p<0.002) compared to VEH, following initial and

repeated administration respectively. Compared to diazepam, open arm time decreased with GIN 0.5 ($p<0.001$, $p<0.001$) and GIN 1.0 ($p<0.001$, $p<0.001$) with initial and repeated administration.

Two factor ANOVA of the number of open arm visits revealed a significant effect of treatment {$F_{(3, 36)} = 5.19$, $p<0.040$}, no significant effect of duration of administration {($F_{(1, 36)} = 2.10$ $p<0.410$)}, but significant interactions between treatment and duration of administration {($F_{(3, 36)} = 6.20$, $p<0.003$)}. Tukey HSD tests revealed a significant increase in number of open arm entries with DIZ ($p<0.001$, $p<0.001$), GIN 0.5 ($p<0.011$, $p<0.010$) and GIN 1.0 ($p<0.010$, $p<0.010$) compared to VEH with initial and repeated administration. Compared to DIZ, open arm entry decreased with GIN 0.5 ($p<0.005$, $p<0.004$) and GIN 1.0 ($p<0.002$, $p<0.001$) following initial and repeated administration respectively.

3.3. Effect of Ginsomin on Locomotor Activity

Figure 3 shows the effect of ginsomin on horizontal locomotion (upper panel) measured as the number of lines crossed within 10 minutes. Two-factor ANOVA, revealed a significant effect of treatment ($F_{(3, 36)} = 15.01$, $p<0.001$) and a significant effect of repeated administration ($F_{(1, 36)} = 23.1$, $p<0.001$), with significant interactions between treatment x repeated administration ($F_{(3,36)} = 10.2$, $p<0.001$). Tukey HSD analysis revealed a significant increase in horizontal locomotion with GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) compared to vehicle, following initial administration. With repeated administration, horizontal locomotion increased significantly with DIZ ($p<0.001$) GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) compared to vehicle. Compared to DIZ, horizontal locomotion increased with GIN 0.5 ($p<0.003$, $p<0.001$) and GIN1.0 ($p<0.002$, $p<0.001$) following initial and repeated administration respectively. Pairwise comparisons of the effect of repeated administration revealed a significant increase in horizontal locomotion with repeated administration of GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) compared to initial administration.

3.4. Effect of Ginsomin on Rearing Activity

Figure 3 shows the effect of ginsomin on rearing (lower panel) measured as the number of times the mouse stood on its hind legs either with its fore-arms against the walls of the observation cage or free in the air, within a 10 minute period. Two-factor ANOVA revealed a significant effect of treatment ($F_{(3, 36)} = 12.03$, $p<0.001$) and a significant effect of repeated administration ($F_{(1,36)} = 10.3$, $p<0.001$), with significant interactions between treatment x repeated administration ($F_{(3, 36)} = 9.10$, $p<0.001$). Tukey HSD analysis revealed a significant increase in rearing with GIN 0.5 ($p<0.0001$, $p<0.001$) and GIN 1.0 ($p<0.001$, $p<0.001$) compared to vehicle, following initial and repeated administration. Compared to DIZ, rearing increased with GIN 0.5 ($p<0.001$, $p<0001$) and GIN 1.0 ($p<0.002$, $p<0.010$) with initial and repeated administration respectively. Pairwise comparisons of the effect of repeated administration and initial administration revealed a significant increase in rearing with repeated administration of GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) compared to initial administration.

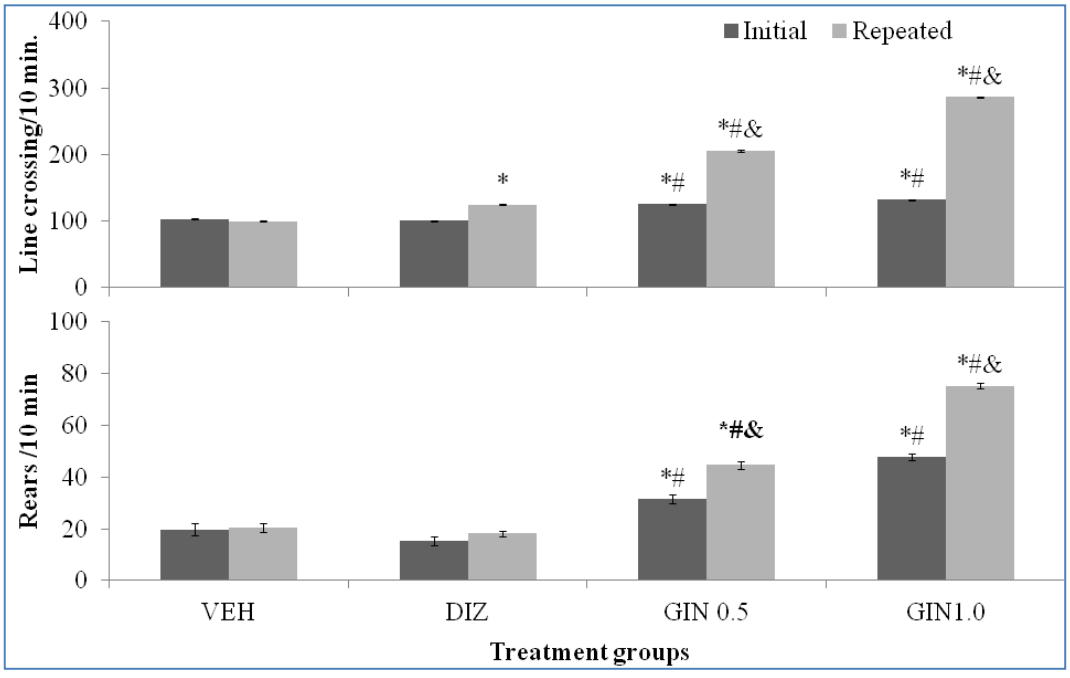

Figure 3. Effect of ginsomin on horizontal locomotion (upper panel) and rearing activity (lower panel). Values are means ± S.E.M. ($^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from DIZ, $^{\&}p < 0.05$ repeated administration significantly different from initial administration). VEH: vehicle, DIZ: diazepam, GIN: ginsomin, number of animals per group=10.

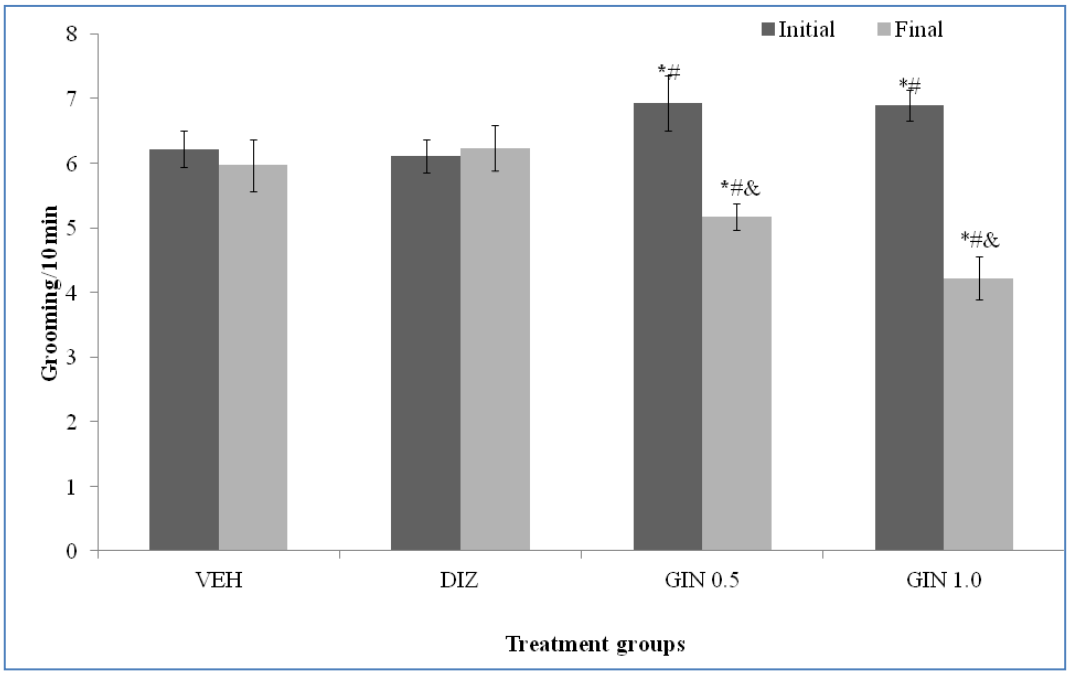

Figure 4. Effect of ginsomin on grooming. Values are means ± S.E.M. ($^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from DIZ, $^{\&}p < 0.05$ repeated administration significantly different from initial administration). VEH: vehicle, DIZ: diazepam, GIN: ginsomin, number of animals per group=10.

3.5. Effect of Ginsomin on Self-grooming

Figure 4 shows the effect of ginsomin on grooming behaviour. Two-factor ANOVA revealed a significant effect of treatment ($F_{(3, 36)} = 14.4$, $p<0.001$) and a significant effect of repeated administration ($F_{(1,36)} = 7.14$ $p<0.003$), and significant interactions between treatment x repeated administration ($F_{(3, 36)} = 7.29$, $p<0.001$). Tukey HSD analysis revealed a significant increase in grooming with GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) compared to vehicle, following initial administration and a significant decrease in grooming with GIN 0.5 ($p<0.001$) and GIN1.0 ($p<0.001$) with repeated administration. Compared to DIZ, grooming increased with GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) following initial administration, and decreased with GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$) following repeated administration. Pairwise comparisons of the effect of repeated administration revealed a significant decrease in grooming with repeated administration of GIN 0.5 ($p<0.001$) and GIN 1.0 ($p<0.001$), compared to initial administration.

3.6. Effect of Ginsomin on Y-maze Working Memory

Figure 5 (upper panel) shows the effects of ginsomin on spatial working-memory in the Y-maze, measured as the percentage alternation in 5 minutes. Two-factor ANOVA revealed a significant effect of treatment ($F_{(3, 36)} = 10.2$, $p<0.005$), significant effect of repeated administration ($F_{(1,36)} = 11.31$, $p<0.001$) and significant interactions between treatment x repeated administration ($F_{(3, 36)} = 9.12$, $p<0.001$). Tukey HSD analysis revealed a significant decrease in spatial memory with SCOP ($p<0.001$, $p<0.001$) and a significant increase with GIN 0.5 ($p<0.001$, $p<0.001$) and GIN 1.0 ($p<0.001$, $p<0.001$) compared to vehicle, following initial and repeated administration. Compared to SCOP, spatial memory increased significantly with GIN 0.5 ($p<0.001$,$p<0.001$) and GIN 1.0 ($p<0.001$,$p<0.001$) following initial and repeated administration. Pairwise comparisons of the effect of repeated administration revealed no significant difference in spatial working-memory with repeated administration of ginsomin compared to initial administration.

3.7. Effect of Ginsomin on Radial-arm Maze Working Memory

Figure 5 (lower panel) shows the effect of ginsomin on spatial working-memory in the radial-arm maze, measured as arm entry before first error in 5 minutes. Two-factor ANOVA revealed a significant effect of treatment ($F_{(3, 36)} = 11.40$, $p<0.001$), repeated administration ($F_{(1, 36)} = 8.20$, $p<0.001$), and significant interactions between treatment x repeated administration ($F_{(3, 36)} = 5.20$, $p<0.005$). Tukey HSD analysis revealed a significant decrease in spatial memory with SCOP ($p<0.001$, $p<0.001$) and a significant increase with GIN 0.5($p<0.001$, $p<0.001$) and GIN1.0 ($p<0.001$, $p<0.001$) compared to VEH, following initial and repeated administration. Compared to SCOP, spatial memory increased significantly with GIN 0.5 ($p<0.001$,$p<0.001$) and GIN 1.0 ($p<0.001$,$p<0.001$) following initial and repeated administration. Pairwise comparisons of the effect of repeated administration revealed no significant difference in spatial working memory with repeated administration of ginsomin compared to initial administration.

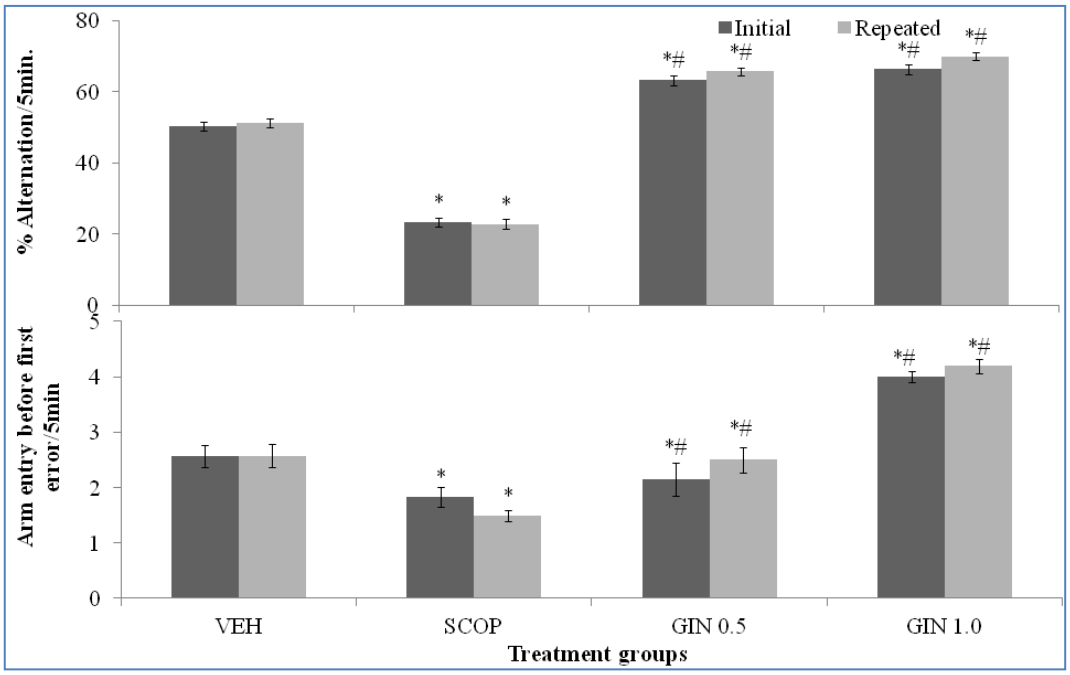

Figure 5. Effect of ginsomin on Y-maze (upper panel) and radial arm maze (lower panel) spatial working-memory. Values are means ± S.E.M. ($^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from DIZ, $^{\&}p < 0.05$ repeated administration significantly different from initial administration). VEH: vehicle, DIZ: diazepam, GIN: ginsomin, number of animals per group=10.

4. Discussion

A number of health benefits relating to the brain are generally ascribable to Panax ginseng-containing products like ginsomin. However, studies examining the behavioural effects of such products in health are uncommon. This study set out to examine the effects of initial dose and repeated administration of ginsomin on behaviours of healthy mice in the EPM, open-field, Y-maze and radial arm maze. From the study, we deduced that administration of ginsomin was associated with the following changes: (1) a significantly reduced weight gain at both doses (2) a decrease in anxiety-related behaviours, compared to vehicle but not diazepam (3) a dose/time-related, increase in open-field behaviours such as horizontal locomotion and rearing (4) a dose/time-dependent initial increase in self-grooming; but a reduction after repeated dosing (5), and a dose-related improvement in working-memory in both models used, compared to vehicle.

In this study, administration of ginsomin at both doses was associated with a significant reduction in weight gain, in comparison to mice administered vehicle. Previous studies have demonstrated the effects ginseng on weight [32-34]. Consumption of ginseng saponins has been shown to inhibit increase in body weight and plasma triacylglycerols following a high-fat diet in mice; via inhibition of lipid metabolism through reduced pancreatic lipase activity [32]. Song et al. [33] also demonstrated a down-regulation of genes associated with lipid-metabolism after feeding mice a high-fat diet containing red ginseng for thirteen weeks. The result from the present study corroborates those of studies on ginseng extract showing that the effects of ginsomin on body weight changes are comparable to that of ginseng extract, which is known to prevent excess weight.

In this study, initial dose and repeated administration of ginsomin was anxiolytic (compared to animals administered vehicle); however, its effects are not comparable to those observed with diazepam. A number of studies have reported that panax ginseng is anxiolytic [15, 35]. Anxiolysis has also been reported with ginsenosides like Rg_3 and Rh_2 which are two of the bioactive compounds found in panax ginseng [15]; although in a few other studies, ginseng was reported to have no effects on anxiety-related behaviours [36]. A significant anxiolytic effect of ginsomin in this study implies the possible preservation of ginseng's anxiolytic effects as documented in other studies, although the presence of minerals like zinc which has also been reported to have anxiolytic effects in rodents [16] may contribute to the anxiolysis observed with ginsomin.

In the open field paradigm, administration of ginsomin was associated with significant increase in both horizontal locomotion and rearing. Initial dose and repeated administration of ginsomin resulted in a dose-related increase in locomotor and rearing activity. Also, the use of a higher dose was associated with a stronger locomotor response (horizontal locomotion and rearing). Mixed locomotor responses have been reported with the use of ginseng; studies have reported a decrease in locomotor activity following chronic oral administration of ginseng [36], or shown that pre-treatment with ginseng total saponins reversed (in a dose-related manner) nicotine-induced behavioural hyperactivity, although it had no influence on resting levels of locomotor activity or extracellular striatal dopamine [37]. Ginseng saponins are believed to counteract

nicotine-induced hyperactivity via inhibition of nicotine-induced dopamine release in the striatum [37]. In another study, pre-treatment and concurrent administration of ginseng extract with rotenone resulted in a reversal of rotenone-induced locomotor impairment in rats [38]. In this study, the summary of the effects (of initial dose and repeated administration) of ginsomin in the open field was central excitation, which affirms ginseng's ability to modulate cortical excitation or inhibition.

Grooming behaviours in rodents is known to be sensitive to factors such as novelty, stress and drugs [39]. Also, dopaminergic (via D1 and D2 receptors), GABAergic [40] and glutamatergic neurotransmission [41] are important in the regulation of grooming behaviours. In this study, repeated administration (but not initial dose) of ginsomin was associated with a significant reduction in the frequency of self-grooming at both doses. Increase in self-grooming is the usual behavioural effect associated with the introduction of rodents to novel environments [42], and this has been attributed to a stress response. Numerous neurotransmitters and/or receptors have been implicated in the grooming response; the activation of D_2 receptors inhibits grooming [43]. Activation of GABA-$_A$ [44] and GABA-$_B$ [45] receptors has been reported to cause a decrease in grooming, which could be the case in this study. A few other studies have also associated the anti-stress properties of ginseng with a reduction in repetitive behaviours such as marble-burying, as observed by Gonzales et al., [7]

Learning and memory responses are modulated by different neurotransmitter systems, and these systems can be altered by effects of drugs or disease, resulting in memory-deficits. In the present study, initial dose and repeated administration of ginsomin improved performances in the working-memory tasks in both the Y maze and radial arm maze. A number of studies [22, 23, 46, 47] have reported memory-enhancing properties of ginseng preparations in subjects with or without cognitive impairment [48], as well as ginseng's ability to ameliorate amyloid plaques [49] in Alzheimer's disease. The memory-enhancing properties have been largely attributed to the presence of ginsenosides, although other components like polysaccharides, peptides and polyacetylenes have also been suggested [50]. In the ginsomin preparation of panax ginseng, the presence of multivitamins and minerals may also add to the memory-enhancing effects observed.

5. Conclusion

This study shows that ginsomin administration in healthy mice is associated with behavioural changes that are similar to those attributable to ginseng extract.

Conflict of Interest

Both authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

REFERENCES

[1] A.Y. Onaolapo, O.J. Onaolapo, S. Bamidbola, O.O. Adegoke. Hepatotoxicity due to acetaminophen overdose in mice: Assessing the protective potentials of ginsomin® supplementation. App. Sci. Report, 15(1) 4–11, 2016.

[2] Y-S. Keum, K-K. Park, J-M. Lee, K-S. Chun, J.H. Park, SK Lee, et al. Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett 150: 41–8, 2000.

[3] E. Nocerino, M. Amato, A.A Izzo. The aphrodisiac and adaptogenic properties of ginseng. Fitoterapia 71: S1–5, 2000.

[4] K.E. Ahmed, W.Q. Chen, J.P John, S.U Kang, G. Lubec, Complete sequencing of the recombinant granulocyte-colony stimulating factor (filgrastim) and detection of Anti-Stress and Ginseng biotinylation by mass spectrometry. Amino Acids, 38, 1043- 1049, 2010

[5] J.H. Kim, D.H. Hahm, D.C. Yang, J.H. Kim, H.J. Lee, I. Shim. Effect of crude saponin of Korean Red Ginseng on high fat diet-induced obesity in the rat. J Pharmacol Sci 97: 124–31, 2005.

[6] D-J Jang, MS Lee, B-C Shin, Y-C Lee, E. Ernst Red ginseng for treating erectile dysfunction: a systematic review. Br J Clin Pharmacol 66: 444–50, 2008.

[7] E.L.T Gonzales, J-h Jang, D.F.N. Mabunga, J-w. Kim, M.J Ko, K.S Cho, G.H. Bahn, M. Hong, J.H. Ryu, H.J. Kim, J.H. Cheong, C.Y. Shin, Supplementation of Korean Red Ginseng improves behavior deviations in animal models of autism. Food and Nutr Res, 60: 29245, 2016.

[8] T.K Yun Brief introduction of Panax ginseng C.A. Meyer," J Korean Med Sci 16: S3- 5, 2001.

[9] J.D. Park, D.K. Rhee, Y.H. Lee. "Biological activities and chemistry of saponins from Panax ginseng C.A. Meyer," Phytochem Rev 4(2-3):159-75, 2005.

[10] P.Y.K. Yue, N.K. Mak, Y.K.Cheng, K.W. Leung, T.B. Ng, D.T.P. Fan, et al. Pharmacogenomics and the Yin/Yan actions of ginseng: anti-tumour, angiomodulating and steroid-like activities of ginsenosides. Chinese Med 2:6, 2007.

[11] H.M. Park, H.K. Go, A.R. Mun. The Korean Society of Ginseng, Jeju, Korea, p. 93. 2012.

[12] D.O. Kennedy, A.B. Scholey, L. Drewery, V.R. Marsh, B. Moore, H. Ashton Electroencephalograph effects of single doses of Ginkgo biloba and Panax ginseng in healthy young volunteers. Pharmacol Biochem Behav. 75:701–709, 2003. doi: 10.1016/S0091-3057(03)00120-5.

[13] J.H. Heo, M.H Kim The efficacy of ginseng on the cognitive function. J Ginseng Res. 3:161–164, 2009. doi: 10.5142/JGR.2009.33.3.161

[14] M.R. Lee, B.S. Sun, L.J. Gu, C.Y. Wang, E.K. CY, S.A. Yang, S.Y. Lee, C.G. Sung Effects of white ginseng and red ginseng extract on learning performance and acetylcholinesterase activity inhibition. J Ginseng Res. 32:341–346, 2008. doi:

10.5142/JGR.2008.32.4.341

[15] T.W. Kim, H.J. Choi, N.J Kim, D.H Kim Anxiolytic-like effects of ginsenosides Rg_3 and Rh_2 from red ginseng in the elevated plus-maze model. Planta Med. 75:836–839, 2009. doi: 10.1055/s-0029-1185402

[16] O.J. Onaolapo, T. Ayanwale, O. Agoi, C. Adetimehin, A.Y. Onaolapo Zinc tempers haloperidol-induced behavioural changes in healthy mice. International J Neurosci Behav Sci. 4:21-31 2016.

[17] G.Z. Zhang, A.L. Liu, Y.B. Zhou, X. San, T.W. Jin, Y. Jin *Panax Ginseng* ginsenoside-Rg (2) protects memory impairment via anti-apoptosis in a rat model with vascular dementia. J Ethnopharmacol, 115: 441-448, 2008.

[18] M.J. Lee, E.H. Kim, D.K. Rhee. Effects of *Panax ginseng* on stress. J. Ginseng Res. 32:8-14, 2008.

[19] T. Ramesh, S.W. Kim, J.H. Sung, S.Y Hwang, S.H Sohn, S.K Yoo, et al. Effect of fermented *Panax ginseng* extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats. Exper Gerontol, 47:77-84, 2012.

[20] A. Karadeniz, A.Yildirim, A. Karakoc., Y. Kalkan, F. Celeb. Protective effect of *Panax ginseng* on carbon tetrachloride induced liver, heart and kidney injury in rats. Revue Méd Vét 16 (5):237-43, 2009.

[21] B. Mohamed, H Ezar, T. Ehab, M Ahmed, E. Samar, E. Shimaa. Beneficial role of Panax ginseng root aqueous extract against Cisplatin induced blood toxicity in rats. American J. Biol. Chem. 3(1): 1-7, 2015.

[22] S-H. Jin, J-K. Park, K-YNam, S-N.Park, N-P. Jung. Korean red ginseng saponins with low ratios of protopanaxadiol and protopanaxatriol saponin improve scopolamine-induced learning disability and spatial working memory in mice. J Ethnopharmacol 66: 123–9, 1999.

[23] H.J. Kim, P. Kim, C.Y. Shin. A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system. J Ginseng Res 37: 8, 2013.

[24] J.S. Lee, H.S. Choi, S.W. Kang, J-H, Chung, H.K. Park, J.Y. Ban, et al. Therapeutic effect of Korean red ginseng on inflammatory cytokines in rats with focal cerebral ischemia/reperfusion injury. American J Chin. Med. 39: 83–94, 2011.

[25] C.S Kim, J.B. Park, K-J Kim, S.J Chang, S-W. Ryoo, B.H. Jeon. Effect of Korea red ginseng on cerebral blood flow and superoxide production. Acta Pharmacol Sin 23: 1152–6, 2002.

[26] O.J. Onaolapo, A.Y. Onaolapo, M.A. Akanmu, G. Olayiwola. Changes in spontaneous working-memory, memory-recall and approach-avoidance following "low dose" monosodium glutamate in mice. AIMS. Neurosci. 3:317-337, 2016.

[27] A.Y. Onaolapo, O.J., Onaolapo, P.U. Nwoha Aspartame and the hippocampus: Revealing a bi-directional, dose/time-dependent behavioural and morphological shift in mice. Neurobiol Learn Memory 139:76–88, 2017.

[28] O.J. Onaolapo, A.Y. Onaolapo, O.R. Akinola, T.O. Anisulowo. Dexamethasone regimens alter spatial memory and anxiety levels in mice. J Behav Brain Sc. 4:159-167, 2014.

[29] O.J Onaolapo, A.Y. Onaolapo, M.A. Akanmu, G. Olayiwola. Foraging enrichment modulates open field response to monosodium glutamate in mice. Annals of Neuroscience 22: 162-170, 2014.

[30] A.Y. Onaolapo, O.J Onaolapo, P.U Nwoha. Alterations in behaviour, cerebral cortical morphology and cerebral oxidative stress markers following aspartame ingestion. J. Chem.Neuroanat. 78: 42–56, 2016.

[31] O.J. Onaolapo, A.Y. Onaolapo, E.O. Awe, N. Jibunor, B. Oyeleke. Oral artesunate-amodiaquine combination causes anxiolysis and impaired cognition in healthy Swiss mice. IOSR Journal of Pharmacy and Biological Sciences **7**: 97-102, 2013. http://dx.doi.org/10.9790/3008-07297102.

[32] N. Karu, R. Reifen, Z. Kerem Weight gain reduction in mice fed Panax ginseng saponin, a pancreatic lipase inhibitor. J Agric Food Chem. 18; 55(8):2824-8. 2007.

[33] Y.B. Song, Y.R. An, S.J. Kim, H.W. Park, J.W. Jung, J.S. Kyung, S.Y. Hwang, Y.S. Kim Lipid metabolic effect of Korean red ginseng extract in mice fed on a high-fat diet. J. Sci. Food Agric. 30:388–396, 2012.

[34] H. Lee, J. Choi, S.S. Shin, M. Yoon Effects of Korean red ginseng (Panax ginseng) on obesity and adipose inflammation in ovariectomized mice. J Ethnopharmacol. 3; 178: 229-37, 2016. doi: 10.1016/j.jep.2015.12.017

[35] S.K Bhattacharya, S.K Mitra Anxiolytic activity of Panax ginseng roots: an experimental study. J Ethnopharmacol. 34(1):87-92, 1991.

[36] H. Einat Chronic oral administration of ginseng extract results in behavioral change but has no effects in mice models of affective and anxiety disorders. 21, 62–66, 2007.

[37] S.E Kim, I. Shim, J-k Chung, M.C. Lee. Effect of ginseng saponins on enhanced dopaminergic transmission and locomotor hyperactivity induced by nicotine. Neuropsychopharmacol. 31, 1714–1721. 2006. doi:10.1038/sj.npp.1300945

[38] P. Euaruksakul, R. Tansawat, R. Rodsiri. Ginseng extract G115 improves locomotor function in rotenone-induced parkinsonism rats via an antioxidant effect Songklanakarin J. Sci. Technol. 37 (2), 163-169, 2015.

[39] A.Y. Onaolapo, A.N Adebayo, O.J. Onaolapo. Exogenous daytime melatonin modulates response of adolescent mice in a repeated unpredictable stress paradigm Naunyn-Schmiedeberg's Arch. Pharmacol. 390:149–161, 2017.

[40] A.A. Kalueff, A.M. Stewart, C. Song, K.C. Berridge, A.M Graybiel, J.C. Fentress Neurobiology of rodent self-grooming and its value for translational neuroscience Nature Rev. Neurosc.17:45–59, 2016.

[41] W. Hong, D-w. Kim, D.J. Anderson Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. Cell, 158:1348–1361, 2014.

[42] A.J Stoessl. Dopamine D1 receptor agonist induced grooming is blocked by the opioid receptor antagonist naloxone. European Journal Pharmacology 259: 301-303, 1996.

[43] B.S Starr, M.S Starr. Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Implications for the roles of D1 and D2 receptors. Neuropharmacology 25: 455-463, 1986

[44] M.S Nin, N.S Couto-Pereira, M.F Souza, L.A Azeredo, M.K Ferri, W. L Dalprá, et al. Anxiolytic effect of clonazepam in female rats: Grooming microstructure and elevated plus maze tests. European Journal of Pharmacology 684: 95–101,2012

[45] J.L Silverman, M.C Pride, J.E Hayes, K.R Puhger, H.M Butler-Struben, S. Baker et al. $GABA_B$ receptor agonist r-baclofen reverses social deficits and reduces repetitive behaviour in two mouse models of autism. Neuropsychopharmacology 40: 2228–2239, 2015.

[46] H-B. Yeo, H-K. Yoon, H-J. Lee, S-G Kang, K-Y. Jung, L Kim. Effects of Korean Red Ginseng on Cognitive and Motor Function: A Double-blind, Randomized, Placebo-controlled TrialJ Ginseng Res. 36(2): 190–197, 2012.

[47] Y. Lee, S. Oh. Administration of red ginseng ameliorates memory decline in aged mice. J Ginseng Res. 39(3), 250–256, 2015

[48] H. Zhao, Q. Li, Z, Zhang, X. Pei, J. Wang, Y. Li. Long-term ginsenoside consumption prevents memory loss in aged SAMP8 mice by decreasing oxidative stress and up-regulating the plasticity-related proteins in hippocampus. Brain Res. 1256:111–122, 2008.

[49] C. Tohda, N. Matsumoto, K. Zou, M.R. Meselhy, K. Komatsu, Abeta(25-35)-induced memory impairment, axonal atrophy, and synaptic loss are ameliorated by M1, A metabolite of protopanaxadiol-type saponins. Neuropsychopharmacol. 29:860–868, 2004

[50] A.S. Attele, J.A Wu, C.S. Yuan. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol. 58:1685–1693, 1999.