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Research Article

Behavior and Biochemical changes of nanoginkgoba (*Ginkgo biloba* gold nano-particles) on restraint stress-induced male albino mice

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ABSTRACT

Ginkgo biloba (GB), is known for its antidepressant effect. In the present study we investigated Ginkgo *biloba* gold nanoparticles (Nano*ginkgoba* -GBGNPs) protective role against restraint stress-induced behavioral and biochemical alterations in mice. Animals were immobilized for a period of 6 hrs/ day. *Ginkgo biloba* Extract (GBE) (100 mg/kg), Nanoginkgoba (10 mg/kg) were administered 30 minutes before the animals were subjecting to acute immobilized stress. Behavioral test parameters for anxiety, spatial memory were assessed followed by biochemical parameters (lipid per oxidation, super oxide dismutase, catalase, glutathione per oxidase, reduced glutathione) subsequently. The Behavior study showed severe anxiety and memory loss compared to unstressed animals. Biochemical analyses revealed an increase in lipid per oxidation, depletion of super oxide dismutase, reduced glutathione , catalase activity and glutathione per oxidase as compared to unstressed animal. Twenty one days *Ginkgo biloba* Extract and Nano*ginkgoba* treatment in a dose of 100 mg/kg and 10mg/kg significantly attenuated restraint stress-induced behavioral and oxidative damage. In conclusion Nanoginkgoba prove the modest activity than the *Ginkgo biloba* Extract.

Keywords: *Ginkgo biloba;* Nanoginkgoba; goldnanoparticles; T-maze; stress.

INTRODUCTION

Stress, depression, and associated mental problems have become increasingly important (Chrousos GP , 1992). Lack of satisfactory treatments of the cognitive deficits usually accompanying these states presents a constant challenge for psychopharmacological research. It is well known that glucocorticoid (GK) hormone levels rise in response to stress (Belanoff JK, 2001; Marcilhac A,1998) . Evidence that the exposure to stress (and GKs) causes subsequent impairment of hippocampus-dependent forms of memory in both humans and animals has been repeatedly shown (Mc Even BS, 1995). The hippocampus, a part of the medial temporal lobe necessary for the formation of stable declarative memory in humans and spatial memory in rodents (Belanoff JK, 2001, De Kloet ER, 2000) has one of the highest densities of the GK receptors in mammalian brain and participates in the GK-mediated negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis (Belanoff JK, 2001; Joels M 1992; Marcilhac A,1998; Mc Even BS, 1995) In the rat hippocampus, corticosterone has been shown to regulate neuronal metabolism, physiological functions, genomic expression, and to alter the cell morphology (Baker KB, 2002; Mc Even BS, 1995). Consequently, certain hippocampal functions (such as learning and memory) appear to be susceptible to uncontrollable stress (Baker KB, 2002; Belanoff JK, 2001) Stress- or corticosterone-dependent behavioral changes are paralleled by both neurochemical and neuroanatomic alterations. Stress and stress hormones have been shown to impair performance in spatial memory tasks (De Quervain DJF , 1998) and also nonspatial hippocampal memory in rats (Baker K B, 2002). and electrophysiological studies indicate that stress and GKs impair hippocampal long-term potentiation (LTP), a putative synaptic mnemonic mechanism in the mammalian brain (Diamond DM , 1994) .

Antianxiety or hypno-sedative agents are commonly used for the management of stress but their use has several disadvantages (Shah ZA , 2003) and replacing them with safe natural products (e.g. medicinal plants) can be an ideal choice. Extracts from the green leaves of the G. biloba tree appear to be clinically effective with beneficial effects on neuroprotection, cardiovascular function and cerebral information processing. In accordance, a variety of studies have been published showing the learning- and memory-enhancing effects of standardized G. biloba extracts (GBEs) (containing 24% flavonoid and 6% terpenoid) in animal research (Continella G , 1985 ; Porsolt RD, 1990), as well as in clinical and healthy subjects (Kleijnen J,1992) . Numerous studies have shown that GBE has an antioxidant (Guidetti C, 2001), free radical scavenging (Tendi EA ,

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2002) neuroprotective (Ni Y , 1996; Smith PF, 1996), and anti platelet effects (Smith PF, 1996). The cellular mechanisms underlying these multiple effects of can be attributed to the different components of the extract, which may act independently or synergistically. Its protective effects include upregulation of mitochondrial ND1 gene expression,which is crucial in meeting the high-energy demands of neurons (Tendi EA , 2002) . Mitochondrial dysfunction implicated in aging and several neurodegenerative disorders, such as Parkinson's, Alzheimer's and Huntington's diseases (Kanowski S,1996; Tendi EA , 2002).

Nanotechnology has recently gained attention as one of the critical research endeavors of the 21st century. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco friendly alternatives to chemical and physical methods (Mohanpuria P, 2008). Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis (Shankar SS , 2004). So, the present study has been designed to evaluate the effects of GBGNPS against stress induced mice.

In the present study, our aim to investigate the effect of GBE and GBGNPS adult male mice on restraint induced male mice relation to behavior and biochemical studies.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (*Mus musculus*), weighing 20-30 g, were procured from the central animal house, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalainagar. The animals were maintained at the central animal house and were fed on a standard balanced diet (Hindustan Lever, Bangalore) and provided with water ad libitum. All studies were conducted in accordance with the National Institute of Health Guide.

Chemicals

Material used for the synthesis of gold nanoparticles are chloroauric acid ($HAuCl_4$, Loba Chemicals), GB leaves used in this study were received from local herbal market, Chidambaram, Tamilnadu. Deionised Double Distilled Water was used in this study.

Plant material and preparation of extracts

GB leaves were purchased from local herbal market, Chidambarm, Tamil Nadu .The leaves were air dried at room temperature, finely powdered with auto-mix blender and stored in a deep freezer until the time of use. The methanolic extract was prepared using Soxhlet apparatus.

Biosynthesis of Gold nanoparticles

1 mM solution of 40 ml Chloroauric acid at concentration of 10^{-3} M was prepared by dissolving DDW, kept in a 250 mL Erlenmeyer flask. 10ml of GB supernatant was added to the chloroauric acid solution. The yellow colored solution which it turned purple red slowly within 30min, indicating the formation of gold nanoparticles.

Experimental design

The animals were divided into six groups, each consisting of six mice. Group I served as control, Group II received 100 mg/kg GBE , Group III received 10mg/Kg of GBGNps Group IV served as restraint stress Group V 100 mg/kg GBE restraint Group VI 10 mg/Kg of GBGNps restraint respectively. *GBE*, GBGNPs (100 and 10 mg/kg, i.p.) were administered for 21 days and animals were subjected to restraint stress on 21 days . The doses were selected based on dose dependent study

Restraint stress

Animals were restraint for 6-hr by taping all the four limbs on a board after putting them on their backs using zinc oxide hospital tape. Release was affected by unraveling the tape after moistening with acetone in order to minimize pain or discomfort. In unstressed group, the mice were kept in animal cage with soft bedding in the experimental room (Sur TK, 1994) .

Behavioral assessments

T-Maze test

Spatial learning was recorded by T –maze on 21st day of experimental period. The T-maze has a start arm and a left and right arms and all arms are painted black inside. At the end of the two arms, a depression having a depth of 0.5 cm and the diameter of the depression is 5 cm. A food cup was placed in the depression situated to the right arm of the animal. The T-maze was located in a dimly illuminated room with a weak light (25w).The animals were trained with the maze, food and food containers on two consecutive days before the commencement of the experiments held over a period of again two consecutive days. Three trials per animals were carried out. The animals were then deprived of food for 24 hours to make the animals for food motivation. During the experimental session, the number of errors and average time taken for each trial were noted. On the second day, the number of trials runs to reach the criterion of 9 correct responses, and the number of errors, and the average time taken for each trial run were noted (Sudha S, 2000) .

Measurement of anxiety: Mirror Chamber Test

The mirror chamber consisted of a wooden chamber having a mirror cube enclosed within it. The container box was (40 × 40 × 30.5) cm. Animal was placed at the distal corner of the mirror chamber at the beginning of the test. During the 5 min test session, following para-

meters were noted- a) latency to enter the mirror chamber, b) average time spent per entry in mirror chamber. An anxiogenic response was defined as decreased the number of entries and time spent in the mirror chamber (Kulkarni SK, 1999).

BIOCHEMICAL ANALYSIS

Estimation of lipid peroxidation

The quantitative measurement of lipid peroxidation was assessed as per method of Wills (1966).

Assay of superoxide dismutase

SOD was assessed by the inhibition of formation of NADH- phenazine methosulphate nitroblue tetrazolium formazon (Kakkar P , 1984) . The reaction was initiated by the addition of NADH after incubation for 90s and stopped by the addition of glacial acetic acid. The colour formed at the end of the reaction was extracted into the butanol layer and measured at 520 nm.

Assay of catalase

CAT was assayed colorimetrically as per the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate when heated in the presence of H_2O_2 . The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of a dichromate-acetic acid mixture and the remaining H_2O_2 was determined colorimetrically as chromic acetate.

Assay of glutathione peroxidase

GPx was estimated as described by Rotruck et al., (1973). A known amount of brain homogenate was allowed to react with H2O2 in the presence of GSH for a specified time period, then the remaining GSH was allowed to react with DTNB and the developed yellow colour was measured at 412 nm.

Estimation of reduced glutathione

GSH in brain homogenate was measured according to the method of Ellman (1959). This method is based on the development of a yellow colour when 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is added to compounds containing sulfhydryl groups.

Table 1: Effect of Ginkgo biloba extract and GBGNPs on behavior changes in stressed and unstressed animals

S. No	Groups	T maze test (time taken in sec)	Mirror chamber test (Average time taken in sec)
1	Control	2.97± 0.75	50.53± 1.75
2	GBE	3.64 ± 0.59[a]	48.32 ± 0.41[a]
3	GBGNP	3.71 ± 0.21[a]	46.63 ± 0.41[a]
4	Restrain stress (RS)	41 ± 0.64[a]	10.16 ± 0.64[a]
5	GBE + RS	20.22 ± 0.51[b]	31.35 ± 0.45[b]
6	GBGNP + RS	10.39.± 1.49[b, c]	41.34± 1.36[b, c]

Values are expressed as mean ± SD for eight animals in each group.
Values not sharing a common superscript differ significantly at p<0.05.
a – compared to control
b – compared to control restraint
c – compared to *G. biloba*

Table 2: Effect of Ginkgo biloba extract and GBGNPs on biochemical changes in stressed and unstressed animals

S. No	Groups	TBARS (n moles/mg of protein	SOD (u/mg of protein)	CAT (u/mg of protein)	Gpx (u/mg of protein)	GSH(ug/100 g t issue)
1	Control	3.53± 0.25	3.94± 0.26	0.53± 0.35	13.84±0.75	0.65± 0.05
2	GBE	3.45± 0.65[a]	3.53± 0.29[a]	0.57± 0.58[a]	13.87± 0.45[a]	0.59± 0.05[a]
3	GBGNP	3.43± 0.72[a]	3.76± 0.61[a]	0.55± 0.36[a]	13.73± 0.31[a]	0.61± 0.03[a]
4	Restrain stress	7.13± 0.63[a]	1.79± 0.57[a]	0.29± 0.17[a]	9.61± 0.03[a]	0.30± 0.07[a]
5	GBE + RS	6.43± 0.51[b]	2.82± 0.39[b]	0.39± 0.42[b]	10.63± 0.06[b]	0.45± 0.03[b]
6	GBGNP + RS	4.37± 0.29[b, c]	3.70± 0.12[b, c]	0.46± 0.31[b, c]	12.71± 0.03[b, c]	0.56± 0.02[b, c]

Values are expressed as mean ± SD for eight animals in each group.

Values not sharing a common superscript differ significantly at p<0.05.

a – compared to control

b – compared to control restraint

c – compared to *G. biloba*

Statistical analysis

The statistical significance of the results was computed by one-way analysis of variance (ANOVA I) followed by Dunnet test. All the values are expressed as mean ± SEM. The data were analyzed by using one way analysis of variance followed by Dunnet test. $P < 0.05$ was considered statistically significant.

RESULTS

Behavioral measurements

Table 1. shows the T maze test and mirror chamber test . The control animals showed the normal spatial memory and anxiety behavior effect.. The restraint stressed animal showed significantly reduction in spatial memory and anxiety behaviors, as compared to unstressed group ($P < 0.05$). 21 days GBE and GBGNPs treatment significantly improved spatial memory and anti-anxiety-like behavior as compared to control animals. GBGNPs treated group significantly improved the activities as compared to GBE treated group ($P < 0.05$).

Table 2 shows the TBARS and antioxidant status in the control and experimental groups. TBARS was significantly ($P<0.05$) increased in stress induced group compared to the control group. GBE and GBGNPs treatment group, significantly ($P<0.05$) decrease the TBARS level compared to control group . HPGNPs treated group significantly decrease TBARS level as compared to GBE treated group ($P < 0.05$). SOD, CAT, GPx and GSH activities were significantly decreased ($P<0.05$) in stress induced animals as compared to the control group. These activities were significantly restored ($P<0.05$) in GBE and GBGNPs treatment group. GBGNPs treated group significantly restored the activities as compared to GBE treated group ($P < 0.05$).

DISCUSSION

In the present study, 6-hr restraint stressed animal showed significant loss of memory and anxiety behavior, impaired memory activity indicating stress induced neurobehavioral alterations (Sevgi S, 2006). The main purpose of this study was to determine if GBE can improve memory processes in control and stressed treated mice. Extensive studies have demonstrated that GBE exerts beneficial effects in a multitude of disease states, including cardiovascular and cerebrovascular disease and neurodegenerative disorders (Cheng, S , 2003). In the present study the spatial memory and anxiety were assessed by T-maze test and mirror chamber test. So it is evident from this study as well as previous reports that GBE and GBGNPs has definite neuroprotective action. Present study suggests its therapeutic potential against these stress related altered behavioral states.

The study also reveals the existence of quantitative behavioural responses in twenty one days stress induced animals for the administration of GBE and GBGNPs. Stress induced mice, subjected to the T maze test and mirror chamber test, revealed a significant loss of spatial working memory and anxiety behavior. GBE and GBGNPS treatment for twenty one days significantly improved behavior alterations, suggesting its neuroprotective effect against stressful conditions.

In the present study, Stress treated groups increase level of lipid per oxidation is indicated that the stress caused significant oxidative damage and depletes SOD, catalase, Gpx. and GSH activity.

Tsuboi et al reported an increased oxidative damage and weak antioxidant defense events are implicated in major depression (Tsuboi H , 2006).

Kabuto et al., reported that the Enzymic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymic reduced glutathione (GSH) play important roles during the process by scavenging reactive oxygen species (ROS) or preventing their formation (Kabuto, H, 2003). Numerous studies have demonstrated that GBE exerts potent antioxidant activity by acting as scavenger of free radicals, e.g., superoxide anions, hydroxyl radicals and nitric oxide, to protect antioxidant defence system (Bridi, R., ,2001).

Le Bars PL et al ; Stoll S et al reported that the compounds in ginkgo act to varying degrees as scavengers for free radicals, which have been considered the mediators of the excessive lipid peroxidation, decline of membrane fluidity, and cell damage observed in Alzheimer's disease (Le Bars PL , 1997). Pharmacological effects of the extract related to its free-radical scavenging properties include inhibition of lipid peroxidation, helping to maintain integrity and permeability of cell walls (Turan I, 1995), and protection of brain neurons against oxidative stress and post-ischemic injury induced by free radical production (Seif-el-Nasr M , 1995; Oyama Y, 1996).

In the present study GBE and GBGNPs improved the antioxidant status in stressed mice.

In the present study, GBE and GBGNPs were effectively improved the antioxidant enzyme activities such as SOD, CAT , GPx and GSH in stress treated animals. So, it is concluded that both the GBE and GBGNPs can exert a significant behavior and neuroprotective effect. But, the GBGNPs is has a significant behavior and neuroprotective effect than compared to the GBE.

CONCLUSION

In conclusion, the present study proved the neuroprotective activity of GBGNPs and GBEs against acute restrain stress causes neurobehavioral alterations and oxidative damage.

GBGNPs treated group had modest activity as compared to GBE treated group.

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