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Neurotherapeutic effects of *Ganoderma lucidum*: Biochemical characterization and behavioral assessment in animal models

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Abstract
Neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, are characterized by progressive neuronal loss, oxidative stress, and impaired cognitive and motor functions. Natural compounds with antioxidant and neuroprotective properties offer promising therapeutic potential. Rich in biologically active compounds such as triterpenoids and polysaccharides, *Ganoderma lucidum* may modulate key molecular pathways involved in neurodegeneration, positioning it as a promising agent for further investigation. This study aims to investigate the effects of *Ganoderma lucidum* on aging-related neurodegenerative processes, with a focus on its biochemical pathways, behavioural outcomes, and potential therapeutic relevance. The chosen dose of *G. lucidum* extracts is 1000mg/kg/day for all in present study. The Morris water maze test and Cook's pole climbing for passive avoidance were used to assess spatial memory. Using a Total Amyloid beta ELISA kit, estimated the amount of Aβ (1-40 and 1-42) in the brain preparation. This is followed by the estimation of the levels of *in-vivo* antioxidant enzymes, such as the levels of lipid peroxidation (LPO) or malondialdehyde (MDA), glutathione content (GSH), superoxide dismutase (SOD) activity, and catalase (CAT) activity in the brain preparation. In comparison to the STZ control group and the sham, sham+aCSF, and vehicle treated groups, the ICV STZ rats treated with *G. lucidum* extract shown a notable improvement in the ICV STZ groups. These findings suggest that *Ganoderma lucidum* exerts multi-targeted neuroprotective effects and holds potential as a natural therapeutic agent for the management of neurodegenerative disorders.

Keywords: Neurodegeneration, alzheimer, *Ganoderma lucidum*, streptozocin (STZ), *in-vivo* antioxidant, biochemical, behavioural studies

Introduction
Neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and amyotrophic lateral sclerosis (ALS), are characterized by progressive loss of structure and function of neurons, leading to cognitive, behavioral, and motor dysfunction. These conditions predominantly affect the elderly, posing significant medical, social, and economic challenges due to the aging global population. According to the World Health Organization, Alzheimer's disease alone accounts for 60-70% of dementia cases worldwide, and its prevalence is expected to triple by 2050 [1, 2].

Despite ongoing advancements in understanding the pathophysiology of these disorders including amyloid-β accumulation, tau protein hyperphosphorylation, oxidative stress, mitochondrial dysfunction, and chronic neuroinflammation-effective disease-modifying treatments remain elusive. Most current pharmacological therapies only offer symptomatic relief and do not halt or reverse disease progression. Moreover, long-term use of conventional drugs often comes with side effects and limited efficacy [3]. This therapeutic gap has sparked growing interest in alternative treatments, particularly natural compounds derived from medicinal plants and fungi, which may offer multi-target neuroprotective effects with fewer side effects. Natural agents often possess antioxidant, anti-inflammatory, anti-apoptotic, and neuroregenerative properties, making them promising candidates for combating the complex etiology of neurodegenerative diseases.

Among these, *Ganoderma lucidum*-a traditional medicinal mushroom used for centuries in East Asian medicine-has attracted scientific attention for its potential in promoting brain health, longevity, and cognitive resilience. Rich in biologically active compounds such as triterpenoids and polysaccharides, *Ganoderma lucidum* may modulate key molecular pathways involved in neurodegeneration, positioning it as a promising agent for further investigation [4]. The enhanced capacity to create medical mushrooms artificially has led to their continued

widespread usage as traditional medicinal components for the treatment of numerous illnesses and related health issues. *Ganoderma lucidum*, commonly known as Reishi or Lingzhi, is a medicinal mushroom that has been revered for over two millennia in traditional Chinese and Japanese medicine. It has been traditionally used to promote longevity, enhance vitality, improve immune function, and combat age-related ailments. Often referred to as the "mushroom of immortality," *Ganoderma lucidum* is known for its adaptogenic and restorative properties, especially in aging populations [5, 6].

This study aims to investigate the effects of *Ganoderma lucidum* on aging-related neurodegenerative processes, with a focus on its biochemical pathways, behavioural outcomes, and potential therapeutic relevance. The findings could contribute to the development of evidence-based, natural interventions for age-associated neurological decline.

2. Materials and Methods

2.1 Plant Material

The fine powder of fruit body of 30-days old matured *G. lucidum*, trade named RG (from Reishi Gano) in the form of capsules RG 270mg each, procures from DXN, Daehsan Trading (India) Pvt. Ltd. 600g of powdered form of Reishi Gano (RG) has taken. Fractionation has been done according to the polarity of the solvent in the batch of 200g at a time.

2.2 Drugs and Chemicals Required

Streptozocin, β-Amyloid (1-40 and 1-42) Estimation Kit were obtained from Sumit Biosciences Pvt Ltd., Mumbai, India. All other chemicals and reagents were purchased commercially by local vendors and were of analytical grade.

2.3 Acute Toxicity Studies

Acute toxicity is involved in the estimation of LD_{50} (the dose which has proved to be lethal to 50% of the tested group of animals). LD_{50} evaluation was conducted in two stages for every fraction. In phase one, 48 fresh rats with four groups of three rats each, were administered with 10, 100 and 1000 mg extract/kg body weight orally (p.o.) accordingly. 1% gum acacia was given to the control groups, which were the fourth group for each fraction. Within 24 hours, the rats were monitored for clinical indications of poisoning, and within 72 hours, they died. 60 fresh rats, three per group, were administered with 500, 1000, 1500, and 3000 mg extract/kg (p.o.) accordingly, based on the extracts' phase one results. One percent gum acacia was given to the control groups, which were the fifth group in each fraction. After then, for seven days, clinical indicators of toxic consequences and death were monitored [7].

2.4 Experimental Animals

Male Wistar rats (8-10 months old, 220-250 g) were obtained from R.V. Northland Institute, Lucknow, and housed in polyacrylic cages under standard conditions (22±2°C, 60-65% humidity, natural light-dark cycle). The animals were divided into twelve groups of seven, with a maximum of four per cage, and had free access to dry food and water. Behavioral tests were conducted between 9 a.m. and 5 p.m. All procedures were approved by the IAEC (protocol no. RVNI/IAEC/24-25/22) and adhered to CPCSEA guidelines.

3. Experimental Procedure

3.1 Method to Induce Alzheimer's Disease

Male Wistar rats (220-250 g) were anesthetized with xylazine (5 mg/kg, I.P) and ketamine (100 mg/kg, I.P.), and placed in a stereotaxic apparatus. A midline scalp incision was made, and bilateral holes were drilled in the skull using coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm ventral from the brain surface. Streptozotocin (STZ), dissolved in citrate buffer (pH 4.4), was injected into the lateral ventricles (10 µl, 1 µl/min) on days 1 and 3 using a Hamilton syringe.

The study involved twelve different groups of seven rats each with different treatment protocols

Table 1: Groups of Animal Design According to their Treatment Schedule

Groups	Treatment
Group I	Sham Operated (SH) (Sham-operated rats wherein the surgery was performed minus drilling of holes and placement of the cannula).
Group II	(SH+ aCSF) artificial cerebrospinal fluid (aCSF) was infused ICV in a volume of 10 µl in each ventricle on day 1 and 3.
Group III	STZ Control (STZ with 1% gum acacia, used as a vehicle for G. lucidum extracts) (On days 1 and 3, rats were given ICV STZ (3 mg/kg) dissolved in aCSF in 10 µl volumes in each ventricle. The animals were also given 1% gum acacia as a vehicle for G. lucidum extracts.) 21 days.
Group IV	STZ+RGHW: Rats treated with 1000 mg/kg/day, p.o. hot water extract of RG (RGHW) for 21 days was given ICV STZ on days 1 and 3 as well as just after the initial STZ infusion.
Group V	STZ+RGHA: Hydroalcoholic extract of RG (RGHA) was administered to rats infused with ICV STZ at a dosage of 1000 mg/kg/day, p.o., for 21 days after the first STZ infusion.
Group VI	STZ+RGCH: For 21 days after the first STZ infusion, rats given ICV STZ were given chloroform extract of RG (RGCH) at a dosage of 1000 mg/kg/day, p.o.
Group VII	STZ+RGPEt: Rats given ICV STZ on days 1 and 3 as well as just after the initial STZ infusion were given 1000 mg/kg/day of RG petroleum ether extract (RGPEt) for 21 days.
Group VIII	*Per Se* group of normal animals were treated with 1% Gum acacia (as vehicle for the extracts of *G. lucidum*)
Group IX	*Per Se* group of normal animals were treated with 1000 mg/kg/day, p.o. of RGHW
Group X	*Per Se* group of normal animals were treated with 1000 mg/kg/day, of RGHA
Group XI	*Per Se* group of normal animals were treated with 1000 mg/kg/day, p.o. of RGCH
Group XII	*Per Se* group of normal animals were treated with 1000 mg/kg/day, p.o. of RGPEt

*Every group containing 7 rats in each i. e. n=7 for each group.

- RGPEt- Petroleum Ether Extracts
- RGCH- Chloroform Extract
- RGHA- Hydro alcoholic Extract
- RGHW- Hot Water Extract

3.2 Behavioural Studies

3.2.1 Passive Avoidance Task (Step-down latency)

This test assesses learning and memory in rodent models of neurodegeneration using a modified Cook's Pole-Climbing

setup, where a wooden platform replaces the pole. Male Wistar rats (~250 g) were trained in a low-light, sound-absorbing chamber with a 25×25×40 cm area and an electrified grid floor. A light and sound served as the conditioned stimulus (CS), followed by a 1.5 mA scrambled foot shock (unconditioned stimulus, US). Test sessions consisted of a maximum of 25 trials or 60 minutes, whichever occurred first, with a minimum intertrial interval of 90 seconds. If a rat climbed the pole before the 30-second response window ended, the remaining time was added to the inter-trial interval. While no consequences were assigned to responses during the intertrial period, rats that made more than ten such responses were excluded from the study. To qualify for experimental substance testing, rats were required to demonstrate at least 80% avoidance responses without any escape failures. Additionally, on the first day, animals underwent the passive avoidance test, during which step-down latency was recorded using a cut-off time of 60 seconds.

3.2.2 Morris Water Maize (MWM)

The Morris water maze test was conducted between days 14 and 18 after ICV STZ injection using a modified white circular tank (165 cm circumference, 100 cm deep) filled with opaque water (20-22°C) to a depth of 37 cm. The tank was divided into four quadrants with starting points at N, S, E, and W. A hidden escape platform (10 cm diameter) was placed in the center of quadrant II. Visual cues such as a door, windows, and an air conditioner were present in the room, and a ceiling-mounted camera recorded the trials. Rats' heads were marked with black dye for tracking, and the tank was cleaned daily. Each rat is first placed on the platform for 20 seconds to observe room cues, then released into a randomly selected quadrant with its head facing the wall. If the rat doesn't find the platform within 60 seconds, it's guided to it. After locating the platform, the rat remains there for five seconds before being removed. This process is repeated across trials, ensuring each rat starts from a different quadrant each time. Trials continue until all rats consistently find the platform in under five seconds. Data is recorded and learning curves are charted for each animal, plotting trial number against time to platform (TTP) [8].

3.3 Biochemical Characterization

3.3.1 β-Amyloid (1-40 and 1-42) Estimation

Using the use of the COVANCE BetaMark Total Beta Amyloid ELISA kit, Catalogue No.: SIG-38966-kit, which is imported and provided by Sumit Biosciences Pvt Ltd, located in Mumbai, India. The BetaMark Total Beta Amyloid ELISA kit (SIG-38966-kit) methodology was followed for the preparation of the brain sample and the Total Beta Amyloid ELISA process. This kit included all the chemicals and solutions that were required [9].

Protocol for the preparation of brain samples for BetaMark™ Beta Amyloid ELISA

Soluble and Insoluble Tissue Fractionation

To extract brain Aβ, homogenize 1 g of tissue in TBS with protease inhibitors (5 mL) using a glass/Teflon homogenizer on ice. Centrifuge at 350,000 g for 20 minutes and collect the supernatant (S1). Resuspend the pellet using either 1% Triton/0.6% SDS or 70% formic acid, incubate, then spin again to collect the detergent or acid-soluble extract (E1). Adding 2 mM EDTA can enhance the buffer [10].

Whole Tissue Extraction

This protocol produces a total Aβ extract by homogenizing tissue in TBS with protease inhibitors and EDTA. After homogenization, Triton X-100, SDS (30%), or formic acid (1%) is added, mixed gently to avoid foaming, and centrifuged at 350,000 g for 20 minutes. The supernatant (whole brain extract) is then collected for analysis.

3.3.2 Chemiluminescent BetaMark™ Total Beta Amyloid ELISA Kit

Preparation of Standard Intermediates

Label label two microcentrifuge tubes as #1 and #2 and add 990 µL of standard diluent to each. Reconstitute a 20 mg Beta Amyloid standard in 80 µL of diluent (final concentration 250 µg/µL) and use the same day. Add 10 µL of this to tube #1, mix gently, then transfer 10 µL from tube #1 to tube #2 and mix. The final concentration in tube #2 will be 25 ng/mL.

Preparation of Working Incubation Buffer

To prepare the working incubation buffer, label a 50 mL centrifuge tube accordingly. Mix 10 mL of 2X incubation buffer with 10 mL of RODI water to make a 1X solution. Then, add 5 mL of HRP detection antibody to the buffer and mix thoroughly using a vortex.

Preparation of Standard Curve

Label eight 1.5 mL microcentrifuge tubes (#1-8). Add 960 µL of working incubation buffer to tube #1 and 400 µL to tubes #2-8. Add 40 µL from intermediate tube #2 (25 ng/mL) to tube #1 to create the top standard point (1000 pg/mL), and mix gently by inversion. Prepare 2-fold serial dilutions by transferring 400 µL from each preceding tube into the next, mixing by inversion between each step.

Sample Preparation

Dilute samples in working incubation buffer. Mix well by inversion. Run samples in duplicate or triplicate.

Running the Assay

The ELISA plate was unwrapped and washed with 1X wash buffer prepared by diluting 125 mL of 5X buffer in 500 mL RODI water. After washing and drying, 100 µL of each standard was added in triplicate according to the plate layout.

3.4 *In-vivo* Antioxidant Screening

3.4.1 Lipid peroxidation (LPO) or Malondialdehyde (MDA) levels

Brain tissues were homogenized in 0.1 M sodium phosphate buffer (pH 7.4). To 0.1 mL of tissue, acetic acid, thiobarbituric acid, and SDS were added, then heated at 100°C for 60 minutes. After cooling, distilled water and n-butanol:pyridine were added, vortexed, and centrifuged. The organic layer was collected, and absorbance was measured at 532 nm. A standard curve was prepared using tetramethoxypropane (1-10 nmol/mL). [11][12]

3.4.2 Glutathoione Content

Brain tissues were homogenized and centrifuged with 5% trichloroacetic acid to extract proteins. The homogenate was mixed with phosphate buffer, DTNB [5-dithiobis-(2-nitrobenzoic acid)], and water, then incubated at room temperature for 15 minutes before reading absorbance at 412 nm. A standard curve using known GSH concentrations was plotted to calculate the reduced glutathione content [12].

3.4.3 Superoxide Dismutase Activity (SOD)
Brain tissues were incubated with sodium pyrophosphate buffer, phenazine methosulphate, nitro blue tetrazolium, NADH, and homogenized samples for 90 minutes at 30°C. After adding acetic acid and n-butanol, the mixture was centrifuged, and the organic layer was measured for absorbance at 560 nm. A standard curve using known superoxide dismutase (SOD) concentrations was plotted to calculate the SOD content [13].

3.4.4 Catalase Activity (CAT)
Brain homogenate was mixed with phosphate buffer, distilled water, and H_2O_2 to start the reaction, which was allowed to proceed for one minute. The reaction was stopped with potassium dichromate-acetic acid reagent and heated in a boiling water bath for 15 minutes. After cooling, absorbance was measured at 570 nm, with a control sample processed without H_2O_2 for comparison [13, 14].

3.5 Statistical Analysis
The data were expressed as mean ±SEM. Statistical differences at $p<0.05$ between the groups were analyzed by one-way ANOVA using Graph Pad Prism software package.

4. Results and Discussion
4.1 Percentage Yield of the Extract
After the calculating of percentage yield there were 2.32, 3.28, 7.1 and 27.22% of extract had been found for petroleum ether, chloroform, hydroalcoholic and hot water fractions respectively.

4.2 Acute Toxicity Studies (LD_{50})
The LD_{50} were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose that is the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase. It was observed that the dose of 3000mg/kg of extract was tolerated by the experimental rats. The maximum dose is given and well tolerated for the human has been seen as 10g/day in case of serious illness. So, the chosen dose of *G. lucidum* extracts is 1000mg/kg/day for all in present study. [13]

4.3 Behavioual Studies
4.3.1 Passive Avoidance Task (step-down latency)
Extracts of *G. lucidum* treatment in ICV STZ infused rats had been shown the significantly increased retention latencies as compared with ICV STZ group in order of STZ+RGHW> STZ+RGHA> STZ+RGCH> STZ+RGPEt with compared to the RGHW Per Se, RGHA Per Se, RGCH Per Se, RGPEt Per Se respectively [$P<0.05$ ICV STZ vs. ICV STZ+RGHW group and $P<0.05$ ICV STZ vs ICV STZ+RGHA]. [7]
The decreased mean retention latency in ICV STZ rats was significantly attenuated by RGHW and RGHA treatment (49.98±3.03 and 61.06±3.77 respectively, $P<0.05$) indicating improved acquisition or retention of memory. Maximum improvement in retention latency on day 15 was observed with RGHW and RGHA treatments in ICV STZ rats at a dose of 1000g/kg having improved acquisition retention on memory as 33.32±2.041 and 36.07±2.101 ($P<0.001$). On the other hand, normal rats treated with RGHW per se and RGHA per se did not show any significant change in retention latency, as compared with those of sham, sham+aCSF and vehicle treated groups ($p>0.05$).

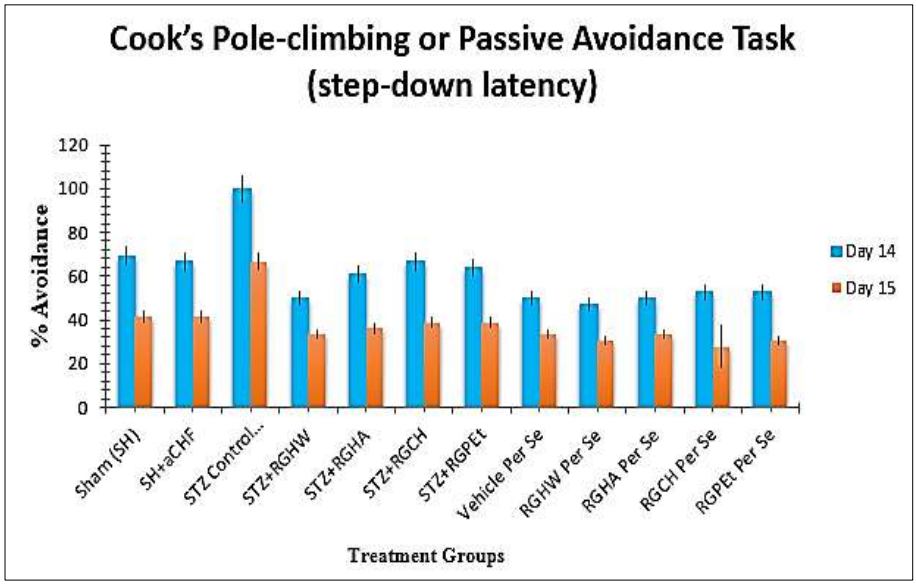

Fig 1: Effect of extracts of *G. lucidum* on memory performance in passive avoidance task in ICV STZ infused rats.

4.3.3 Morris Water Maize (MWM)
The mean latencies were found to be significantly prolonged even on day 18 =56; day 19=49 and day 38 in the ICV STZ group of animals as compared to those of sham, sham+aCSF and vehicle groups, (on day 18 =21, 22, & 22; day 19=11, 10 & 10 and day 20=6, 7 & 6) showing poorer learning performance. Amongst the extracts of *G. lucidum* were used in the present study, the RGHW and RGHA were found to be most effective in ICV induced STZ spatial memory deficit. On the other hand, administration of RGHW per se and RGHA per se at a dose of 1 µg/5µl/ ventricle in normal animals had been significantly modify basal learning performance of animals during 4 days of training as compared to sham, sham+ aCSF and vehicle treated groups ($P>0.05$).

Time Spent in Target Quadrant

Table 2: Effect of *G. lucidum* extracts on percentage of time spent in target quadrant in ICV STZ infused rats.

Groups	Time spent in Target Quadrant (Sec)			
	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Sham (SH)	9	6	11	13
SH+aCHF	11	10	8	11
STZ Control (STZ+Vehicle)	8	17	14	13
STZ+RGHW	14	13	16	18
STZ+RGHA	7	6	11	13
STZ+RGCH	8	19	12	11
STZ+RGPEt	10	6	13	12
Vehicle *Per Se*	8	7	12	11
RGHW *Per Se*	11	13	12	12
RGHA *Per Se*	10	8	13	15
RGCH *Per Se*	9	11	14	13
RGPEt *Per Se*	11	15	12	16

Result expressed as mean value of time (sec) spent in each quarter respectively

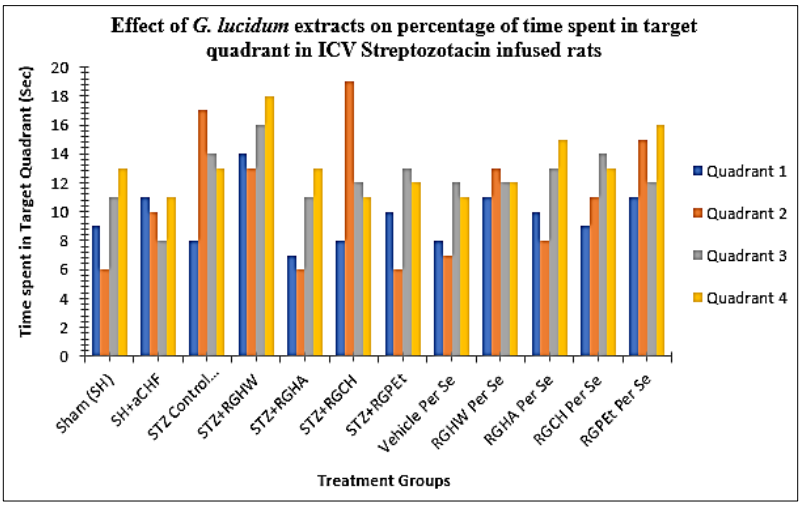

Fig 2: Effect of *G. lucidum* extracts on the percentage time spent in target quardrant in ICV STZ infused rats

G. lucidum extracts treatment in ICV STZ infused rats significantly attenuated STZ induced memory deficits compared with ICV STZ group [$P<0.05$ vs. ICV STZ group, $p< 0.05$ vs. ICV STZ and RGHW, RGHA, RGCH & RGPEt groups on day 21] and $p< 0.05$ vs. ICV STZ and RGHW *per se*, RGHA *per se*, RGCH *per se* & RGPEt *per se* groups on day 21]. *G. lucidum* extracts significantly improved memory in ICV STZ-infused rats, as shown by increased time spent in the target quadrant during the Morris water maze probe trial. Compared to the STZ control group, treated rats showed reduced memory deficits, with RGHW and RGHA groups demonstrating the most notable improvements, indicating enhanced memory consolidation.

4.4 Biochemical Characterization

4.4.1 β-Amyloid (1-42) Estimation

The Aβ40 and Aβ42 levels were analyzed by multi point one-way analysis of variance (ANOVA) between the control groups (sham, sham+aCSF and vehicle treated) and ICV STZ control vs. ICV STZ induced *G. lucidum* extracts treatment groups as compared with per se groups respectively and within the ICV STZ treated with extracts groups followed by Dunnet test. $P<0.05$ was considered statistically significant.

Table 3: Estimation of $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in homogenize brain preparation of treatment groups with different extracts of *G. lucidum.*

S. No.	Groups	Concentration	Luminescence Count	
		Pg/ml	$A\beta_{40}$	$A\beta_{42}$
1.	Sham (SH)	200	8.1	2.7
2.	SH+aCHF		8.6	2.6
3.	STZ Control (STZ+Vehicle)		6.5	8.9
4.	STZ+RGHW		9.1	2.8
5.	STZ+RGHA		8.9	2.9
6.	STZ+RGCH		8.2	3.2
7.	STZ+RGPEt		7.8	4.1
8.	Vehicle *Per Se*		8.9	2.3
9.	RGHW *Per Se*		9.2	1.8
10.	RGHA *Per Se*		8.5	2.1
11.	RGCH *Per Se*		8.4	2.3
12.	RGPEt *Per Se*		8.1	2.5

The animals treated with *G. lucidum* extracts, such as RGHW, RGHA, RGCH and RGPEt significantly decreased Aβ42 levels as compared with those of ICV STZ injected control rats [STZ control=8.9 vs. STZ+RGHW, STZ+RGHA, STZ+RGCH & STZ+RGPEt=2.8, 2.9. 3.2 & 4.1 respectively; $P<0.001$]. RGHW Per Se, RGHA Per Se, RGCH Per Se & RGPEt Per Se treatments in normal rats having normal Aβ40 and Aβ42 levels which remained similar to those of sham control, sham+aCSF and vehicle treated groups ($P>0.05$). Significant value with ANOVA analysis for the decrease in Aβ42 level shown between the ICV STZ induced extracts treated groups were found to be the STZ+RGHW and STZ+RGHA had been better result than STZ+RGCH and STZ+RGPEt, treatment groups. Presence of the increased level of Aβ42 confirm the pathogenesis of AD; but in treatment groups; ICV STZ control group shown the increased level of Aβ42 levels then the other groups and it was found to be significantly decreases in the extract treated ICV STZ groups.

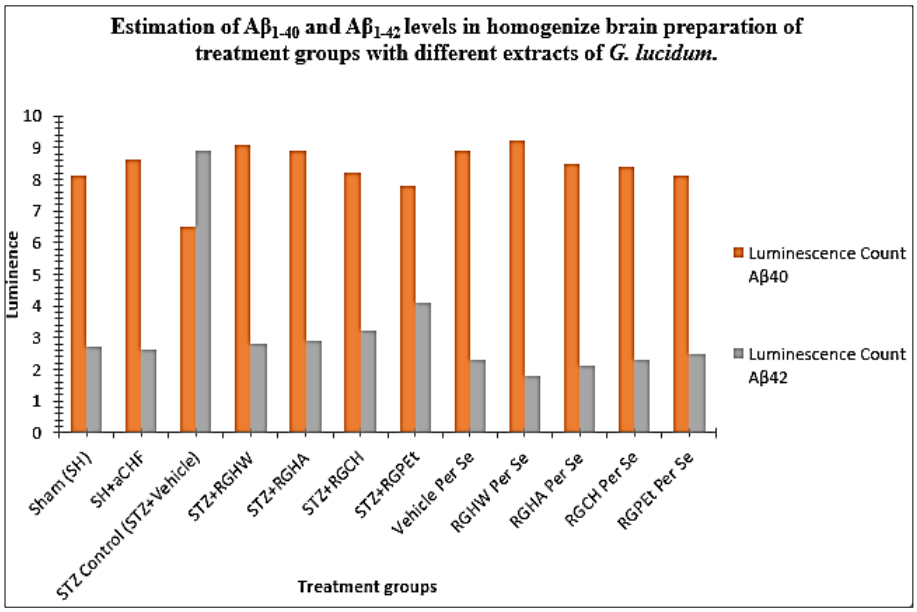

Fig 3: Estimation of Aβ1-40 and Aβ1-42 levels in homogenize brain preparation of treatment groups with different extracts of *G. lucidum*

4.5 *In-vivo* Antioxidant Screening

4.5.1 Lipid Peroxidation (LPO) or Malondialdehyde (MDA) Levels

The levels of LPO significantly in ICV STZ injected rats as compared to those of sham control, sham+aCSF and vehicle treated rats ($P<0.001$). But the treatment of these animals with *G. lucidum* extracts, such as RGHW, RGHA, RGCH and RGPEt significantly increased LPO levels as compared with those of ICV STZ injected control rats [STZ control=8.9 vs. STZ+RGHW, STZ+RGHA, STZ+RGCH & STZ+RGPE t=2.8, 2.9. 3.2 & 4.1 respectively; $P<0.001$]. RGHW Per Se, RGHA Per Se, RGCH Per Se & RGPEt Per Se treatments in normal rats having normal Aβ40 and Aβ42 levels which remained similar to those of sham control, sham+aCSF and vehicle treated groups.

Significant value with ANOVA analysis for LPO activity shown between the ICV STZ induced extracts treated groups were found to be the STZ+RGHW STZ+RGHA and STZ+RGCH was found to be almost same, but in STZ+RGPEt, group, LPO level was slightly down than other treatment groups.

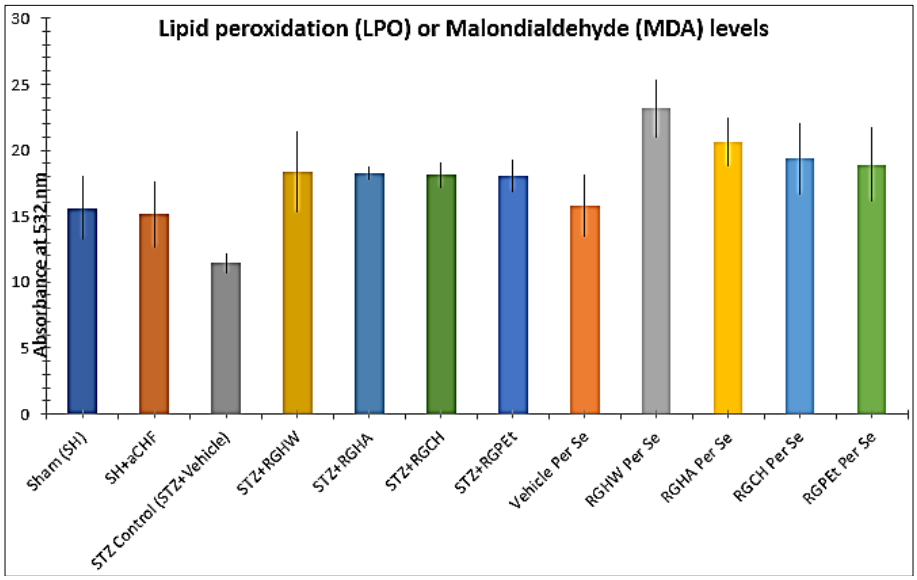

Fig 4: Lipid Peroxidation (LPO) or Malondialdehyde (MDA) levels in brain sample of treatment groups

4.5.2 Glutathione Content

Glutathione (GSH) levels in brain tissue were quantitatively measured by recording absorbance at 412 nm using a spectrophotometer, and the results were expressed in nmol/mL of supernatant. The levels of GSH significantly in ICV STZ injected rats as compared to those of Sham control, Sham+aCSF and vehicle treated rats (P<0.001). But the treatment of these animals with *G. lucidum* extracts, such as RGHW, RGHA, RGCH and RGPEt significantly increases GSH levels as compared with those of ICV STZ injected control rats [STZ control=11.92±0.65 vs. STZ+RGHW, STZ+RGHA, STZ+RGCH & STZ+RGPEt=16.61±2.32, 16.25±1.16, 16.02±2.36 & 15.96±2.71 respectively; P<0.001]. RGHW Per Se, RGHA Per Se, RGCH Per Se & RGPEt Per Se treatments in normal rats having normal GSH levels which remained similar to those of sham control, sham+aCSF and vehicle treated groups.

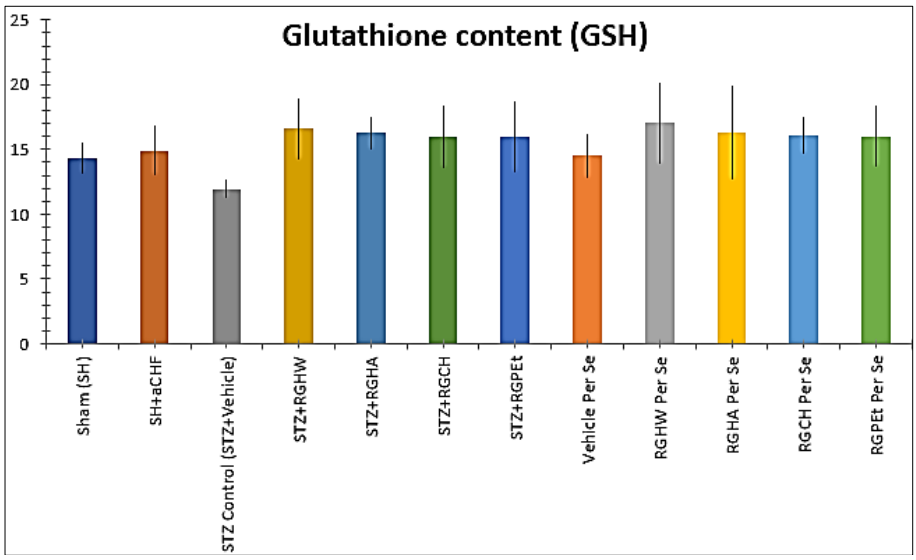

Fig 5: Glutathione content (GSH) levels in brain sample of treatment groups. Absorbance read at 412 nm of spectrophotometer

4.5.3 Superoxide Dismutase (SOD) Activity

The levels of SOD significantly in ICV STZ injected rats as compared to those of sham control, sham+aCSF and vehicle treated rats (P<0.001). But the treatment of these animals with *G. lucidum* extracts, such as RGHW, RGHA, RGCH and RGPEt significantly increases SOD levels as compared with those of ICV STZ injected control rats [STZ control=4.03±0.74 vs. STZ+RGHW, STZ+RGHA, STZ+RGCH & STZ+RGPEt=7.42±0.57, 6.99±1.73, 6.36±2.31 & 6.03±1.26 respectively; P<0.001]. RGHW Per Se, RGHA Per Se, RGCH Per Se & RGPEt Per Se treatments in normal rats having normal SOD levels which remained similar to those of sham control, sham+aCSF and vehicle treated groups.

Significant value with ANOVA analysis for SOD activity shown between the ICV STZ induced extracts treated groups were found to be the STZ+RGHA, STZ+RGPEt, and STZ+RGCH had been almost same results, but STZ+RGHW group shown somewhat better result than other treatment groups.

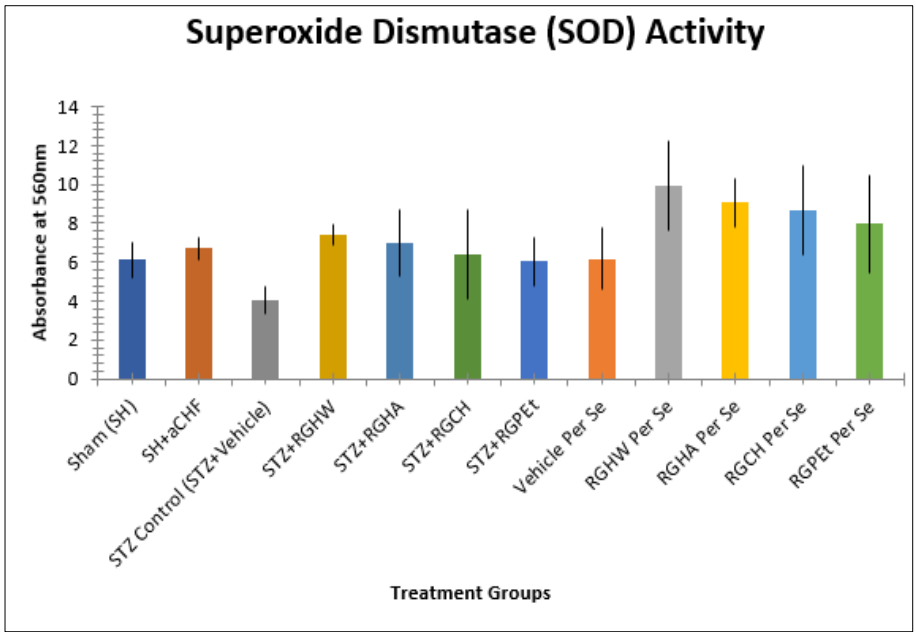

Fig 6: Superoxide dismutase (SOD) content levels in brain sample of treatment groups. Absorbance read at 560 nm of spectrophotometer

4.5.6 Catalase (CAT) Activity

The levels of CAT significantly in ICV STZ injected rats as compared to those of sham control, sham + aCSF and vehicle treated rats (P<0.001). But the treatment of these animals with *G. lucidum* extracts, such as RGHW, RGHA, RGCH and RGPEt significantly increased CAT levels as compared with those of ICV STZ injected control rats [STZ control=13.59±1.03 vs. STZ + RGHW, STZ+RGHA, STZ+RGCH & STZ + RGPEt=13.59±1.03, 13.06±2.05, 12.86±1.45 & 12.68±2.36 respectively; P<0.001]. RGHW Per Se, RGHA Per Se, RGCH Per Se & RGPEt Per Se treatments in normal rats having normal CAT levels which remained similar to those of sham control, sham+aCSF and vehicle treated groups.

Significant value with ANOVA analysis for CAT activity shown between the ICV STZ induced extracts treated groups were found to be the STZ + RGPEt and STZ+RGCH had been slightly better result than STZ+RGHW and STZ+RGHA groups.

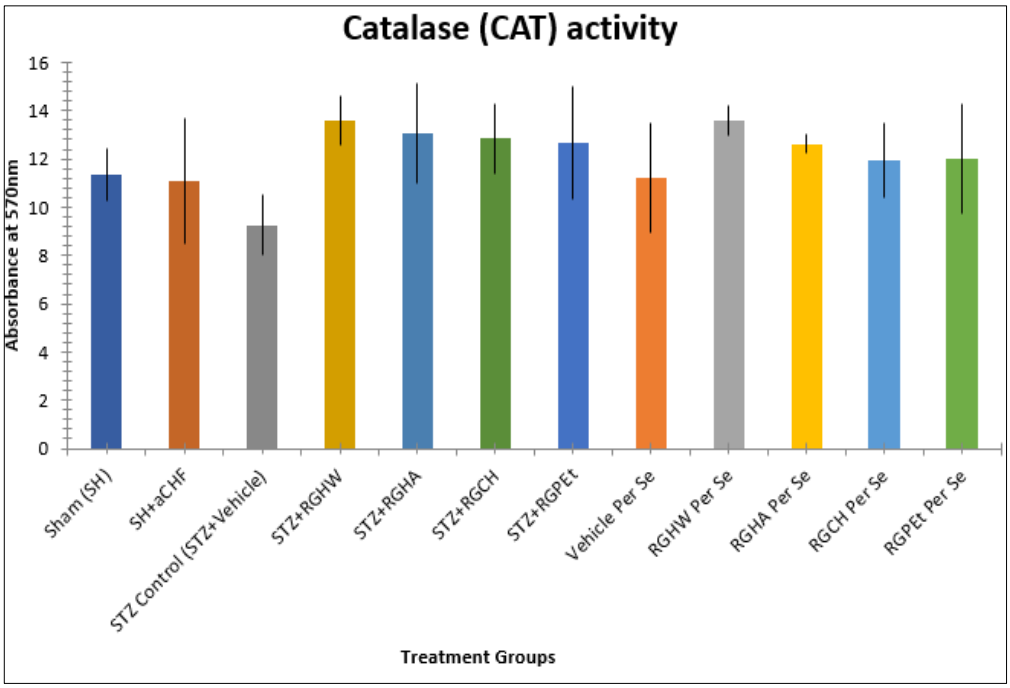

Fig 7: Catalase (CAT) Activity content levels in brain sample of treatment groups. Absorbance read at 570 nm of spectrophotometer

5. Conclusion

Acute toxicological studies are conducted in two stages to determine the appropriate dosage for treatment. *G. lucidum* had been well tolerated at the higher dosage, the treatment dose in this trial was 1000 mg/kg/day p.o. The Morris water maze test and Cook's pole climbing for passive avoidance were used to assess spatial memory. Using a Total Amyloid beta ELISA kit, estimated the amount of Aβ (1-40 and 1-42) in the brain preparation. This is followed by the estimation of the levels of *in-vivo* antioxidant enzymes, such as the levels of lipid peroxidation (LPO) or malondialdehyde (MDA), glutathione content (GSH), superoxide dismutase (SOD) activity, and catalase (CAT) activity in the brain preparation. In comparison to the STZ control group and the sham, sham+aCSF, and vehicle treated groups, the ICV STZ rats treated with *G. lucidum* extract shown a notable improvement in the ICV STZ groups. Petroleum ether, chloroform, hydroalcoholic, and hot water were used in order to further fractionate the fine powder of *G. lucidum* that was obtained from the DXN for this investigation. These findings suggest that *G. lucidum* exerts its therapeutic effects through a combination of reducing amyloid plaque accumulation, enhancing antioxidant defences, and mitigating oxidative stress and neurodegeneration. However, further research, including clinical trials, is essential to fully understand the molecular mechanisms underlying these effects and to confirm its clinical applicability as a therapeutic intervention for AD and other neurodegenerative diseases.

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