

## Full Length Research Paper

# Therapeutic role of glucogalactan polysaccharide extracted from *Agaricus bisporus* on trimethyltin chloride induced neuropathy in rats

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Trimethyltin (TMT) chloride induces limbic system neuro-degeneration, resulting in behavioral alterations like cognitive deficits. This study investigates the effect of glucogalactan polysaccharide (GA) extract, which was purified from the roots of *Agaricus bisporus* mushroom, on trimethyltin chloride (TMT) induced neuropathy in rats. Adult male rats (200 ±10 g) were divided into four groups that were fed with basal diet throughout the experiment (28 days). The first group (G I) was control group, the second group (GII) was treated with 300 mg/kg BW GA intraperitoneally (i.p.) daily for 28 day. The third group (GIII) was administered i.p. with 8.0 mg TMT /kg body weight (BW), and the fourth (G IV) was treated like the third group and was injected with GA 300 mg/kg BW daily for 28 day after 48 h of TMT. Many bioactive compounds, which were found in GA did not cause any changes in the second group compared to normal control group. The results reveal that GA given 48 h after TMT treatment has excellent neuropathy effect, lowers the average of MDA, HSP70, homocystein and the neurotransmitters in brain tissue homogenate; they were markedly reduced by the administration of GA to almost normal levels. Neurotransmitters and nitric oxide were significantly increased in the group given GA treatment compared to TMT group. The comet assay for DNA revealed that, TMT induced statistically significant (P<0.05) increase in the mean value of the tail length and tail moment of the rats. They significantly decreased after GA treatment, suggesting alleviated oxidative stress mediated by TMT. GA administered TMT-treated rats had improved brain histology, diminished level of MDA and nitric oxide (NO) in brain tissue and enhanced total antioxidant capacity in serum compared to TMT group. It could be concluded that GA plays a positive role in the improvement of brain function after TMT-induced neuropathy. Taken together, our results suggest that GA will be useful in developing strategies for protecting nervous system and improving the brain.

**Key words:** Trimethyltin, neuro-degeneration, *Agaricusbisporus*, glucogalactan.

## INTRODUCTION

Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. Nowadays, biochemical and nutritional researchers pay more attention to mushroom polysaccharide due to its various biological functions in food, health care or

medicine; it has antioxidant, immunostimulatory and antitumor effects (Li et al., 2009). Basidiomycetes present different kinds of glucans and heteropolysaccharides. The common monosaccharide composition of these polysaccharides is glucose, galactose, xylose, mannose

and fucose. Generally, (1→3), (1→6)- $\beta$ -glucans are extracted from these mushrooms, and also galactomannans, heteroglycans, and fucogalactans (Moradali et al., 2007). Mushrooms included in the same genera show more similarities in their composition, including the structure of carbohydrates (Zhang et al., 2007). Mushroom polysaccharides have traditionally been used for the prevention and treatment of a multitude of disorders like infectious illnesses, cancers and various autoimmune diseases. Bioactive polysaccharides are recognized by membrane receptors in leukocytes and macrophages, leading to proliferation and differentiation of immune cells (Moradali et al., 2007). These activities are responsible for enhancing the innate and cell-mediated immune responses, and consequently, for the induction of antitumoral and bactericidal effect (Lull et al., 2005). Besides the well-known antitumor  $\beta$ -(1→3)-glucans, a wide range of biologically active glucans with other structures have been described. These polysaccharides have linear or branched molecules in a backbone composed of  $\alpha$ - or  $\beta$ -linked glucose units, and they contain side chains that are attached in different ways. Heteroglucan side chains contain glucuronic acid, xylose, galactose, mannose, arabinose, or ribose as a main component or in different combinations. Glycans, in general, are polysaccharides containing units other than glucose in their backbone. They are classified as galactans, fucans, xylans and mannans by the individual sugar components in the backbone. Heteroglycan side chains contain arabinose, mannose, fucos, galactose, xylose, glucuronic acid and glucose as a main component or in different combinations. Mushroom polysaccharides exert their antitumor action mostly via activation of the immune response of the host organism. These substances are regarded as biological response modifiers (Wasser and Weis, 1999). This basically means that: (1) they cause no harm and place no additional stress on the body; (2) they help the body to adapt to various environmental and biological stresses; and (3) they exert a nonspecific action on the body, supporting some or all of the major systems, including nervous, hormonal, and immune systems, as well as regulatory functions (Brekman, 1980).

The number of mushrooms with known pharmacological qualities is much lower still. Isolation and purification of polysaccharides from mushroom material is relatively simple and straightforward, and can be carried

out with minimal effort. Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and submerged culture is the best technique for obtaining consistent and safe mushroom products (Borchers et al., 1999; Wasser et al., 2000). Therefore, discovering novel structurally and biologically polysaccharides from mushroom, especially those unexploited species has become a hot spot of great interest. The present study was carried out in an attempt to investigate the neuro-protective effect of GA polysaccharide from *Agaricus bisporus* in rats.

## MATERIALS AND METHODS

### Laboratory animals and experimental design

Dawley rats (weighing  $180 \pm 20$  g) were purchased from the laboratory animal colony at the Institute of Ophthalmology, Cairo University, Egypt. All rats were provided with food and water *ad libitum* throughout the experimental period. The experiment was carried out in accordance with the guidelines of the experimental animal ethics. After seven days of acclimatization, rats were randomly assigned into four groups (eight per group); the first group (GI) acting as a control received saline (0.5 ml) for 28 days. The second group (GII) received GA (300 mg/kg) intraperitoneally (i.p.). The third group (GIII) rats were injected intraperitoneally (i.p.) with TMT (8.0 mg/kg, body weight) dissolved in 0.9% saline and then received saline after 48 h of TMT for 28 days. The fourth group (GIV) was administered GA (300 mg/kg, i.p.) like the second group after 48 h of TMT. Anesthetic procedures and handling with animals were approved by and complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt (Approval number 10082).

### Chemicals

Trimethyltin chloride (TMT) was purchased from Sigma-Aldrich Co. (St. Louis MO), and solvents were from E. Merck (Darmstadt, Germany).

### Extraction and purification of the polysaccharide

Fresh fruit bodies (1 kg) of *A. bisporus* were collected from Bloschia Mushroom Company in Dokki, Egypt (Mahmoud et al., 2014). The sample was washed with distilled water and boiled in a water bath for 6 h. The mixture was kept overnight at 4°C and filtered through fresh linen cloth. The filtrate was centrifuged at 5000 rpm (Sigma-Laborzentrifugen, 2K 215, Sigma Co., and D37520 Osterode-am-Harz, Germany) for 45 min at 4°C. The supernatant solution was collected and the polysaccharide (CAB) was precipitated with EtOH

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**Abbreviations:** CA1, Cornu ammonis 1; CA3, cornu ammonis 3; AB, water soluble; ABI, water insoluble; CAB, crude of aqua's polysaccharide; CSF, cerebrospinal fluid; AchE, acetylcholine esterase; DA, dopamine; Da, dalton; EtOH, ethanol; EPS: exopolysaccharide; 5-HT, serotonin; MDA, malondialdehyde; NO, nitric oxide; IL-1, interleukin 1; IL-3, interleukin 3, NaOH, sodium hydroxide; GMA, glucogalactan from mushroom (*Agaricus bisporus*); FTIR, Fourier transforms infrared; KBr, potassium bromide; i.p, intraperitoneal.

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(1:5, v/v). After keeping the precipitated material in the mixture overnight at 4°C, it was centrifuged at 4°C for 1 h, and then the residue was freeze-dried (1.5 g). The dried material was dissolved in 4% NaOH solution and re-precipitated with ethanol. The re-precipitated material was collected through centrifugation and dissolved in minimum volume of water. The solution was then dialyzed against distilled water for 30 h (3x 1000 mL) to remove alkali and low molecular weight materials. During this dialysis, one portion got precipitated from this solution. The whole dialyzed solution was centrifuged at 8000 rpm and 4°C. The water soluble (AB) and insoluble (ABI) parts were lyophilized separately (Hana et al., 2010).

### Isolation and purification of exopolysaccharide

Inoculum was prepared by transferring one loop full of culture from *A. bisporus* slant to an Erlenmeyer flask (250 mL) containing 50 mL seed medium containing (g/L) sucrose, 20; yeast extract, 2; peptone and 75% sea water (Jiang et al., 1999). The seed cultures were grown at 37°C on a rotary shaker incubator at 150 rpm for 24 h. After incubation, 3 mL of the seed culture was transferred into an Erlenmeyer flask (250 mL) containing 50 mL of fermentation medium (g/L) sucrose, 50; peptone, 4; yeast extract, 2 in 75% sea water pH 7.0 (Read and Costerton, 1987). The fermentation cultures were then incubated at 37°C with shaking at 150 rpm for three days. The exopolysaccharide (EPS) sample was prepared from *A. bisporus* culture in the fermentation medium. The fermented broth was collected and centrifuged at 5000 rpm and 4°C for 20 min. It was dialyzed three times (1000 mL x 3) under a flowing tap-water, in a dialysis tube (MWCO 2000) for 24 h. The dialyzed solution was precipitated with four volume chilled ethanol; the precipitate was washed with acetone, diethyl ether and dried at 50°C until constant weight. The crude EPS was re-dissolved in deionized water and forced through a filter (0.45 mm); it was then applied to a column (2.5 x 70 cm, i.d.) of DEAE-cellulose. After loading with sample, the column was eluted with gradient NaCl solution (0.0 to 1.0 M), and the procedure was monitored by the phenol-sulfuric acid method mentioned above. One polysaccharide active fraction (GA) was collected, dialyzed and lyophilized. GA was used for activity assessment and structural analysis (Asker et al., 2009). Nevertheless, other macromolecules, such as proteins, may be also present in the medium. Therefore, several purification steps must be carried out to remove other substances. The samples were subjected to methanolic extraction in order to remove phenolic compounds, monosaccharides, amino acids and other related molecules. The elimination of phenolic derivatives was successful (Palacios et al., 2011), and it increased the effectiveness of the extraction (Park et al., 2009). Proteins were removed by precipitating with trifluoroacetic acid (20%, w/v) or treating with enzyme protease at 40°C for 1 h (pH 7.5). The proteins were separated by centrifugation. After protein removal, polysaccharides were precipitated from the supernatants by the addition of ethanol in 2:1 ratio (v/v). Concentrated sodium chloride solutions were added to favor the precipitation, and the solid was washed with organic solvents, such as acetone or ethanol. Once the polysaccharides were separated from other compounds, pure carbohydrate fractions were obtained; however, each fraction contains several polysaccharides showing different molecular sizes. Fractionation was performed by precipitating with ammonium sulfate.

### Chemical analyses

The purified GA (50 mg) was subjected to hydrolysis with 6N HCl for 4 h at 100°C in a sealed tube. Excess acid was removed by evaporation in a water bath at a temperature of 40°C and co-distilled with water (1 mL x 3) (Sudhamani et al., 2004). Uronic acid

contents were determined by measuring the absorbance at 525 nm using the m-hydroxybiphenyl colorimetric procedure and with glucuronic acid as the standard (Filisetti-Cozzi and Carpita, 1991). Sulfate was measured using the turbidimetric method (Dodgson and Price, 1962) with sodium sulfate as standard. N-acetyl glucose amine was estimated by the Morgan and Elson reaction (1934). UV-vis spectroscopy analyses were conducted on ultraviolet-visible-near-infrared spectrophotometer (2401PC (Shimadzu, Japan). The polysaccharide solution was prepared by suspending the sample in distilled water to a concentration of 1.0 mg/mL for UV-vis measurement in the wavelength range of 190 to 700 nm. Monosaccharide compositions of the GA were determined by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm x 30 cm, i.d.), using deionized water as the mobile phase (flow rate 0.5 mL/min), as described by El-Sayed et al. (2005).

### Molecular weight determination

The molecular weight of the polysaccharide was determined by gel permeation chromatography (GPC) on Agilent 1100 series, Germany, Detector: Refractive Index FPI gel particle size (5µm), 3 columns of pore type (100, 104 and 105 Å) on series, length 7.5 x 300 mm (1000, 5000000 Da) for DMF solvent Styrogel HR-DMF, 3 µm (7.8 x 300 mm), Water Company Ireland; one column (5000 to 600000 Da) for water solvent (polyethylene oxide/glycol standard) PL aqua-gel-OH 7.5 mm and 30 µm pore type; 8 µm particle size; PL aqua-gel-OH 7.5 mm, 50 µm pore type and 8 µm particle size, in series Mw from 100 to 1250000 g/mol. The sample (0.01 g) was dissolved in 2 ml of solvent, and then filtrated by siring filter (0.45). Then the sample was put in GPC device. The polydispersity index was calculated from the Mw/Mn ratio (You et al., 2013).

### Infrared spectroscopy

The Fourier-transform infrared (FT-IR) spectrum of the polysaccharide was measured using a Bucker scientific 500-IR Spectrophotometer. The exopolysaccharide was mixed with KBr powder, ground and pressed into 1 mm pellets for FTIR measurements in the range of 400 to 4000 cm<sup>-1</sup> (Ray, 2006).

### In-vitro antioxidant studies

#### Free radical scavenging effect

The free radical scavenging activity of AB and standard compounds at different concentrations (75, 150, 300 and 600µg/ml) was measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) using the method of Yamaguchi et al. (1998). Briefly, 0.1 mM solution of DPPH in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of samples and standard solution Vitamin C (VC) and Butylated hydroxytoluene (BHT) at various concentrations of the polysaccharide. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophotometer (Schimadzu UV/VIS-2401PC). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical concentration in the reaction medium was calculated from the following equation: DPPH scavenging effect (%) = 100 - [(A0-A1)/A0] x 100, Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample of polysaccharide (Oktay et al., 2003).

### In-vivo study

#### Collection of blood and tissue samples

At the end of the 28th day of the trial, blood samples were collected

from all groups by cardiac puncture into clean dry centrifuge tubes. Subsequently, the brain tissues of the animals were excised. The blood samples collected were centrifuged for 15 min at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wasserman and kept frozen at  $-20^{\circ}\text{C}$  until analysis. The brain tissues were washed with deionized water for the removal of blood. Homogenization was performed in a phosphate buffer solution with pH value adjusted to 7.4, and later the supernatant was separated by means of centrifugation at 20,000 rpm for 1 h. The supernatant and hemolysate obtained were used for the analyses of certain parameters.

### Biochemical analysis

Lipid peroxide concentration was determined by measuring brain-malondialdehyde (MDA) according to the method of Yoshioka et al. (1979). Nitric oxide was determined according to Miranda et al. (2001). The acetylcholine esterase (AChE) was measured by the method of Ellman et al. (1961), which involves the formation of a yellow colour due to the reaction of thiocholine with dithiobisnitrobenzoate ions. Determination of homocysteine was carried out using HPLC (Varian Inc., CA, USA) attached to fluorescent HPLC detector according to the method of Dimitrova et al. (2001). Noradrenaline (NA) was measured using appropriate [ $^{125}\text{I}$ ] radioimmunoassay kit (IBL, Hamburg, Germany) while dopamine (DA) and serotonin (5 HT) were estimated in the brain tissues of rats according to Zagrodzka et al. (2000). Serum total antioxidant capacity was estimated in serum by colorimetric method according to the method of Koracevic et al. (2001). Total heat shock protein 70 (HSP70) levels were determined by ELISA technique (Life Science Inc.).

### Detection of oxidative DNA damage (comet assay)

To investigate the *in vivo* genotoxicity effect of the TMT, a single-cell gel electrophoresis analysis (comet assay) was used, which is a simple, rapid, and sensitive technique for detecting DNA damage at the level of individual eukaryotic cells (Singh et al., 1988; Zegura and Filipic, 2004). Crushed samples of 2 gm were transferred to 1 ml of ice-cold PBS. This suspension was stirred for 5 min and filtered. The cell suspension was mixed with an equal volume of 1% low-melting-point agarose at  $37^{\circ}\text{C}$  and quickly pipetted onto the first agarose layer in the same manner. Finally, 70ml of 0.5% low-melting-point agarose was added to cover the cell layer. The slides sandwiched without coverslips were immersed in freshly prepared cold lysing buffer [2.5 mol/l NaCl, 100 mmol/l  $\text{Na}_2\text{-EDTA}$ , 10 mol/l Tris, 1%N-lauroyl sarcosine sodium salt (pH 10) with 1% Triton X-100 added just before use] and kept at  $4^{\circ}\text{C}$  for 45 min to 1 h. The slides were placed on a horizontal gel electrophoresis platform and covered with cold alkaline buffer (300 mmol/l NaOH and 1 mmol/l  $\text{Na}_2\text{-EDTA}$ ) for 8 to 20 min in the dark at  $4^{\circ}\text{C}$  to allow DNA unwinding and expression of the alkali-labile sites. Electrophoresis was conducted at  $41^{\circ}\text{C}$  in the dark for 20 min at 25 V and 300 mA. The slides were then rinsed gently twice with neutralizing buffer (0.4 mol/l Tris, pH 7.5). Each slide was stained with 50ml of ethidium bromide at a concentration of 2 mg/ml and covered with a coverslip. They were then stored at  $4^{\circ}\text{C}$  in sealed boxes until analysis. A total of 100 randomly captured comets from each slide were examined at X 400 magnification using a fluorescence microscope connected to a CCD camera in an image analysis system [Comet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK)]. A computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components, and then evaluates the range of derived parameters. To quantify the DNA damage tail length (TL), the percentage of migrated DNA (tail DNA %) and tail moment (TM) were evaluated. TL (length of DNA migration) is related directly to

the DNA fragment size and presented in micrometers. It was calculated from the center of the cell. Finally, the program calculates TM.

### Histological examination

Autopsy samples were taken from the brain of rats in different groups and fixed in 10% formal saline for 24 h. Washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stain for routine examination through light electric microscope (Banchroft et al., 1996).

### Statistical analysis

The results were expressed as mean  $\pm$  SD. The differences among means were analyzed through one way analysis of variance (ANOVA) followed by Duncan's post hoc analysis and the values  $\leq 0.05$  were considered significant. SAS software version 9.0 was used for the statistical analysis.

## RESULTS

### Analysis of monosaccharide composition of *A. bisporus*

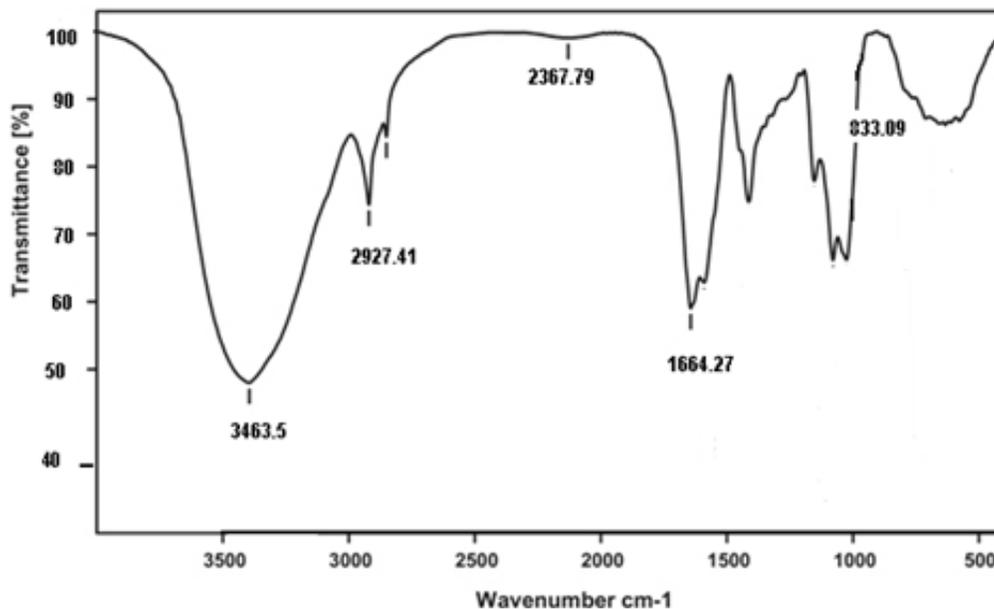
The semi-purified polysaccharide extract was analyzed for monosaccharide composition. The monosaccharide composition was analyzed by HPLC. The main monosaccharide of the AB and AB-I are glucose and galactose in a molar ratio of 6:1, respectively.

### Structural characterization of GA extract

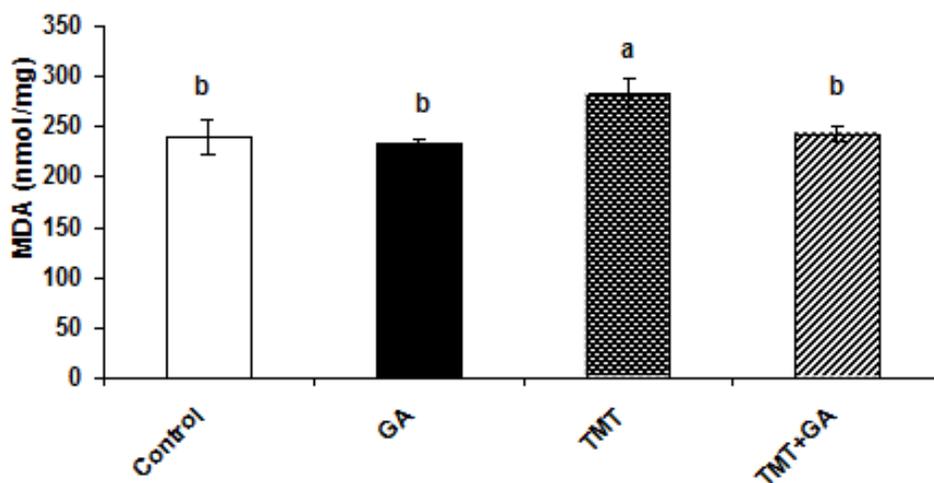
GABP extract from *A. bisporus* was confirmed by IR as shown in Figure 1. The bands in the region of  $3463.53\text{ cm}^{-1}$  were due to the hydroxyl stretching vibration of the polysaccharides. The bands in the region of  $2927.41\text{ cm}^{-1}$  were due to C-H stretching vibration, and the bands in the region of  $1664.27\text{ cm}^{-1}$  were due to associated water. The characteristic absorptions at  $833.09\text{ cm}^{-1}$  in the IR spectra indicated that  $\alpha$ - configurations were simultaneously present in *A. bisporus*. The actual molecular weight and distribution of the AB were determined by gel permeation chromatography (GPC). The polysaccharide in the GPC chromatogram (Figure 1) was widely dispersed molecules polydispersity index of 1.79 and had an overall weight average molecular weight (Mw) of  $2.08 \times 10^6\text{ g/mol}$  and number average molecular weight (Mn) of  $1.61 \times 10^6\text{ g/mol}$ .

### Effect of GA on lipid peroxidation

The results of the present study showed significant



**Figure 1.** Structural characterization of glucogalactan polysaccharide (GA) extract by FT-IR.



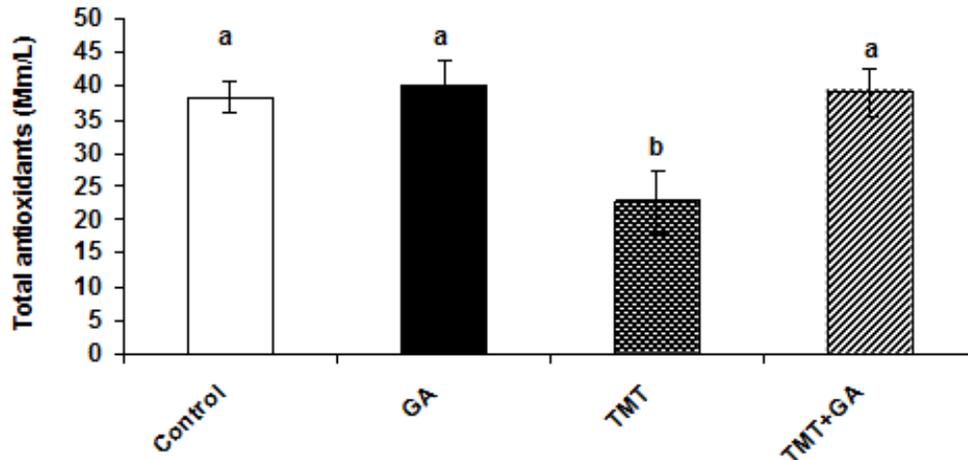
**Figure 2.** Impact of Trimethyltin (TMT) or glucogalactan polysaccharide (GA) on malondialdehyde (MDA): **a and b**: Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 3.347$ ,  $P < 0.05$ .

increase ( $p < 0.05$ ) of MDA contents in brain tissues of group of rats administrated with TMT compared to control group ( $282.69 \pm 15.6$  and  $239.32 \pm 17.42$ , respectively) (Figure 2). The elevation of MDA was significantly ( $p < 0.05$ ) decreased in group of rats administrated with TMT and treated with GA ( $243.15 \pm 9.50$ ). On the other hand, there was significant ( $p < 0.05$ ) decrease in the total antioxidants in brain tissues of group of rats administrated with TMT compared to control group ( $22.75 \pm 4.70$  and  $38.25 \pm 2.46$ , respectively); and this led to

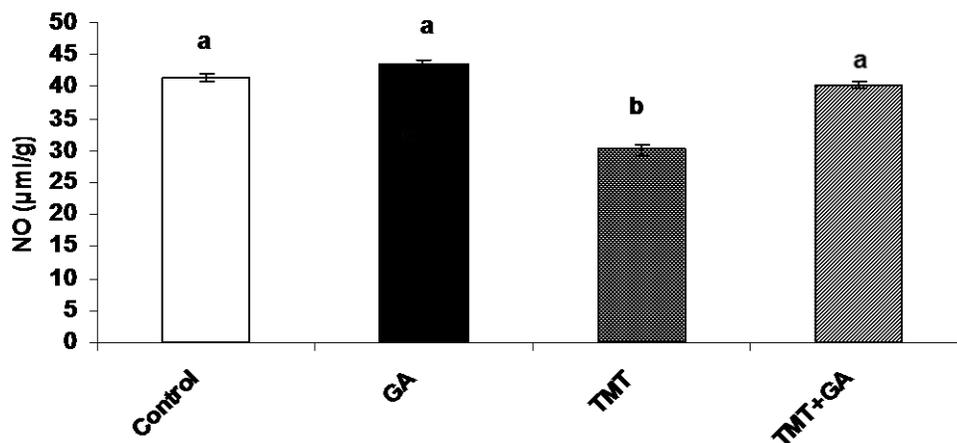
close to normal in the treated group ( $39.15 \pm 3.53$ ) (Figure 3).

#### **Effect of GA on NO and AchE in control and experimental groups**

The results revealed that TMT reduced NO and AchE concentrations in brain tissues compared to the control group. In contrast, the results indicate significant ( $p <$



**Figure 3.** Effect of treatments on total antioxidants in brain of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 4.46^*$ ,  $P < 0.05$ .



**Figure 4.** Effect of treatments on NO in brain of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 3.347$ ,  $P < 0.05$ .

0.05) increase of NO and AchE concentrations in brain tissues in group of rats administrated with GA after 48 h of TMT; and this concentration increased to close to normal group (Figures 4 and 5).

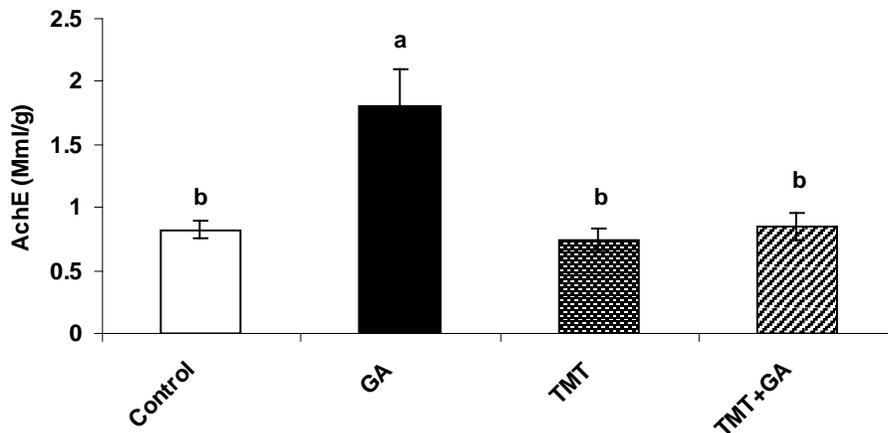
#### Effect of GA on neurotransmitters in control and experimental groups

The NA, DA and 5-HT concentrations in brain tissues were investigated. The results reveal that the concentrations of these neurotransmitters decreased significantly ( $p < 0.05$ ) in group of rats administrated with TMT as compared to control group. These concentrations increase significantly ( $p < 0.05$ ) in group of rats treated

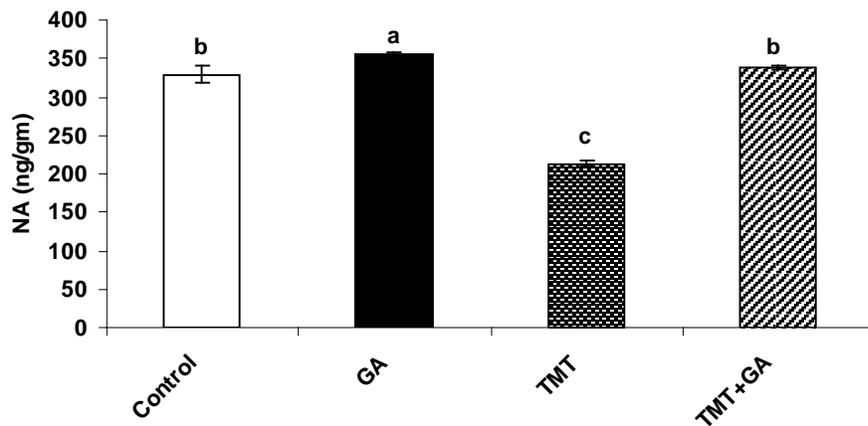
with GA for 28 days to close to normal as compared with group of rats administrated with TMT only (Figure 6a, b and c, respectively).

#### Effect of GA on tissues homocysteine and HSP70 in control and experimental groups

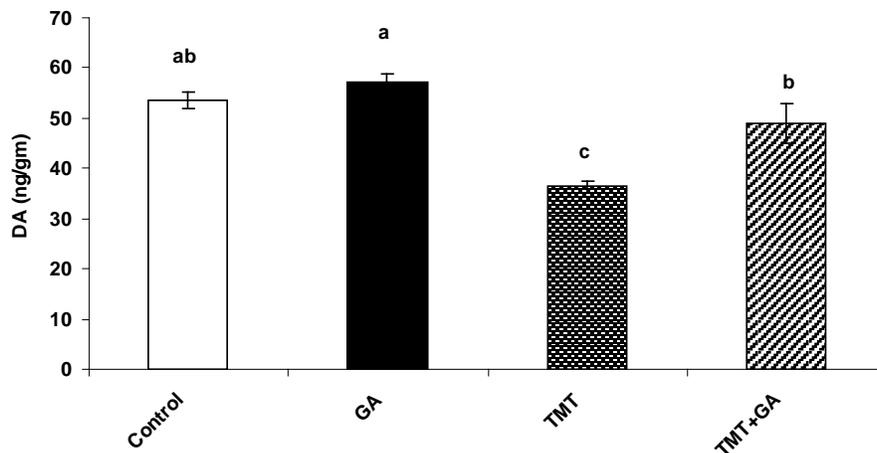
Homocysteine and HSP70 concentrations in brain tissues of the present study showed significant increase ( $p < 0.05$ ) in group of rats administrated with TMT compared to control group, and this increase normalized significantly ( $p < 0.05$ ) in group of rats administrated with TMT and treated with GA compared to group of rats administrated with TMT only (Figures 7 and 8, respectively).



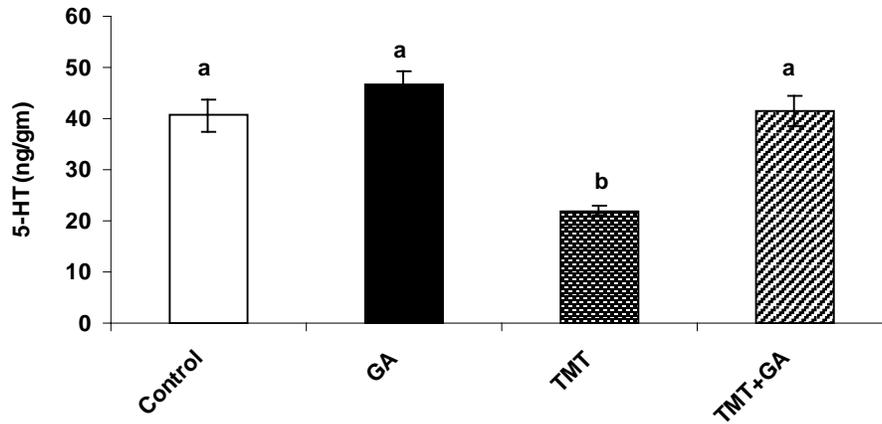
**Figure 5.** Effect of treatments on AchE in brain of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 9.638$ ,  $P < 0.05$ .



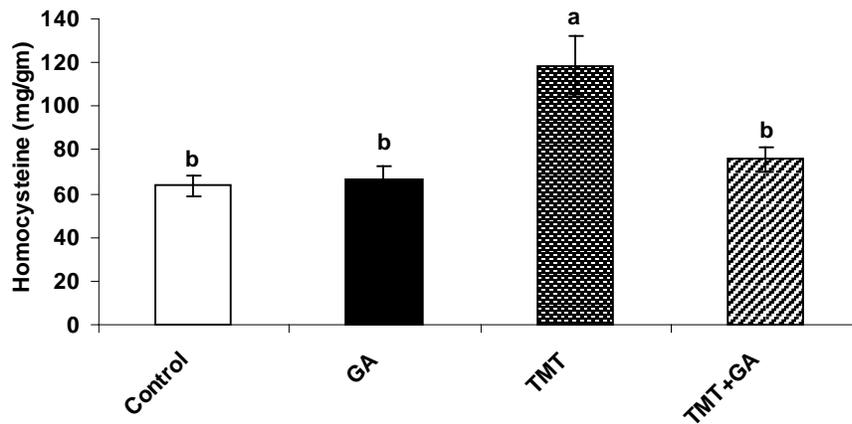
**Figure 6a.** Impact of treatments on NA in brain tissues of rats: **a, b and c:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 28.09$ ,  $P < 0.05$ .



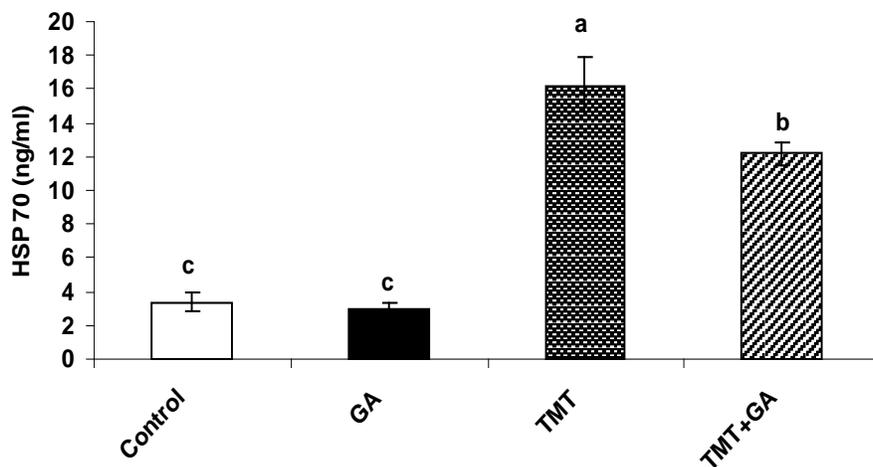
**Figure 6b.** Impact of treatments on DA in brain tissues of rats: **a, b and c:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 16.70$ ,  $P < 0.05$ .



**Figure 6c.** Impact of treatments on 5-HT in brain tissues of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 18.52$ ,  $P < 0.05$ .



**Figure 7.** Impact of treatments on homocystein in brain tissues of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 9.64$ ,  $P < 0.05$ .

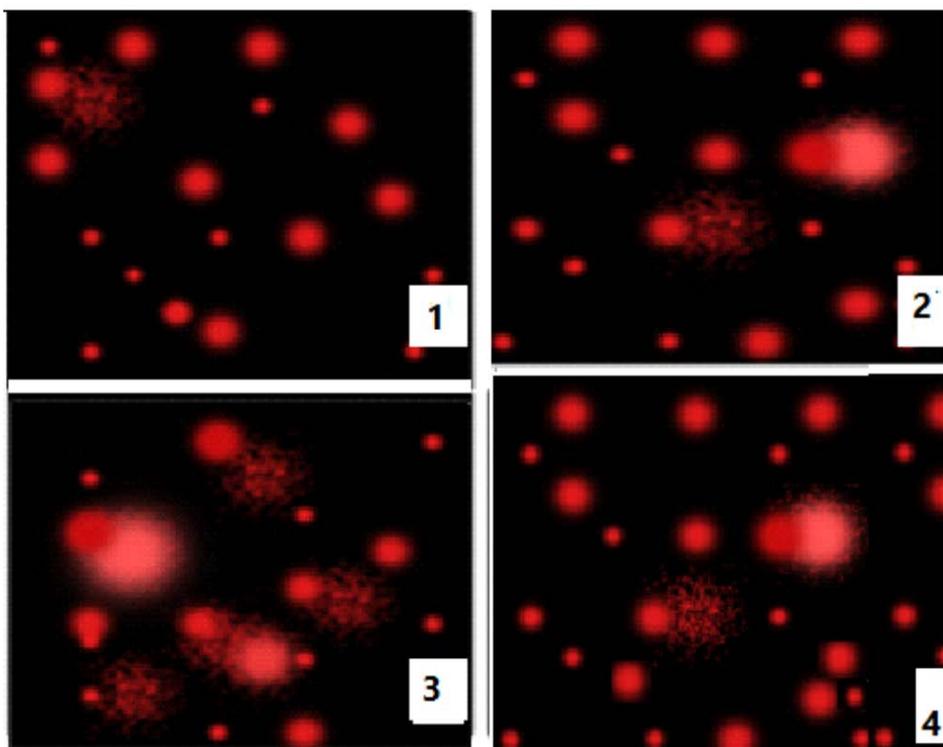


**Figure 8.** Impact of treatments on HSP70 in brain tissues of rats: **a and b:** Statistically significant from control or experimental groups at  $P < 0.05$  using one-way ANOVA followed by Duncan as a post-hoc test;  $F = 28.36$ ,  $P < 0.05$ .

**Table 1.**Effect of GA on genomic DNA in the control and experimental groups.

Groups	Control	GA	TMT	TMT+GA	F
Tail DNA (%)	1.79±0.11 <sup>c</sup>	1.95±0.18 <sup>c</sup>	5.90±0.03 <sup>a</sup>	3.37±0.72 <sup>b</sup>	27.38*
Tail length(µm)	1.76±0.07 <sup>c</sup>	1.96±0.01 <sup>c</sup>	5.96±0.72 <sup>a</sup>	3.05±0.6 <sup>b</sup>	30.63*
Tail moment(Units)	3.16±0.30 <sup>c</sup>	3.82±0.25 <sup>c</sup>	35.80±3.24 <sup>a</sup>	8.06±1.42 <sup>b</sup>	77.41*
Untailed DNA (%)	95.67±1.53 <sup>a</sup>	96.33±0.58 <sup>a</sup>	77.33±3.06 <sup>c</sup>	90.33±3.51 <sup>b</sup>	38.26*

Data expressed as mean ± SE. a, b and c: Statistically significant from control or experimental groups at P<0.05 using one-way ANOVA followed by Duncan as a post-hoc test; \* F (P<0.05).



**Figure 9.** Photographs showing the effect of GA treatment against TMT-induced DNA damage in the brain of rat as measured by the comet assay represented by Tail length (µm) and % of DNA damage in the brain cells from control (1), GA only treated rats (2), TMT treated rats (3) and TMT + GA treated rats (4).

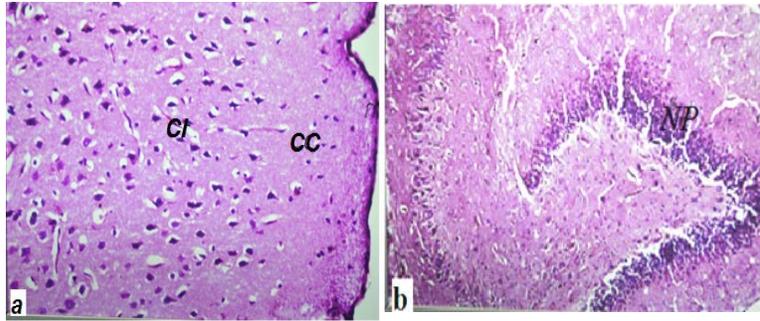
### DNA damage detected by the comet assay

Concerning the brain genotoxic potential of TMT using the comet assay, there was a significant increase in the tail length of DNA, tail intensity (DNA %) and tail moment in the TMT treated rats compared to the control (Table 1 and Figure 9). On the other hand, the treatment with GA significantly decreased DNA tail length, intensity and moment as compared to the TMT treated rats (Figure 9).

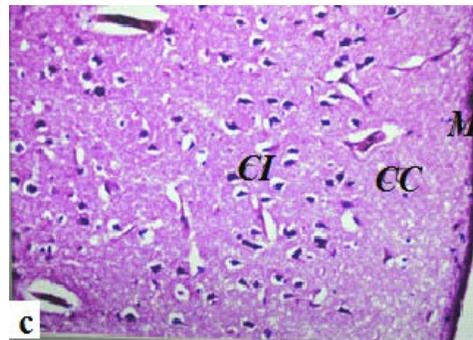
### Histopathological findings

The present study revealed that there was no

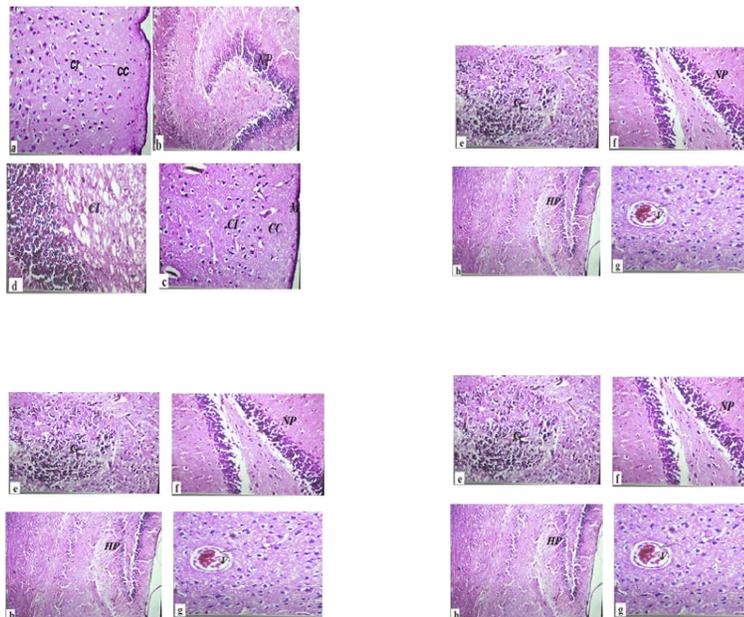
hisopathological alteration, and the normal histological structures of the meninges, cerebral cortex, cerebral striatum and hippocampus were recorded in the control group administrated with GA (Figures 10a, b and c, respectively). In group of rats administrated TMT, the cerebral striatum showed desquamation in the lining endothelium of the congested blood vessels with diffuse gliosis (Figure 10d). Diffuse gliosis was noticed also in between the neuronal cells of the hippocampus (Figure 10e). There was focal area of neuronal degeneration with gliosis in the cerebral striatum (Figure 10f). Encephalomalacia with vacuolization was detected in the cerebellum (Figure 10g); while group of rats administrated TMT and treated with GA showed significant improvement



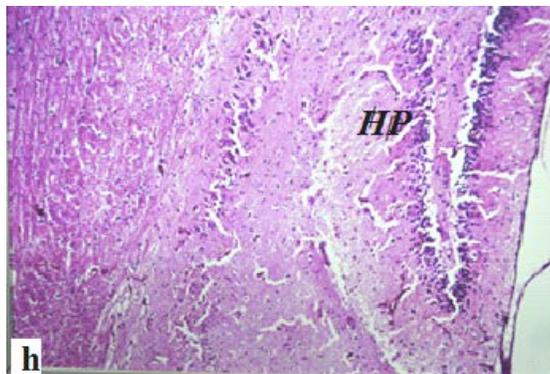
**Figure 10a, b:** Photomicrograph of a section in brain of control rat, there was no hisopathological alteration and the normal histological structure of the meninges, cerebral cortex, cerebral striatum and hippocampus.



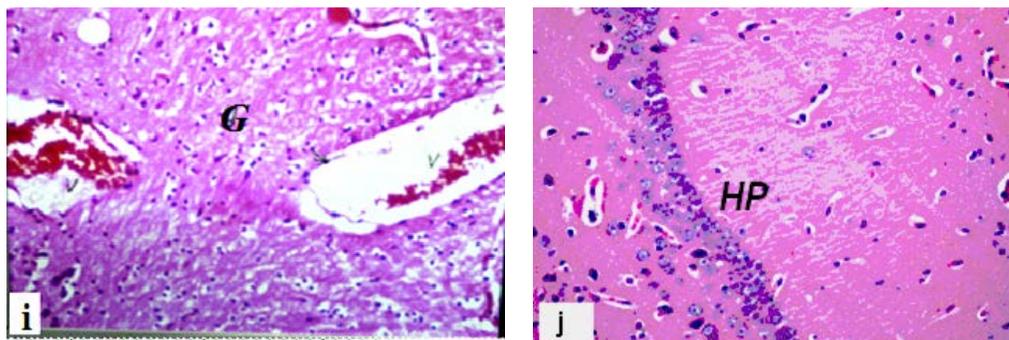
**Figure 10c:** Rats treated with GA only there was no histopathological alteration.



**Figure 10d, e, f and g.** Histopathology of brain TMT only treated rats. **(d).** Photomicrographs showing the cerebral striatum showed desquamation in the lining endothelium of the congested blood vessels with diffuse gliosis. **(e).** Diffuse gliosis was noticed also in between the neuronal cells of the hippocampus. **(f).** There was focal area of neuronal degeneration with gliosis in the cerebral striatum. **(g).** Encephalomalacia with vacuolization were detected I the cerebellum.



**Figure 10h.** Treated group with TMT+ GA with significant improvement in the brain tissues where the cerebral striatum showed slight congestion in the blood vessels.



**Figure 10i and j.** Treated group with TMT+ GA. (i). The cerebral striatum showed congestion in the blood vessels; while the hippocampus was histological normal. (j). Brain of rat in group four (TMT +GA) showing normal histological structure of the hippocampus (HP).

in the brain tissues where the cerebral striatum showed slight congestion in the blood vessels (Figure 10h), and the hippocampus was histologically normal (Figure 10i and 10j).

## DISCUSSION

Trimethyltin chloride (TMT) is a potent neurotoxicant that causes selective neuronal death specifically localized in the limbic system and particularly in the hippocampus of the mammalian brain (Chang, 1990). The molecular mechanisms by which TMT induces selective neuronal death are still not conclusively clarified; different pathogenetic pathways, probably acting differently *in vivo* and *in vitro* models, seem to be involved, including neuroinflammation, intracellular calcium overload, and oxidative stress (Geloso et al., 2011). In the present study, TMT acute exposure induced brain cell apoptosis and oxidative damage to rat brain, as evidenced by

significant rise in brain MDA (an end product of lipid peroxidation) levels; it also induced significant reduction in total antioxidant activities. The increase in tissue MDA levels in the present study was observed in the group that was administered TMT alone. This demonstrates that lipid peroxidation has developed. The occurrence of a significant increase in brain MDA levels compared to the control group is also indicative of damage to have been caused in the brain tissues examined, as a result of free radicals generated by TMT. The consequence of oxidative stress to nervous tissue is many, as brain is particularly vulnerable to oxidative stress due to its high rate of oxygen consumption. Oxidative stress induces many damaging processes in stress disorders such as mitochondrial dysfunction, dysregulation of calcium homeostasis, disruption of energy pathway (Amoroso et al., 2000), damage to neuronal precursors, impairment of neurogenesis (Papadopolos et al., 1997). After TMT administration, rats develop extensive lesions in the CA, typically localized in the CA3/Hilus and also involving CA1,

while granular neurons in the DG are generally spared (Moradali et al., 2007). Neuronal death shows a delayed onset (two days after treatment) and progressively worsens: it develops over three weeks (Geloso et al., 2011), probably on account of the high affinity of rat hemoglobin for TMT, involving CA3 earlier and more severely than CA1 (Corvino et al., 2012). TMT activates different pathogenic mechanisms leading to cell death; the approach based on gene profiling examination appears to be promising, since it provides a comprehensive snapshot of the molecular scenario. Several studies have demonstrated behavioral abnormalities such as increased seizure susceptibility, aggression, and learning impairment after TMT-induced neurodegeneration, which is consistent with results of this study (Bernaś et al., 2006). GA was found to be a highly potent antioxidant that could inhibit free radical generation in the brain. It has been shown that it inhibits the lipid peroxidation and prevents cell death induced by oxidative stress. Recent research has shown that some polysaccharides rich in mannose, glucose or galactose play important roles as free radical scavengers, ferrous metal ion chelators and reducers for the prevention of oxidative damage in living organisms (Tian et al., 2011).

The glucogalactan chains were composed of (1→6) (1→5)- and (1→5,6); the biologically active polysaccharides mainly in the form of  $\beta$ -D-glucans are extracted from these organisms, and also galactomannans, heteroglycans, and fucogalactans (Bi et al., 2013). Mushrooms included in the same genera show more similarities in their composition, including the structure of carbohydrates (Carbonero et al., 2008). In the present study, an increase in the levels of lipid peroxidation was found in TMT group and this was significantly reduced after treatment with GA. Recently, it has been found that polysaccharides have many potent biological and pharmacological activities, including immunostimulation as well as anti-tumor, anti-virus, anti-inflammatory and hypoglycemic activities (Wang et al., 2010). Mushroom polysaccharides have recently become attractive as food and as sources for the development of drugs. Hence, antioxidant status has been suggested as a useful tool in estimating risk of oxidative damage induced neurodegeneration. The present study shows that the treatment with TMT decreased total antioxidant activities. This observation is in agreement with Jung et al. (2013). While, the administration of GA to TMT – treated rats showed restoration of total antioxidant capacity towards normal. Several polysaccharides from fungi have exhibited strong antioxidant effects that are relevant to their health-protecting functions (Tseng, et al., 2008). The free radical of DPPH is stable and widely used to evaluate the free radical scavenging ability of natural compounds. The DPPH radical-scavenging activity is conceivably due to a hydrogen-donating ability.

In the present study, the biochemical analysis of brain tissue revealed that administration of TMT induced

significant increase in homocysteine and AchE levels and significant decrease in NO level. These results agree with previous findings that chronic experimental hyperhomocysteinemia lead to oxidative stress (Chao and Lee, 2000), decrease in NO bioavailability (Volman et al., 2010) and increase in brain AchE (Monteiro et al., 2005). The oxidative stress results from auto-oxidation of homocysteine and generation of free oxygen radicals (Hartl et al., 2011). Improvement in these parameters was observed in the group which was administered with GA treatment.

In this study, we evaluated the capacity of the mushroom extracts to stimulate the production of the neurotransmitters (NA, DA and 5 HT) in brain tissues. Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism (Reshetnikov et al., 2001). It is now well established that NO is a physiological mediator of the central nervous system. The role of NO in developing brain remains poorly understood, but it seems to be involved in the regulation of cerebral blood flow, and in memory acquisition. In fact, NO appears to be a double-edged sword, simultaneously neurotoxic and neuroprotective. Numerous experimental studies demonstrated the deleterious effects of nitrogen reactive species accumulation in ischemic-reperfusion cerebral injury through depletion of energy, lipid peroxidation, protein nitrosylation, DNA alterations and increased permeability of the blood brain-barrier (Arul and Konduri, 2009). Hypoxia-ischemia results in inflammation, especially in the developing white matter. High concentrations of NO and peroxynitrite produced locally by activated microglia may become toxic to neurons and immature oligodendrocytes *in vitro* (Li et al., 2005). It is also well known that hypoxia-ischemia results in the accumulation of extracellular glutamate, inducing the excitotoxicity cascade that causes neuronal death. More or less effective neuroprotection can be achieved by using NOS inhibitors that inhibit nNOS at the early phase and iNOS during the reperfusion of hypoxic insult (Margaill et al., 1994). An increase in brain infarct volume has been reported in sheep and rat when NO production is decreased by NOS inhibitors (Rosenberg et al., 1999). NO seems to be beneficial to the brain mostly through its vasodilator effects, and has proangiogenic effects potential.

In the current study, TMT induced significant increase in HSP70. This result agrees with Anderson et al. (1997). On the other hand, the administration of GA showed significant decrease in HSP70, but still greater than normal. Hsp70 proteins can act to protect cells from thermal or oxidative stress. These stresses normally act to damage proteins, causing partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially denatured proteins from aggregating, and

allows them to refold. Our data shows that TMT induced statistically significant increase in DNA damage which represented by tail moment in brain tissues. The DNA breaks that are detected in the comet assay and cytogenetic analysis may be resulted from the oxidative stress and enhancement of the intracellular generation of reactive oxygen species (ROS) formed by TMT (Sergent et al., 1999). These ROS can damage DNA and division of cells with unrepaired or misrepaired damage leading to mutations. This DNA damage could also originate from apoptotic cells. Several laboratories have reported that the onset of apoptosis can give comet images whose cell aspect and tail parameter values are the same with cells of moderate DNA damages (Choucroun et al., 2001).

## Conclusion

Mushrooms can be viewed as an important source of bioactive polysaccharides. The most common pattern consists of a  $\beta$ -linked glucose backbone displaying branches at certain sugar residues; nevertheless, other polysaccharides, such as galactans. These carbohydrates have been considered as biological response modifiers due to their ability to ameliorate the neurodegenerative induced by TMT through the improvement of some biochemical parameters in brain tissues and serum such as MDA, neurotransmitters, homocysteine and % tail DNA additionally, total antioxidant capacity and NO. Changes determined in the parameters examined may be reliable indicators of neurodegenerative impairment. Further studies are needed in order to proof its efficacy in the treatment of Alzheimer as a result of neurodegenerative *in vivo*.

## Conflict of interests

The authors did not declare any conflict of interest.

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