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COMPARATIVE EFFECT OF GINSENG ROOT AND VITAMIN E ON LEARNING AND MEMORY BEHAVIOUR IN BIOLOGICALLY AND CHEMICALLY STRESSED OUT CDI MICE EXPERIMENTAL MODEL

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ABSTRACT

The aim of this study was to compare the effects of a herbal remedy Ginseng and vitamin E which has known antioxidant activity and neuroprotective benefits on learning and memory functions in an experimental model of CD1 Swiss mice exposed to biological and chemical stress induction; So as to substantiate or grade the efficacy of ginseng. This is against the background of the enormity of physical as well as socioeconomic burden of neurodegenerative and mental health related challenges. A total of seventy – two pups of post natal day 2 (PND2) obtained from bred timed-pregnant C57BL/6 dams were used for this experiment. They were placed in four sets of three groups, such that each group contained 6 pups with an averagely equal distribution of male and female. For Set 1, group 1 had normal condition as control, group 2 was exposed to chronic unpredictable stress (CUS) including combination of social deprivation, food starvation, hot dry air condition, bright light at night once daily (as biologically stressed mice), and group 3 was exposed to stress by treatment with neurotoxin 3-NP (as chemically stressed mice). For Set 2 (non-stressed set), group 1 had normal condition, group 2 was treated with 0.5 mg/kg Vitamin E orally and group 3 was orally administered 1mg/kg Ginseng. For Set 3(biologically stressed set), group 1 was only exposed to CUS, group 2 in addition to the CUS exposure was treated with 0.5 mg/kg Vitamin E while group 3 was given 1mg/kg Ginseng orally in addition to CUS exposure. Set 4 was designated as chemically stressed set of mice. Its groups 1, 2 and 3 were treated intraperitonealy with 15mg/kg dose of 3-NP daily for 4 days, and in addition group 2 was treated with 0.5mg/kg Vitamin E while group 3 was treated with 1mg/kg Ginseng both orally. The experimental study lasted from PND2 to PND 24 when mice were taken for neurobehavioural study involving T-maze test and Morris water maze, a standard protocol to investigate learning and memory behaviour. The results observed suggest that treatment with ginseng and vitamin E improved visuospatial memory. However, for comparative efficacy, in the biological stress model, vitamin E appears more effective than ginseng, as it showed a higher degree of impact for reversal of memory impairment that was significant at $P<0.05$, whereas this similar difference observed for the chemical stress model was not significant, probably due to the greater extent of damage by the 3-NP neurotoxin on cognition or a mechanism yet to be known, which could be a suggested further study.

KEYWORDS: Ginseng, Vitamin E, biological Stress, chemical stress, neurodegeneration.

INTRODUCTION

There has been a globally increasing concern for disabilities related to neurodevelopment in humans, with their attendant socioeconomic burden which affects the patients, relatives and society at large (Landrigan 2012). Some of these challenges include autism spectrum disorders, mental retardation, schizophrenia, attention-deficit hyperactivity disorder and Alzheimer's disease, which is age related (Landrigan 2012).

It is also known that many or virtually all of these challenges could interfere with cognitive functions in individuals and generally affect the process of learning and memory. The acquiring of information and its storage, especially when it is due to experience can be described as learning, whereas memory refers to phenomena that encompass the process of a more permanent storage of information that was learned (Sandi, 2013).

These neurodevelopmental challenges are even worsened by certain physiological processes such as apoptosis that characterize multicellular organisms and could be linked with neurodegeneration, autoimmune disease and cancer; although it may be beneficial for maintenance of the organism's homeostasis processes, usually under streamlined regulation (Radi et al 2014). Meanwhile, some of these conditions may progress into worst stage, e.g. Alzheimer's disease which can degenerate to dementia. Learning and memory processes are delicate and significant neurological phenomena that could be interfered with during stress (Radi *et al*, 2014).

Stress has been reported to elicit changes in the brain's structural composition, with varying behavioural outcomes and responses from person to person (Lupien *et al*, 2009). It could result in loss of brain weight and atrophy, leading to interference with the ability to respond to stress, thus further reducing cognition and memory processes; especially because it releases glucocorticosteroids with ability to cross the blood-brain barrier (Schlegel *et al*, 2014). Stress may come in different forms such as chemical, biological or traumatic, and can cause dysfunction in memory and judgment by affecting areas in the hippocampus; although with variations between individuals mild stress could improve cognitive process, while beyond a threshold it tends to cause neurodevelopmental disorders (Sandi 2013).

Two examples of experimental model of stress induction are the biological method; wherein rodents are exposed to deprivations, such as isolation for an hour, food starvation, blowing of hot dry air, putting on bright light at night, with all of these done once daily for at least 28 days (four weeks) in an unpredictable sequence (Monteiro *et al*, 2015): and a chemical method in which the substance 3-nitropropionic acid that is a known neurotoxin, is administered to rodents. It exhibits deleterious effect on brain cells' mitochondrial functions, associated with release of reactive oxygen species and ultimately causing dysfunction in cognitive process (Binat *et al*, 2005).

However, the body also has certain ways through which it combats stress. One particular strategy is that which relies on mechanisms of antioxidants such as Catalase, super oxide dismutase, glutathione and glutathione peroxidase (i.e. CAT, SOD, GSH and GSHpx respectively); such that free radicals or reactive oxygen species that cause oxidative damage to cells and tissues can be neutralized (Koracevic *et al*, 2001). Considering the enormous challenges posed by neurodevelopmental disorders, it is worthwhile to continue exploring possible ways of alleviating neurodegenerative conditions that may arise either due to the effects of stress which is capable of interfering with cognitive processes, and progressing into more severe neurodegenerative disorders (Radi ***et al***).

In this regard, several herbal remedies have been experimentally demonstrated in some of our collaborative studies to have beneficial health potential, particularly those with active antioxidant properties (Erigbali *et al*, 2021; Sule *et al*, 2017a; Sule *et al*, 2017b). Ginseng is one medicinal plant that has been reportedly used to treat diabetes, cardiovascular disease, cancer, and has been ascribed for autoimmune booster, stress relief and antioxidant activities (Christensen, 2009; Jung and Jin, 1996). Some researchers reported that ginseng and its active ingredient (ginsenosides) are beneficial to cognitive functions (Ong *et al*, 2015; Qi *et al*, 2010).

But in this current study, a comparative analysis of the effects of the herb Ginseng and vitamin E (which has known antioxidant activity) on learning and memory functions was carried out in an experimental model of biological and chemical stress- induced CDI mice.

METHODOLOGY

A total of seventy – two pups of post natal day 2 (PND2) were used for this experiment. They were obtained as follows; from the University of Calabar Nigeria, Neurobehavioural laboratory, we purchased C57BL/6 timed-pregnant dams, housed them singly in standard cages where they were provided food and clean water under controlled temperature (20 ± 1°C) with light-dark cycle of 12 hours. Seventy-two (72) of their pups - PND2 were then placed in four sets of three groups, such that each group contained 6 pups with an averagely equal distribution of male and female.

Set 1 was designated Control mice containing group 1(control), group 2 (biologically stressed), and group 3 (chemically stressed) mice.

Set 2 designated as Non – stressed mice containing group 1 (control), group 2 (Vitamin E treated) and group 3 (Ginseng treated) mice.

Set 3 designated as biologically stressed mice with group 1 (control), group 2 (Vitamin E treated) and group 3 (Ginseng treated) mice.

Set 4 designated chemically stressed mice containing group 1 (control), group 2 (Vitamin E treated) and group 3 (Ginseng treated) mice. See table 1.

Two separate models of stress induction were activated as follows; A biologically induced stress, wherein the m/ethod of Monteiro *et al*, 2015 was modified, so that mice were subjected to social deprivation by isolation, exposure to generator noise for one hour (1 hr), and longer light hours as well as exposure to cold for twenty minutes (20 mins), all daily for the period of the experimental studies. Then a chemically induced stress in which 3-nitropropionic acid was intraperitonealy administered at a dose of 15mg/kg daily for 4 days to mice group (Binat *et al*, 2005).

The actual exposure of the categories of mice to the various treatments commenced from PND2. All sets of mice were treated as indicated in the experimental design (see table 1). For the control groups of the sets 1 and 2 mice, they had normal mouse feed and water ad libitum, with regulated normal light/night conditions and temperature, but control groups of sets 3 and 4 mice were both exposed to biological and chemical stress respectively. Furthermore, the group 2 and 3 mice in set 1 were subjected to biological and chemical stress respectively, while groups 2 and 3 mice in set 2 were treated with Vitamin E and Ginseng in that order with no exposure to stress. Meanwhile, all the set 3 and 4 mice, were respectively exposed to biological and chemical stress, but in addition, group 2 and 3 in set 3 were treated with Vitamin E and ginseng respectively; just as group 2 and 3 of set 4 were treated with Vitamin E and ginseng in that order. The treatments were in this format for set 1; group 1 or control mice was only allowed access to normal food, water, and conditions. Group 2 was in addition to the normal treatment exposed to biological stress by a modified model of Monterio *et al*, 2015). Then group 3 was exposed to chemically induced stress according to the model of Bizat *et al*, (2005). For the set 2; group 1 mice had normal treatment and conditions, group 2 was in addition to normal conditions administered Vitamin E (0.5mg/kg) orally, while group 3 was administered ginseng (1mg/kg) orally as well. Meanwhile, set 3 and 4 mice were both exposed to biological and chemically induced stress respectively. Then apart from the control of these sets 3 and 4 that had only the stress factors, their groups 2 and 3 were additionally treated respectively with vitamin E (0.5mg/kg) and ginseng (1mg/kg) oral administration once daily. The pups were eventually weaned on PND21 and by PND24 they were then subjected to neurobehabioural tests. The test procedure was in two categories following standard neurobehavioural protocol as follows:

Morris Water maze (MWM): This is a wide-spread model with standard protocol for assessing learning and memory in mice, since it is particularly modeled to investigate spatial learning and memory behaviour (Crawley, 2008;) A circular plastic pool (100cm diameter) of water (30 cm height) demarcated into four quadrants (Northeast - NE, Northwest - NW, Southeast - SE, Southwest - SW), and made opaque by adding non-toxic white tempura paint to it was allowed in a room to reach 28 ± 2˚C temperature. The protocol of Morris, 1984 was followed; the first 3 days of acquisition training, mice were placed at predetermined points, and allowed to use extra maze visual cues to locate a hidden escape platform in one of the quadrants (NW). The next three days was reversal training with the hidden platform placed in the opposite quadrant (NE). Each animal was given 4 trials of 60 seconds each day using a stop clock to measure swim latency and quadrant duration. Any mouse that could not locate the platform within 60 seconds was guided to locate the platform and allowed to explore for about 10 seconds. Each rodent was dried up properly after training session. On day 7 (probe trial), visuo-spatial learning and memory was investigated, by removing the platform and recording each of the quadrant duration (McDonald & White, 1994) and day 8 projecting the platform to be visible. These 7^{th} and 8^{th} days, the rodents were only allowed one trial of 60 seconds each.

The T – Maze Test: This is among the easiest mazes, only having two turns (one to the right or the other to the left) at the upper end away from the T's base, in which either of the two paths could be blocked as a way of alteration (Dao, 2010). When a reward is placed in one arm or both, the path to navigate from decision by the rodent could measure its preference. Multiple repetitions of this, without any reward placed on both arms or with rewards placed in alternating pattern on both arms could be used to observe the rodent's choice of entering arm not previously visited, with every commencement opportunity. This assesses working spatial memory.

Table 1: Experiment Design.

	SET 1 Control	**SET 2 Non-stressed**	**SET 3 Biostressed**	**SET 4 Chemostressed**
Group 1 No of mice	Control 6	Control 6	Control 6	Control 6
Group 2 No of mice	Biostressed 6	+ Vit E 6	+ Vit E 6	+ Vit E 6
Group 3 No of mice	Chemostressed 6	+ Ginseng 6	+ Ginseng 6	+ Ginseng 6

Statistical Analysis

Standard statistical methods were used to collate all data, with the aid of SPSS version 17.0. One way analysis of variance test was utilized for the variance within and among samples, whereas statistical difference between two groups was analyzed using post-hoc least significant difference test. Meanwhile all the results were recorded as mean ± standard error of mean, and illustrated in bar chart with groups.

RESULTS

The results from the neurobehavioural studies was recorded as mean ± standard error of mean, and presented here in illustrated bar chart with groups in figures (See figures 1 - 14).

Comparison of percentage correct entry in the (T-maze) spontaneous alternation test for stressed and non-stressed mice

There was significant (P<0.001) decrease in the mean percentage correct entry for biologically stressed and chemically stressed groups of mice compared to control (figure 1)

Comparison of percentage correct entry in the spontaneous alternation test for non-stressed mice treated with ginseng (1mg/kg) and vitamin E (0.5mg/kg)

In the non-stressed mice (set 2), the mean percentage correct entry was significantly (P<0.05) reduced for ginseng treated group than control, but increased in vitamin E treated group than control significantly (P<0.05), figure 2.

Comparison of percentage correct entry in the spontaneous alternation test for biologically-stressed mice treated with ginseng (1mg/kg) and vitamin E (0.5mg/kg)

The mean percentage correct entry in this test with biologically-stressed mice (set 3) was significantly increased for ginseng and vitamin E treated groups compared to control (P<0.001), figure 3.

Comparison of percentage correct entry in the spontaneous alternation test for chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E (0.5mg/kg)

In this case with chemically-stressed mice (set 4), there was significant (P<0.001) increase in mean percentage correct entry in the spontaneous alternation test by both ginseng and vitamin E treated groups than the control (fig 4).

Swim latencies in Morris water maze test for non-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg) as shown in Learning curves

The swim latencies in Morris water maze as seen from the learning curves did not present any significant difference for the ginseng and vitamin E treated groups as well as control (P<0.05) fig 5.

Swim latencies from learning curves in Morris water maze test for biologically- stressed mice (set 3) treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

There was no significant difference between all the groups during acquisition training (fig 6).

Learning curves showing swim latencies in Morris water maze test for chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

The indices investigated here showed no significant difference in the treated groups as well as control during all the acquisition training, see figure 7.

Quadrant durations in Morris water maze test showing preference for the NW (Reversal) quadrant in chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E (0.5mg/kg)

These indices showed that there was no significant (p<0.05) difference between all groups as presented (figure 8). It is worth noting that there was no significant difference in the preference for NW (reversal) quadrant shown by all the categories; non-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg); biologically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg).

Comparison of retention quadrant duration in non-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

Mean retention quadrant duration was increased in vitamin E and ginseng treated group than control in non-stressed mice; although this increase was significant for vitamin E, it was not for ginseng. Also it was significantly (p<0.05) higher in vitamin E group than ginseng (fig 9).

Comparison of retention quadrant duration in biologically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

In the biologically stressed mice treated with ginseng and vitamin E, during Morris water maze test, the retention quadrant duration increased significantly (P<0.001) for the treated groups than control. Also, this retention duration was significantly higher for vitamin E group than ginseng group at P<0.05 (fig 10).

Comparison of retention quadrant duration in chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

In this set 4 mice, observations of their mean duration in retention quadrant increased significantly (P<0.01, P<0.001) in both treated groups (ginseng, vitamin E) respectively when compared to control (figure 11).

Comparison of annulus reversal crossings in non-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

In the non-stressed mice, mean annulus crossings was significantly (P<0.05) increased in vitamin E than control, and non-significantly reduced in ginseng compared to control at P<0.05 (fig 12).

Comparison of annulus reversal crossings in biologically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

The annulus reversal crossing mean value in biologically stressed mice was increased significantly in both treated groups than control, and this increase was significantly higher for vitamin E group than ginseng group (fig 13).

Comparison of annulus reversal crossings in chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg)

Mean annulus reversal crossings in those chemically stressed mice were significantly ($p<0.001$) increased for both treated mice groups (i.e. ginseng and vitamin E) than control (fig 14).

DISCUSSION

The present study was to investigate the comparative neurobehavioral impact of the herb ginseng and vitamin E with respect to the potential of preservation or recovery of cognitive function such as learning and memory, using an experimental model of white Swiss mice that were subjected to stress induction; since stress to an extent has been reported to affect cognitive behaviour adversely (Radi *et al*, 2014).

Oxidative stress was induced in two separate forms; one was the chronic unpredictable stress protocol involving social and biological (in combination) interferences with circadian rhythm of the rodents. The second was the established mitochondrial toxin 3-NP that was used to cause neuronal degeneration. Meanwhile following stress induction, the neurobehavioural parameters studied focused on learning and memory. The known effect of Vitamin E on improving both neuronal regeneration and activities informed the reason for its use (Dokkaew *et al*, 2009), so as to either grade or substantiate the efficacy of the herb ginseng.

The Morris water maze test is a long standing protocol for studying learning and memory behaviour in rodents as indicated in the methodology session. In the instance for the sets of mice in this experimental design, the learning curves during acquisition and reversal days indicates that there was no significant ($P<0.05$) difference in how all groups (ginseng and vitamin E treated groups as well as control) learned the location of the escape platform as seen in the uniformity of swim latency decrease during each of the 3 days acquisition and reversal trainings respectively. But during days of reversal training, with altering of the escape platform position, ginseng and vitamin E treated groups learned faster in the first two days than the biologically and chemically stressed groups, as seen in longer swim latencies of days 1 and 2 reversal trainings.

The mice were assessed for visuospatial memory during probe trial in which as expected, they would express preference for the north east and north west (retention) quadrants where the escape platform was placed during acquisition and reversal trainings respectively; and if they actually remembered where the escape platform was on the reversal training, then they will spend more time exploring the retention quadrant.

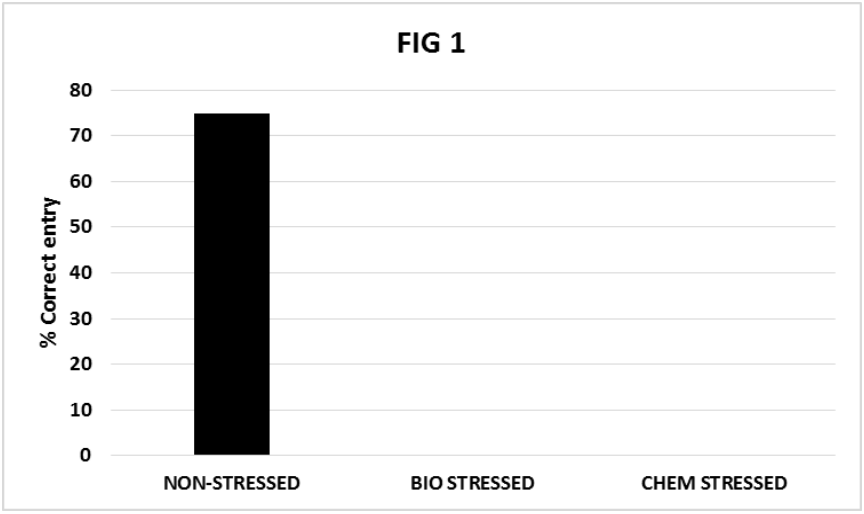
The retention quadrant duration for the chemically stressed mice was decreased significantly and less so for the biologically stressed group of mice. In the same vein, there was decrease in the annulus acquisition and annulus reversal crossings frequency (i.e frequency with which mice crossed the supposed position of the hidden platform during acquisition and reversal trainings respectively) for these groups. And the implication of this behaviour is that there was impaired visuospatial memory in these stressed groups of mice, although the degree of impairment was milder in those mice exposed to biologically induced stress compared to chemically stressed mice.

However, in the ginseng and vitamin E treated groups, higher retention quadrant duration as well as annulus reversal crossings frequency were observed compared to the biologically stressed group, implying a likely reversal in memory impairment. Also, vitamin E treated group's retention quadrant duration was significantly higher than that of ginseng treated mice (Fig ? 34). Similarly, the mean retention quadrant duration in chemically stressed group of mice that were treated with vitamin E and ginseng was significantly ($P<0.001$) increased, compared to control (fig ? 38), indicating visuospatial memory impairment reversal.

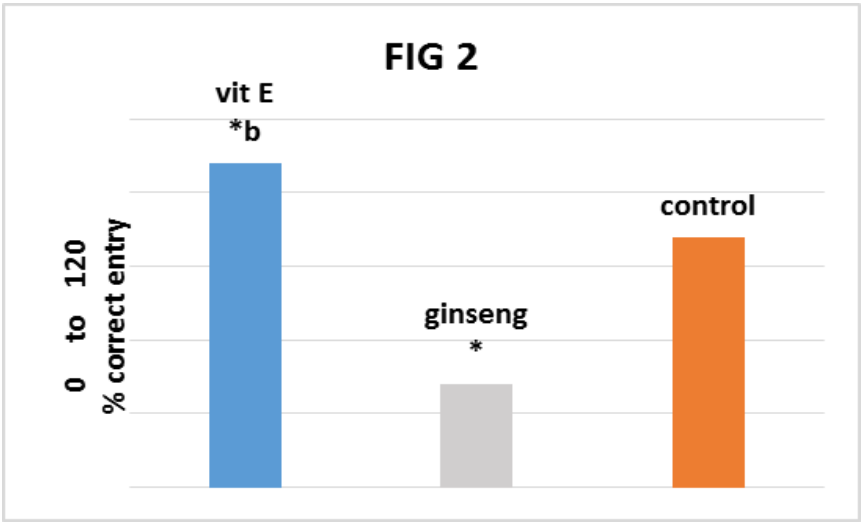
It is worth noting that during visible platform task, swim latencies was not significantly different between the test groups and control, indicating all the mice had good visual cues without visual impairment which could have negatively influenced the rodents' ability to correctly locate the platform. Also, the spontaneous alternation test results show that ginseng and vitamin E improves working memory.

Conclusively, these results suggest that treatment with ginseng and vitamin E improved visuospatial memory. But for comparative efficacy, in the biological stress model, vitamin E appears more effective than ginseng indicated by its higher degree of impact on memory impairment reversal (significant at $P<0.05$, as seen in Fig ? 34), whereas this similar difference observed for the chemical stress model was not significant, probably due to the greater extent of damage by the 3-NP neurotoxin on cognition (fig? 23) or by a mechanism which can be investigated further. Vitamin E is well known for its neuroprotective effect (Alzoubi *et al*, 2012; Rahangadale *et al*, 2012) whereas, ginseng shows a neuroprotective potential that may be attributed to some yet to be elucidated mechanisms recommended for further studies.

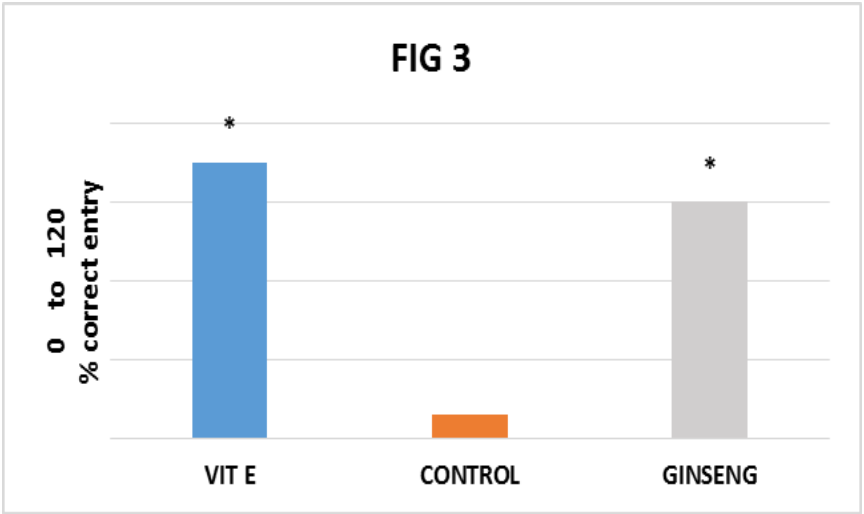
List of Figures



Comparison of percentage correct entry in the (T-maze) spontaneous alternation test for stressed and non-stressed mice, *** significant at p<0.001 vs control



Comparison of percentage correct entry in the (T-maze) spontaneous alternation test for non-stressed mice treated with vitamin E and ginseng, * = significant at p<0.05 vs control; b = significant at p<0.05 vs ginseng



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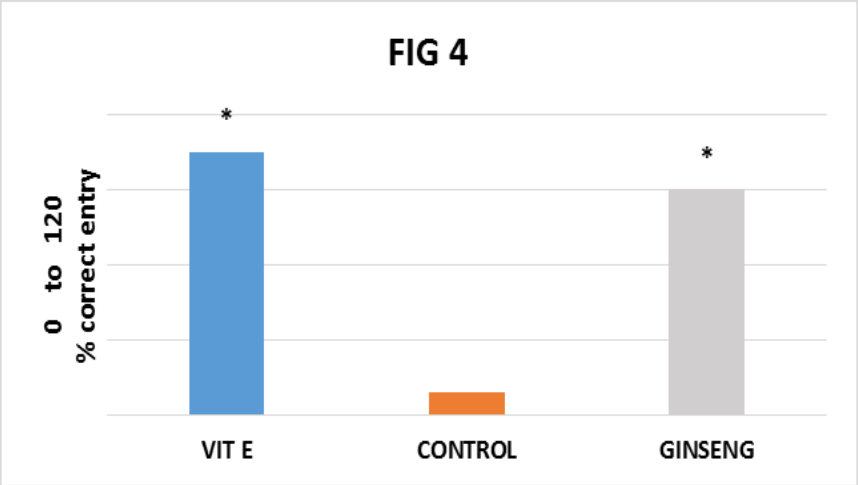

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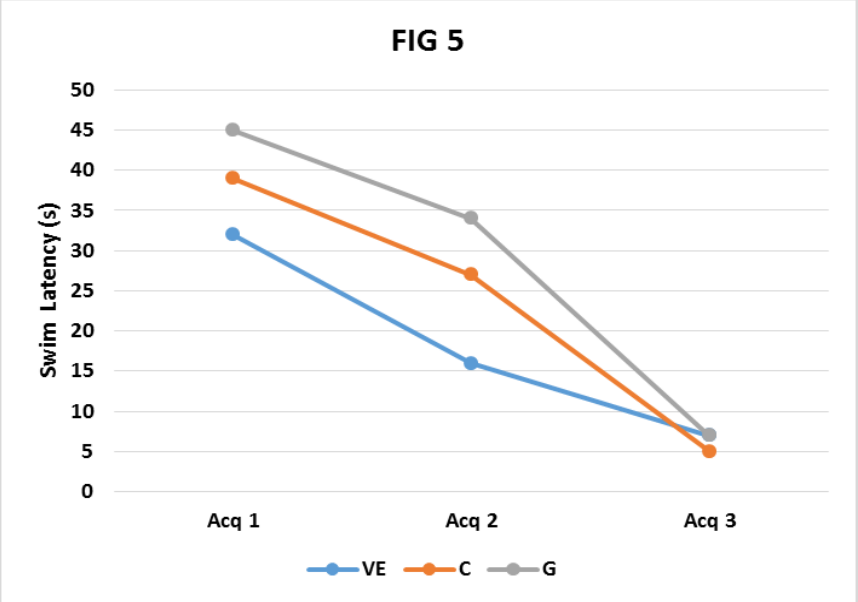
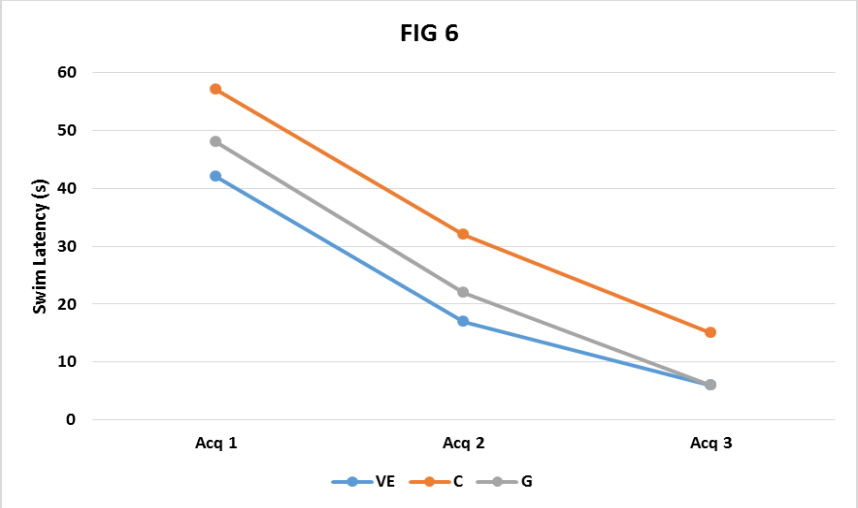


Swim latencies in Morris water maze test for non-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg) as shown in Learning curves: VE = Vitamin E; C = Control; G = Ginseng



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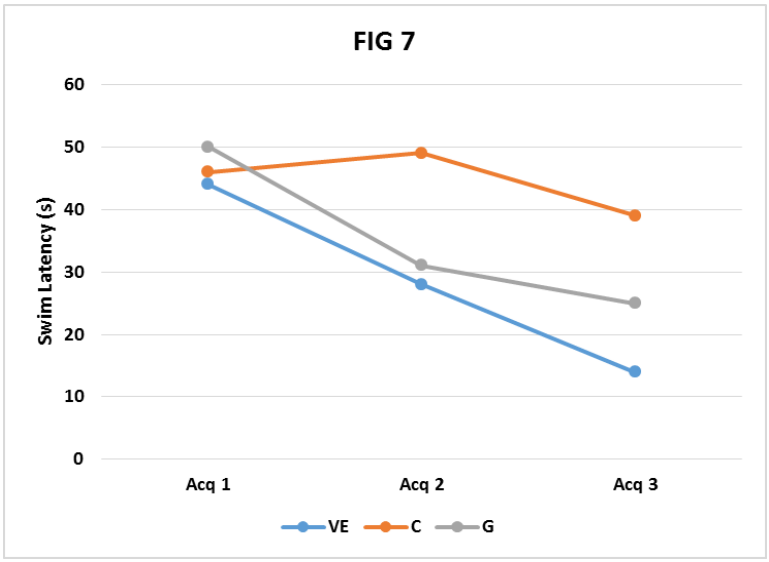
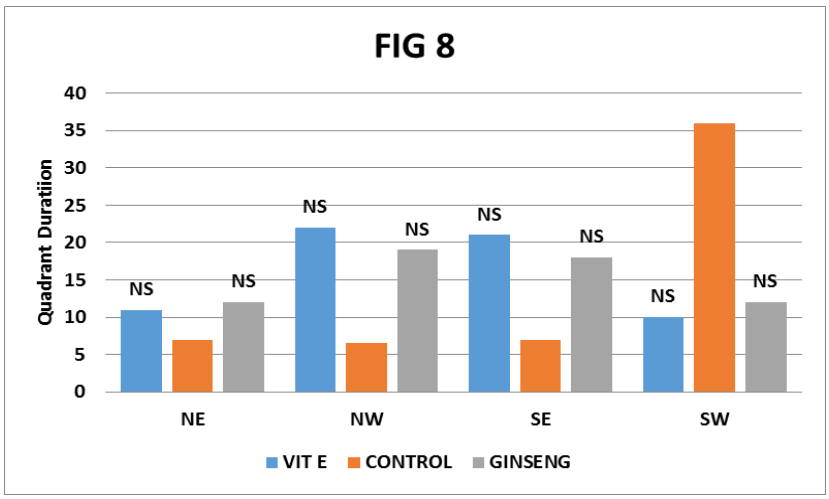
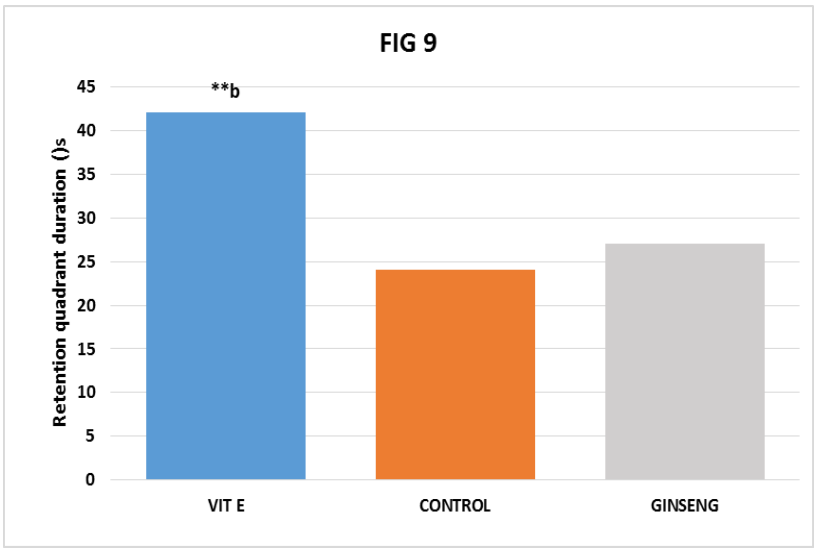


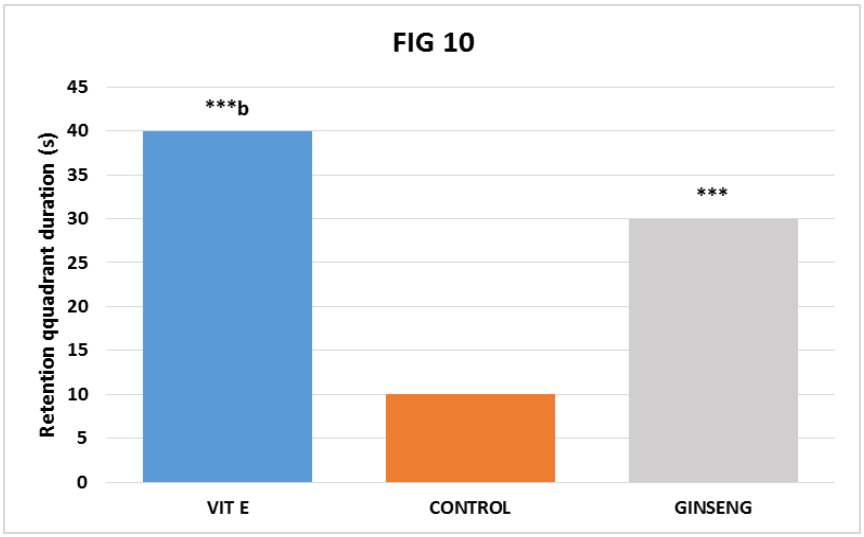
Swim latencies in Morris water maze test for chemically-stressed mice treated with ginseng (1mg/kg) and vitamin E(0.5mg/kg) as shown in Learning curves: VE = Vitamin E; C = Control; G = Ginseng



Quadrant duration in Morris water maze test showing preference for the NW (Reversal) quadrant in chemically-stressed mice treated with vitamin E and ginseng: NS = Not significant vs control at $p<0.05$.



Comparison of retention quadrant duration in Morris water maze test for non-stressed mice treated with vitamin E and ginseng: ** = significant at $p<0.01$ vs control; b = significant at $p<0.05$ vs ginseng



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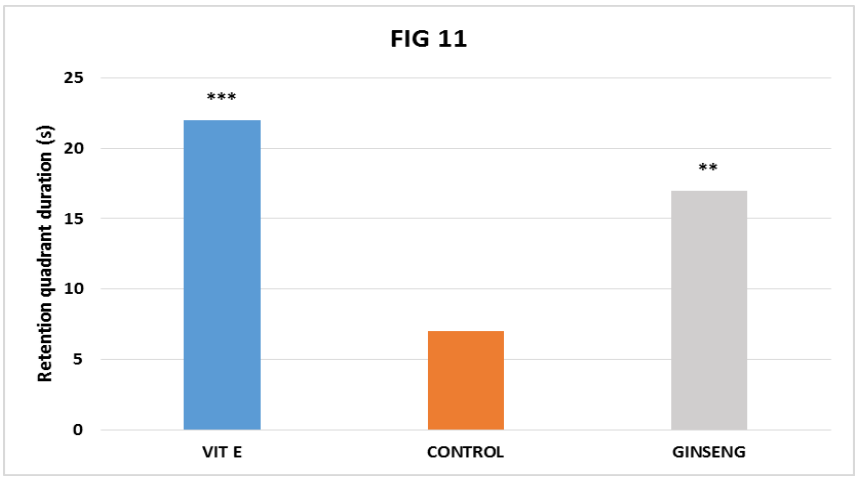

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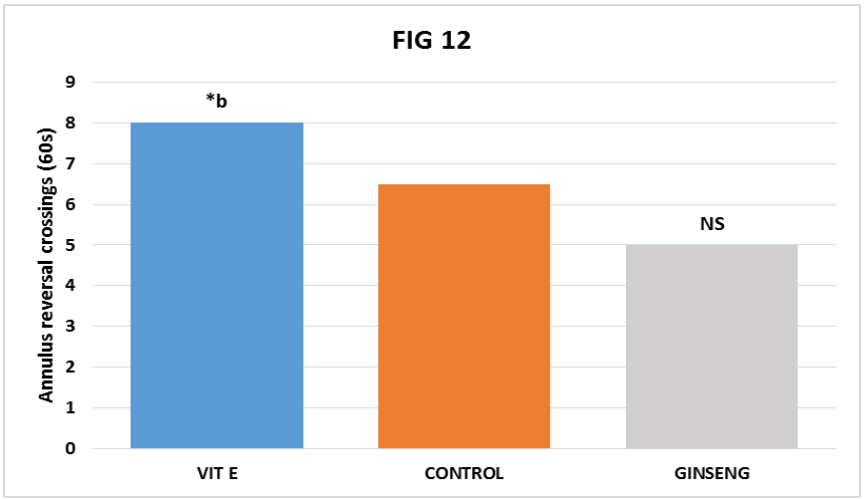
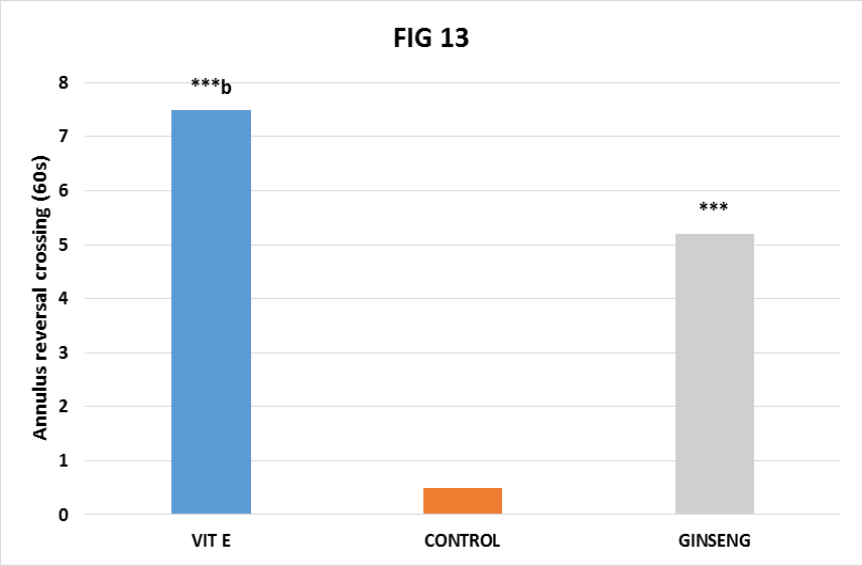
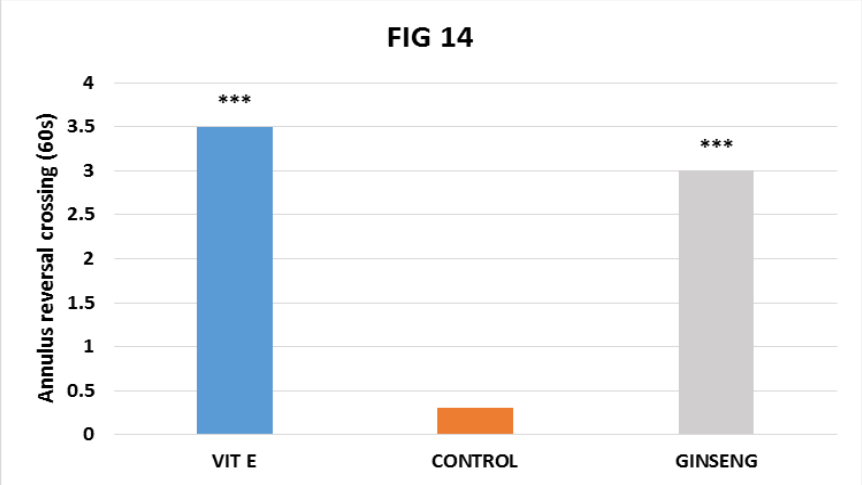


Comparison of annulus reversal crossings in the Morris water maze test for the non-stressed mice treated with vitamin E and ginseng: NS = not significant at p<0.05 vs control; * = significant at p<0.05 vs control; b = significant at p<0.05 vs ginseng



Comparison of annulus reversal crossings in the Morris water maze test for the biologically-stressed mice treated with vitamin E and ginseng: ** = significant at p<0.01 vs control; *** = significant at p<0.001 vs control; b = significant at p<0.05 vs ginseng



Comparison of annulus reversal crossings in the Morris water maze test for the chemically-stressed mice treated with vitamin E and ginseng: *** = significant at p<0.001 vs control

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