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Effects of Slim Green Tea on Some Tissue Oxidative Stress Markers, Lipid Profile and Cognitive Functions in Wistar Rats

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ABSTRACT

Slim green tea is a popular brand of tea that supports weight reduction and maintenance. High consumption of this tea in worldwide populations is a major concern to researchers and medical personnel, as regards its benefits or dangers to human health. Therefore the aim of research was to ascertain impact of slim green tea on some oxidative stress markers in heart and brain tissues, lipid profile, and cognitive functions in Wistar rats. Twenty-five (25) male Wistar rats weighing between 120g and 150g were acclimatized for 2 weeks, they were separated into 5 groups; a control Group (Group 1) and Groups (2, 3, 4 and 5) which served as test groups. Various doses of slim green tea extract (50mg/kg, 100mg/kg and 150mg/kg) and a dose of vitamin E (100mg/kg) were administered orally for four weeks to the different groups of rats (2, 3, 4 and 5) respectively, while control group received normal chaw and water *ad libitum*. The results indicated that slim green tea extract caused dose-dependent significant decrease ($p \leq 0.05$) on Malondialdehyde (MDA) and nitric oxide (NO) levels, and a dose-dependent significant increase ($p \leq 0.05$) on Superoxide Dismutase (SOD), Glutathione (GSH), and Catalase (CAT) levels in tissues of heart and brain in Wistar rats. The result showed high doses of slim green tea extract seemed more functional in increasing antioxidant properties in brain tissue than in heart tissue. Treatment of test groups with varied doses of tea extract on serum lipid profile demonstrated a dose-dependent significant decrease ($p \leq 0.05$) on total cholesterol and HDL levels in every group compared to control group. The extract equally caused a significant decrement on LDL level and an insignificant effect on triglyceride level when administered at 50mg/kg and 100mg/kg respectively, while dose of 150mg/kg increased LDL and triglyceride levels significantly. Results on cognitive assessment in brain showed slim green tea had no cognition-enhancing property. These findings suggest intake of slim green tea has beneficial effect especially as regard cardio-protection and neuro-oxidative protection but no effect on cognition.

Keywords: Slim green tea, Vitamin E, superoxide Dismutase, Glutathione, Catalase, cognition.

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INTRODUCTION

Tea is an aromatic drink prepared by pouring boiling or sizzling hot water on dried leaves of *Camellia sinensis* (green tea) plant. Apart from water, rate of tea consumption is higher amongst humans in comparison to other consumable drinks taken globally (Katiyar and Mukhta, 1996).

Pure tea is classified according to processes its leaves pass through due to exposure to oxygen as non-oxidized green tea, partly-oxidized oolong tea, completely-oxidized black tea, white tea (made from non-oxidized and uncured leaves) and overly-fermented Pu'erh tea (Dong *et al.*, 2011). These varieties of tea are obtained from *Camellia sinensis* plant, except herbal teas which are herbal infusions.

Most herbs used in preparing slimming teas include; green tea extract (rich in antioxidants that protects the cells from pollutants and also improve memory), *Garcinia cambogia* (enhances satiety, by raising serotonin in brain that decreases the feeling of hunger), ginger root (for treating infections and fever), caffeine (CNS stimulant, that reduces fatigue and drowsiness and improves wakefulness and motor coordination), senna leaves (used as laxatives to relieve constipation) etc. Some of these herbs are also used in preparing pure teas and most benefits gotten from pure teas are found in slimming teas because they contain polyphenol and caffeine (Okafor, 2013).

MATERIALS AND METHOD

Experimental Animals

Male Wistar rats (25) weighing between 120g and 150g were raised from the Animal house at the University of Port Harcourt, Nigeria. Rats were kept in cages in an airy experimental room and they had free approach to standard rat feed and clean water, acclimatization lasted for 2 weeks before treatment was commenced. Ethical approval was given by UPH Research Ethics Committee (UPH/CEREMAD/REC/04). This is an independent research, no grant was received from any funding agency/organization and there was no conflict of interest.

Purchase of Drugs and Chemicals

LA Botti Slimming Tea manufactured by Gemi Teas Colombo (PVT) LTD, Homagana Sri Lanka, was purchased from a reputable pharmaceutical company in Ogun State Nigeria. Vitamin E manufactured by Nature's field pharmaceutical, USA was procured from the University of Port Harcourt pharmacy. Chemicals used in this research were of analytical grade.

Preparation of Slim Green Tea Extract

Each slim green tea bag weighed 2g. The slim green tea extract was prepared by infusing a tea bag in 100mls of hot boiling water for 3-5 minutes. Volume of stock given to each animal was calculated using the modified method of Tedong *et al.*, (2007)

Vol. (ml) = dose of extract (mg/kg⁻¹) x weight of rat (kg)/conc. of stock (mg/ml)

Experimental Design

Groups	Treatment	Dosage (four weeks)
Group 1	Normal feed and water <i>ad libitum</i>	4 weeks
Group 2	Slim green tea	(50mg/kg)/day
Group 3	Slim green tea	(100mg/kg)/day
Group 4	Slim green tea	(150mg/kg)/day
Group 5	Vitamin E (a known antioxidant)	(100mg/kg)/day

Specimen Collection

On completing the experiment, the rats were sacrificed by cervical dislocation. 5mls of blood samples were gotten from each rat through cardiac punctures and they were temporarily stored in a plain centrifuge tube and labelled. Sera were separated by centrifuge and placed in aliquots at -70°C till utilized for lipid profile estimation i.e total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, by enzymatic colorimetric techniques utilizing commercial kits. Animals were decapitated to remove the brain and thorax was dissected to remove the heart, the two organs were washed thrice in an ice cold saline and smeared separately on ash-free filter paper in order to prepare tissue homogenates for estimating tissue MDA, NO, CAT, GSH and SOD enzyme levels.

Assessment of Experimental parameters

In this study, the parameters assessed are lipid profile of rats, oxidative stress markers in heart and brain tissues (MDA, SOD, CAT, NO, GSH) and cognitive abilities of the wistar rats. These assessments were done after four weeks of treating the animals with slim green tea.

Preparation of brain and heart tissue homogenates

Samples collected from each of the organs were set apart into two sections. Each sample was weighed and homogenised separately with a potter- Elvenhjem tissue homogeniser. *One part* was homogenised in phosphate buffer saline (PBS) 50 mM pH (7.4) for estimation of SOD, CAT, and GSH levels, while the *other part* was homogenised in potassium phosphate buffer 10 mM pH (7.4) to determine MDA and NO levels.

Estimation of tissue MDA and NO levels

Malondialdehyde (MDA)

Malondialdehyde an indicator of lipid peroxidation was estimated using the technique for Buege and Aust (1978). 1.0 ml of supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent was boiled for a period of 15 minutes at 100°C, and was allowed to cool. Flaky materials were expelled by centrifuge at 3000 rpm for 10 minutes and the supernatant which was detected spectrophotometrically at 532nm was removed. MDA was estimated by molar extinction coefficient for MDATBA- complex of $1.56 \times 10^5 \text{ M}^{-1}\text{CM}^{-1}$.

Nitric oxide (NO)

Nitric oxide (nitrite + nitrate) was detected in tissues by measuring formation of nitrite by a nitrate/nitrite colorimetric assay kit. Concentrations were ascertained by comparison to nitrite standards. The results of NO assays are measured as $\mu\text{mol/g}$ tissue.

Enzymatic assays

Catalase Enzyme Assay: Catalase was estimated by the technique of Sinha, (1972) which depended on formation of chromic acetate from dichromate and glacial acetic acid in presence of hydrogen peroxide. The mixture (1.5ml) had 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2 . Chromic acetate that was produced was estimated colorimetrically at 610 nm.

Superoxide Dismutase Assay: An indirect method of stopping auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activities in blood. Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent prepared as reported by Onwurah, (1999) to a mixture of epinephrine ($3 \times 10^{-4} \text{ M}$), Na_2CO_3 (10^{-3}M), EDTA (10^{-4}M), and 1.0ml of deionized water at a final volume of 6 ml. The auto-oxidation was interpreted in a spectrophotometer at 480 nm each 30 sec for 5 min.

Glutathione (GSH)

Glutathione (GSH) was estimated with the technique of Ellman, (1959). Equal amount of homogenate was combined with 10% trichloroacetic acid and centrifuged to isolate the proteins. The mixture was slightly shaken and absorbance read at 412 nm within 15 min. Concentration of glutathione was expressed as $\mu\text{mol/g}$ tissue.

Biochemical Assays

Lipid profile, which include total cholesterol (TC), triglyceride (TAG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were ascertained using Randox assay kits.

Cognitive Tests

Non-Passive Avoidance Apparatus (LIGHT-DARK BOX TEST)

This test is done to assess cognition recovery potential of small laboratory animals (Ogren and Stiedt, 2010). The non-passive avoidance box comprises of a small dark compartment (one third) and a large illuminated aversive compartment (two thirds). The light part is composed of a bulb while the dark part has no bulb but has an electrified platform. The animal ordinarily prefers the dark compartment but gets shocked and enters into the light compartment, and the time/speed of returning to the dim compartment determines how quickly forgetful the animals are.

Animals with normal learning and memory will avoid entering the chamber where they had been exposed to shock. This is estimated by recording step-through latency (STL) to cross through the gate between the light and dark compartments (Ogren, 1985). Rats were returned to the illuminated part after the conditional phase (learning) and they were expected to return into the dim compartment in test phase. Latency to return to the dim compartment was estimated within 180 seconds (3 minutes) for every rat. The longer or shorter time in STL value indicates an increment or reduction in memory retention.

Navigational Box / Opaque Maze

Opaque maze or navigational box study is extensively used in behavioral neuroscience to evaluate spatial learning and memory. This maze is a tour puzzle in form of an intricate branching labyrinth through which the rat is expected to discover an exit route and time taken to get to exit route is recorded. The interval it takes the rat to explore from the inlet point to the exit point determines how intelligent and cognitively active the rat is (Olton. 1979). The rats were expected to locate the exit point within two minutes interval in this experiment.

Statistical analysis

A Statistical Package for Social Science (SPSS), version 20.0 was used in analyzing the result statistically. Mean value \pm SD was used to present values of the different parameters. A student t-test was used to calculate the differences between 2 groups and p values < 0.05 was taken as statistically significant.

RESULTS AND DISCUSSION

Table 1 Effects of slim green tea and vitamin E administration on oxidative stress markers in heart tissue homogenates of Wistar rats

GROUPS	Malondialdehyde (MDA) ($\mu\text{g/ml}\pm\text{sem}$)	Catalase (CAT) ($\text{u/g}\pm\text{sem}$)	Superoxide dismutase (SOD) ($\text{u/ml}\pm\text{sem}$)	Glutathione (GSH) ($\mu\text{g/ml}\pm\text{sem}$)
1) Control	97.51 \pm 6.88	92.12 \pm 11.13	13.67 \pm 0.32	20.16 \pm 1.18

2) Slim green tea (50mg/kg)	68.95±5.00*	171.58±33.55*	9.52±0.63*	18.09±4.17
3) Slim green tea (100mg/kg)	79.81±7.91*	28.21±1.35*	14.96±0.62	16.52±1.77*
4) Slim green tea (150mg/kg)	79.61±8.26*	43.65±4.58*	15.97±1.14	11.44±0.99*
5) Vit E(100mg/kg)	75.16±1.83*	125.88±0.39*	24.82±2.87*	12.58±0.40*

Values are expressed in mean ± sem. n= 5. $P \leq 0.05$ *means values are statistically significant compared to control.

Table 2: Effects of slim green tea and vitamin E administration on oxidative stress markers in brain tissue homogenates of Wistar rats.

GROUPS	Malondialdehyde (MDA) ($\mu\text{g/ml} \pm \text{sem}$)	Catalase(CAT) (u/g±sem)	Superoxide dismutase (SOD)(u/ml±se)	Glutathione (GSH) ($\mu\text{g/ml} \pm \text{sem}$)	Nitric oxide (NO) (mmol/l±sem)
1) Control	68.93±7.12	39.88±1.63	8.61±0.39	12.11±0.27	132.79±12.29
2) Slim green tea(50mg/kg)	106.39±8.53*	45.78±1.34*	4.03±0.35*	20.01±0.47*	22.33±0.59*
3) Slim green tea(100mg/kg)	91.61±0.80*	88.39 ±14.08*	4.69±0.62*	18.02±0.32*	53.62±18.63*
4) Slim green tea(150mg/kg)	61.22±0.80*	180.80±26.13*	9.73±1.05	12.14±0.83	75.36±22.53*
5) Vitamin E (100mg/kg)	68.06±0.61	50.48±5.58*	10.68±0.52*	20.64±0.96*	72.26±5.45*

Values are expressed in mean ± sem. n= 5. $P \leq 0.05$ *means values are statistically significant when compared to control.

Table 3: Effect of slim green tea and vitamin E administration on serum lipid profile in Wistar rats

GROUPS	CHOLESTEROL (mg/dl±sem)	TRIGLYCERIDE (mmol/l±sem)	HDL (mg/dl±sem)	LDL (mg/dl±sem)
1) Control	140.55±1.49	133.18±3.01	88.62±1.30	25.02±0.55
2) Slim green tea (50mg/kg)	135.82±6.33*	132.66±3.72	70.94±0.79*	26.34±0.44
3) Slim green tea (100mg/kg)	131.94±1.94*	133.90±5.52	73.28±4.34*	22.94±0.40*
4) Slim green tea (150mg/kg)	124.06±1.10*	168.28±11.89*	73.42±5.11*	32.22±2.57*
5) Vit E (100mg/kg)	111.62±1.05*	141.70±1.77*	59.80±2.82*	23.86±3.32*

Values are expressed in mean ± sem. n= 5. $P \leq 0.05$ *means values are statistically significant when compared to control

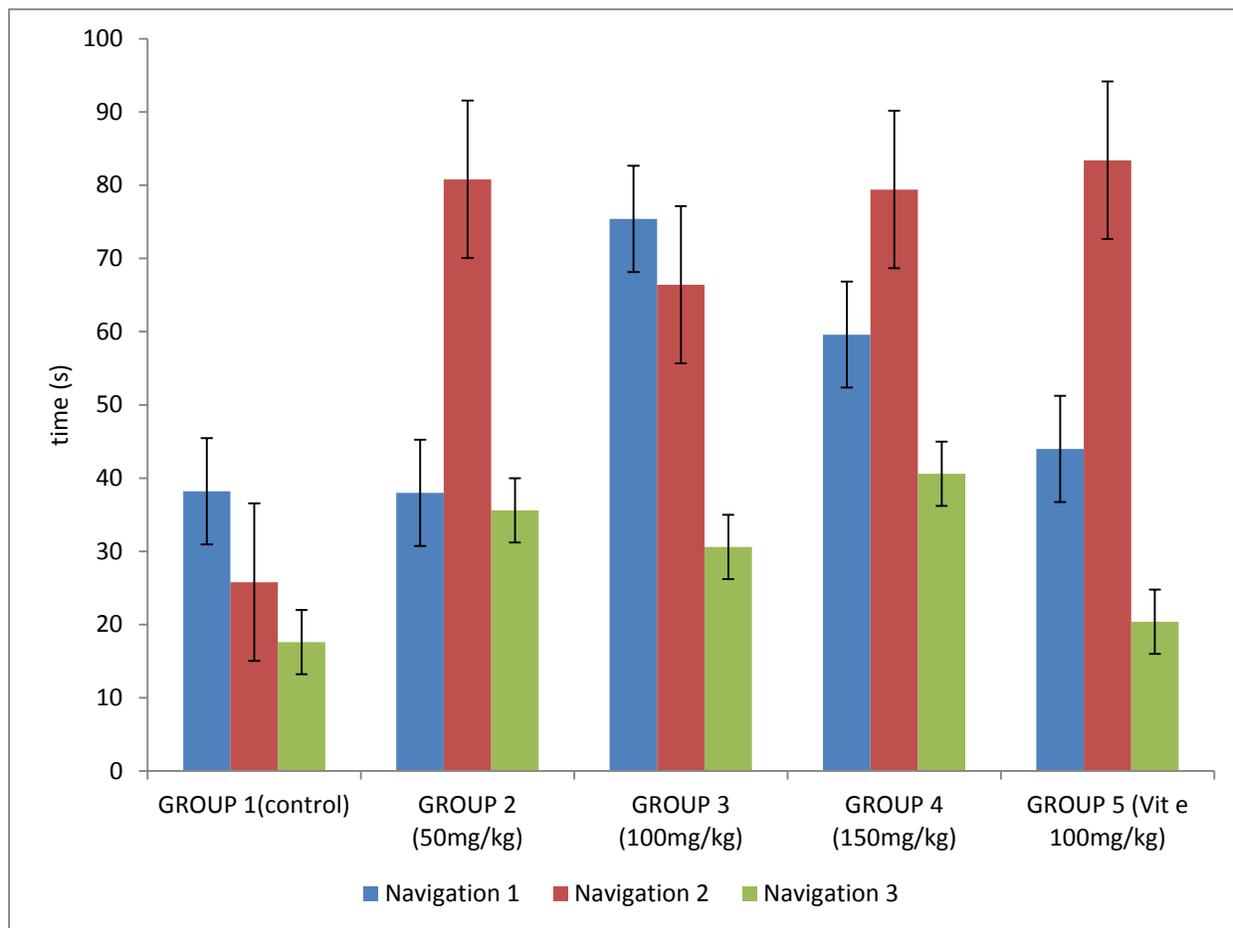


Figure 1 Navigational maze test result by various groups of the Wistar rats in 3 trials at 30min interval after administration of slim green tea and vitamin E.

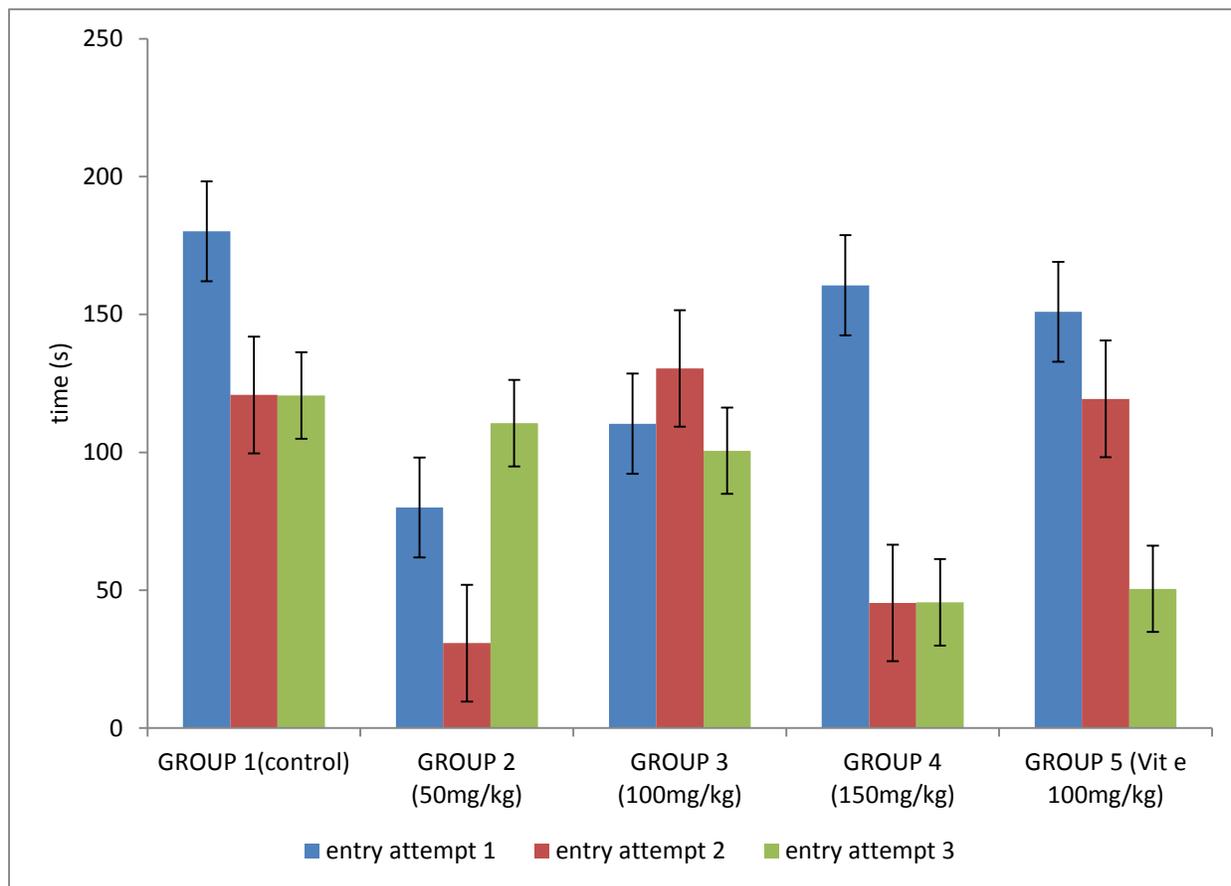


Figure 2: No of entry attempt in Passive Avoidance test by various groups of Wistar rats in 3 trials at 60min interval after administration of slim green tea and vitamin E

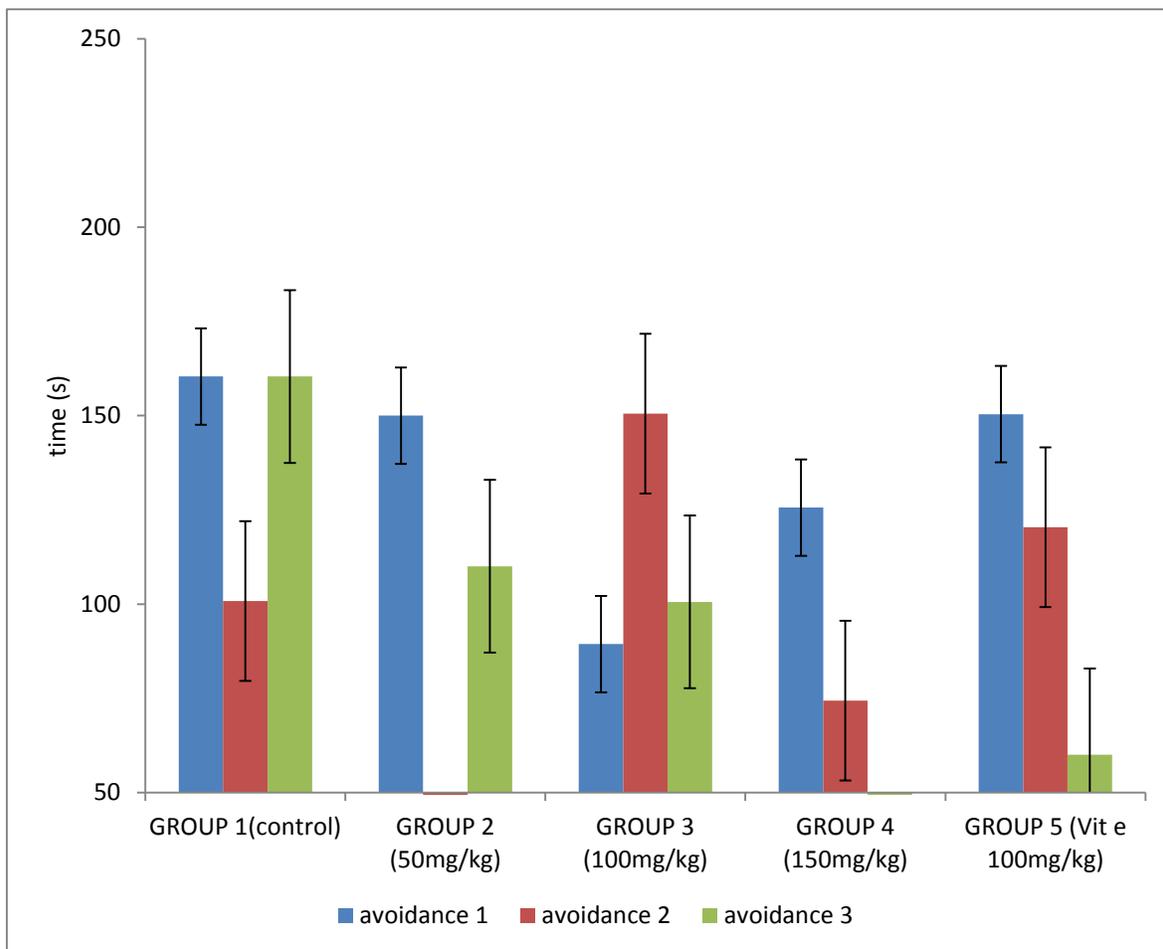


Figure 3: No of avoidance attempt in Passive Avoidance test by various groups of Wistar rats in 3 trials at 60 min interval after administration of slim green tea and vitamin E.

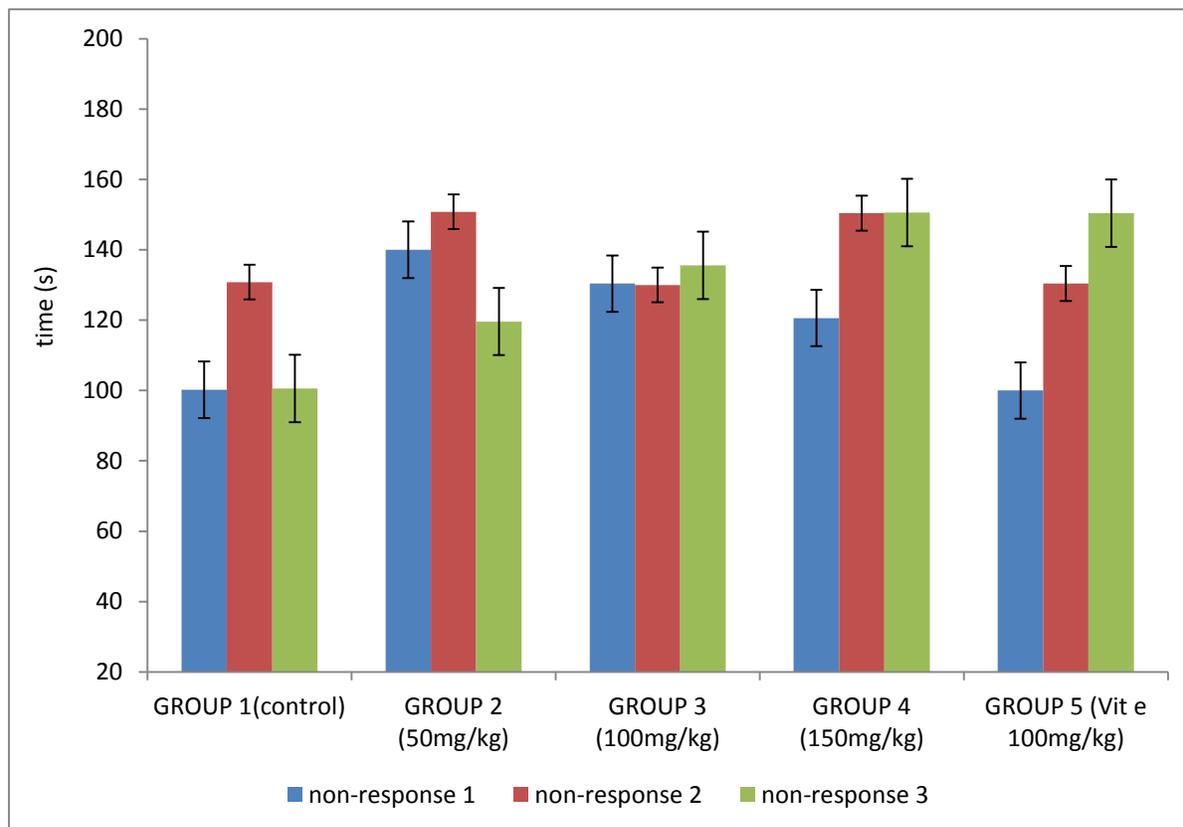


Figure 4: Non-response count in Passive Avoidance test by various groups of Wistar rats in 3 trials at 60 min interval after administration of slim green tea and vitamin E.

Slim green tea is a new popular brand of tea used to maintain or reduce weight and no research is done concerning beneficial or unfavorable impacts of consuming this tea. Therefore this research was done to examine effect of slim green tea on oxidative stress markers in heart and brain tissues, lipid profile and cognitive abilities in wistar rats. Various doses of slim green tea 50mg/kg, 100mg/kg and 150mg/kg and a vitamin E dose 100mg/kg, was given to different groups of rats (2,3,4 and 5) respectively for four weeks compared to control group (Group 1) which received normal chaw and water ad libitum

The present research describes that the slim green tea extract had an increasing effect on antioxidant enzymes in heart and brain tissues. This was demonstrated by its significant decrease ($p \leq 0.05$) on malondialdehyde levels (MDA) and its significant increase ($p \leq 0.05$) on superoxide dismutase, glutathione, and catalase (SOD, GSH, and CAT) levels in heart and brain tissues of Wistar rats, this increasing effect on antioxidant enzymes was discovered to be dose-dependent. The result also indicated high dose (150mg/kg) of slim green tea extract seemed more functional in enhancing antioxidant properties in brain tissue, though it reduced nitric oxide levels, while

moderate doses (50mg/kg and 100mg/kg) seemed more functional in improving antioxidant properties in heart tissue.

Slim green tea in this research affected serum lipid profile, typically causing significant decrease ($p \leq 0.05$) on total cholesterol and HDL levels in every group compared to control group. The tea caused significant decreasing effect on LDL level and an insignificant effect on triglyceride level when administered at 50mg/kg and 100mg/kg respectively, while dose of 150mg/kg increased LDL and triglyceride levels significantly. This gives credence to studies by Nicod *et al.*, (2014), affirming the modulatory impact of moderate intake of green tea on serum lipid levels.

The result also demonstrated that slim green tea exhibited no cognition-enhancing property in all doses administered to the rats. Vitamin E elicited an elevation in antioxidant properties in tissues of heart and brain in Wistar rats, it had significant impact on serum lipid profile by reducing total cholesterol and LDL levels, though it decreased HDL and elevated triglyceride levels. It also had little significant impact on cognition.

Therefore, the combined effect of reducing lipid levels and oxidative stress, suggest a possible cardio-protective role of slim green tea in the Wistar rats because of its polyphenol and caffeine contents, while an increase in antioxidant properties in brain at high dose and non-cognitive properties of this tea could be due to differences in brain and plasma bioavailability, dose-response effect and short-term administration.

CONCLUSION

This research indicated that slim green tea extract increases antioxidant defenses in heart and brain tissues of the Wistar rats, and that moderate dose (50mg/kg and 100mg/kg) of the slim green tea extract seemed more functional in increasing antioxidant defenses in heart tissues while high dose (150mg/kg) enhanced antioxidant defenses in brain tissues. Also, this research demonstrated that varied doses of slim green tea extract on serum lipid profile caused a dose-dependent significant decrease on total cholesterol and HDL levels in every group, significant decrement on LDL level, and an insignificant effect on triglyceride level when administered in low and medium doses, while at a high dose it significantly increased LDL and triglyceride levels, while results on cognitive assessment in brain demonstrated that slim green tea had no cognition-enhancing property.

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