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QUERCETIN NANOPARTICLES REPRESSED LIVER AND BRAIN TOXICITIES INDUCED BY TARTRAZINE IN RATS

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ABSTRACT

Tartrazine (TZ) is an azobenzene artificial yellow dye for foods, drugs, and cosmetics. Quercetin is member of the flavonoid family. The current study investigated the protective effect of quercetin nanoparticles (QNPs) against TZ. Male albino rats were divided into group1: control, group 2: TZ (50 mg/kg), group 3: QNPs (5 mg/kg), and group 4: QNPs+TZ. The results of the present study revealed that, the oral administration of TZ dye caused significantly increased liver biomarkers. Also, a marked effect on lipid profile and blood parameters was shown. In addition TZ induced an elevation in the examined oxidative stress biomarkers and decrease in glutathione peroxidases and acetylcholine esterase. TZ diminished exploration and rearing in open field test as well as elevation in GABA content and apoptosis as well as changes in tissues by histopathological examination. In fact, the results showed good influence of QNPs in improving injuries associated with TZ administration.

Keywords: Tartrazine Dye, Quercetin Nanoparticles, Liver-Brain injuries.

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INTRODUCTION

A wide sort of food additives, running into more than 2,500 items used to preserve, dye or enhance foods [1, 2, 3] are a consequence of industrialization and the development of food processing technology. They are approved for human utilization after studying its acute, sub-acute and chronic toxicities. The individual response varies not only according to dose, age, gender, nutritional status and genetic factors, but also according to long term disclosure to low doses [4, 5, 6, 7].

Numerous metabolites of these substances, such as nitrous compounds, have been found to be carcinogens. Toxicity or benefit depends on to what degree food components affect absorption, excretion or the metabolism as a whole. Because there may be interaction between different substances, the clarity of adequate safety limits for human consumption is further compounded [8, 9].

Tartrazine (TZ) is an azobenzene artificial yellow dye whose structure features a tri-sodium salt of 3-carboxy-5-hydroxy-1 (p-sulfophenyl) -4-(sulfophenylazo) pirazolone. It is extensively used to dye sweets, chewing gum, jellies, puddings, juices, jams, mustard, sodas, drugs and cosmetics. It is reduced in the individual to an aromatic amine which is highly sensitizing. Its main metabolite identified to date is sulfanylic acid [10, 11, 12, 13].

Tartrazine is used as the food additive, most often responsible for allergic reactions. Although urticaria, asthma, purpura and eczema have been described, essentially affecting individuals allergy to aspirin, the underlying immunologic mechanism has proved elusive. Some studies have suggested a humoral immune response brought about by the sulfanyl group [14, 15, 16, 17]. Some countries such as Sweden, Switzerland and Norway have withdrawn TZ on the basis of its anaphylactic potential [18]. Several authors have studied the carcinogenetic and mutagenetic properties of TZ [19, 20, 21, 22, 7].

Quercetin is members of the flavonoid family [23]. It has anti-cancerogenic, antiviral, anti-ischemic, anti-inflammatory and antiallergenic, as well as defensive influence in atherosclerosis and coronary heart disease [24]. Actually flavonoids can prevent oxidative damage as a result of their ability to scavenge reactive oxygen species [25] and metal chelating [26]. Noteworthy, quercetin present in the human diet as vegetables, herbs and edible fruits. Even with the medicinal benefits of quercetin, it has low bioavailability (less than 17% in rats and even 1% in human) [27] due to its reduced aqueous solubility, as a result, the clinical application of this drug greatly restricted. Hence, it becomes necessary to build up a system which could increase the solubility of quercetin.

Nanoparticles are mainly suitable for drug delivery for water insoluble compounds such as quercetin [27]. According to Noyes-Whitney equation a decrease in particle size will lead to an increase in effective surface area which results in enhanced bioavailability [28].

In fact, the body has defense mechanisms against oxidative damage which are composed of enzymatic and non-enzymatic systems [29]. The enzymatic mechanism is made of free radical scavengers like catalase (CAT), superoxide dismutase (SOD) and the glutathione-depend enzymes such as glutathioneperoxidase (GPx), and glutathione-S-transferase [30, 31]. The non-enzymatic mechanism involves certain endogenous compounds in the body as reduced glutathione (GSH) and certain exogenous compounds taken as vitamins E and C, and flavonoids [29, 23].

The aim of this study was to determine the effect of TZ exposure on male albino rats and to assess whether these effects can be ameliorated by quercetin nanoparticles (QNPs) two hours after TZ.

MATERIAL AND METHODS

Materials

Food colors additives Tartrazine yellow (E102) was obtained from the local market and administered orally according to **Walton et al. (1999)** [22]. Chitosan (CH), medium molecular weight and viscosity 190–310 kDa, acetic acid, tripolyphosphate (TPP) and Quercetin nanoparticles powder with an average particle diameter of about 45 nm were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) kits were purchased from Spectrum-diagnostics Co. (Cairo, Egypt). Alkaline phosphatase (ALP), and acetylcholine esterase (AChE) kits were obtained from Diamond diagnostics. Glutathione peroxidase (GPx) kit was obtained from Randox diagnostics. Reactive oxygen species (ROS), lipid peroxidation (LPO), and protein carbonyl (PC), were obtained from Sigma Chemical Company (preparation in lab) according to method in paper.

All other chemicals used throughout the experiments were of the highest analytical grade available.

Preparation of CH /QNPs

A sequence of CH /QNPs beads were prepared based on were prepared by ionic cross-linking as the following method. Typically, 1.0 g of CH was dissolved using magnetic stirring in 50 mL of 0.1M acetic acid at 25°C for 20min until complete dissolved and viscose homogenous solution was obtained. Various amounts of QNPs; 0, 5%, 10% and 15% dispersed in 10 mL of distilled water were added slowly into the above CH solution. The obtained mixture was further stirred for 10min to obtain a homogenous system. Subsequently, the solution was extruded in the form of dewdrops, using a syringe (2 mm diameter), into a solution (50 ml) containing TPP (3.0 g) and NaOH (2.0 g). The beads were remained in the mixture of solution overnight so as to crosslink with TPP. The pH of the suspension was adjusted to 5 by adding 0.1 M NaOH and the stirring was continued for another 30 min. without pH adjustment.

Experimental Animals

Adult male albino rats (40 animals) weighing between 195 ± 10g were used in the present study. The rats were obtained from the animal house of the National Organization for Drug Control and Research Egypt (NODCAR). The animals were kept under standard laboratory conditions of light/dark cycle (12/12 h), temperature (20 -25°C), and allowed free access to food (standard pellet diet), water *ad libitum* for one week. Animals were treated gently; squeezing, pressure and tough maneuver were avoided. The investigation was complied with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Experimental design

Rats were randomly divided into four groups (n=10). Groups were treated as follows:

Control group rats were administrated with one ml saline solution.TZ group was given TZ (50 mg/kg, orally). QNPs group was given (5 mg/kg, orally). Rats of the protective groups were given (50mg TZ + 5mg QNPs, orally). The administered QNPs and TZ were given for 30 days.

1. Behavioral test; open field test:

Open field test was carried out at the end of the treatment period according to **Sethi *et al*, (2008)** [32], where the latency time, ambulation frequency, grooming frequency, and rearing frequency were measured.

2. Sample preparation

At the end of the experimental period, all rats were fasted for 12 hours and then the blood samples were collected from venous sinus in centrifuge tubes to separate serum by centrifugation at 3000 rpm for 15 minutes. Sera were kept at -80^{o}C for biochemical analyses. In addition, the liver and the cerebellum were dissected and isolated immediately, plotted free from adhering blood, and dried between two filter papers. Each tissue sample was then weighed. Parts from liver and cerebellum were kept in 10% formalin to examine histologically and the other parts were kept at -80^{o}C for biochemical examination in liver and cerebellum tissues.

3. Biochemical analysis

3.1- Measurement of blood parameters

Hemoglobin (Hb %) concentration, red blood cell (RBC) count and hematocrit (HCT%) were estimation using a semiautomatic hematological analyzer (SWELAB IEO Model). The auto counter utilized 20 µl of blood in 16 ml of a commercially prepared diluent. The machine's ability to count cells was based on the principle of electronic impedance. In addition, serum glucose level was as described by **Trinder, (1969)** method [33].

3.2- Measurement of liver functions

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were performed according to the method of **Reitman and Frankel, (1957)** [34]. Determination of alkaline phosphatase (ALP) was determined in serum according to the method of was determined by **Belfield and Goldberg, (1971)** [35].

3.3- Measurement of Lipid profile

Serum LDL-cholesterol concentration was estimated by the method of **Wieland and Seidel, (1983)** [36], for enzymatic colorimetric method. Serum HDL concentration was estimated by the method of **Burstein et al, (1970)** [37], for enzymatic colorimetric method. Besides, serum cholesterol and triglyceride concentration were estimated by the method described by **Zollner and Kirsch, (1962)** [38] that is an enzymatic colorimetric method.

3.4- Measurement of reactive oxygen species (ROS) content.

A modified version of a previously described assay for the intracellular conversion of nitro blue tetrazolium (NBT) to form azan by superoxide anion was used to measure the generation of reactive oxygen species [39].

3.5- Measurement of malondialdehyde content

Lipid peroxides formation was determined in liver and cerebellum homogenates as thiobarbituric acid reactive substances (TBARS). It was determined according to the method of **(Uchiyama and Mihara, 1978)** [40].

3.6- Measurement of glutathione peroxidase (*GPx)* activity:

Glutathione peroxidase activity determined in liver and cerebellum homogenates according to the method of **(Brigelius-Flohe, 1999)** [41].

3.7- Measurement of Protein Carbonyl Concentration:

Protein carbonyl concentration was measured in liver and cerebellum by first forming labeled protein hydrazone derivatives using 2, 4-dinitrophenyl hydrazide [42].

3.8- Measurement of Myelopreoxidase (MPO) Activity

Myeloperoxidase activity was evaluated in liver and brain tissues according to **Bradley et al, (1982)** [43].

3.9- Measurement of acetyl cholinesterase (ACHE) activity

The Procedure used for the determination of Cholinesterase activity in the cerebellum of rats is a modification of the method of **Ellman et al, (1961)** [44] as described by **Gorun et al, (1978)** [45]. The principle of the method is the measurement of the rate of production of thiocholine as a result of acetylthiochline hydrolysis.

3.10- Determination of GABA, 5-HT, NE and DA contents

Cerebellum g*amma-aminobutyric acid (*GABA), serotonin (5-HT), norepinephrin (NE) and dopamine (DA) were estimated according to method of **Ciarlone (1978)** [46]. The method is based on fluorometric test in which fluorescent product results from reaction with orthophthalaldehyde solution in case of 5-HT and reaction with alkaline sulfite and iodine solution in case of NE and DA.

3.11- Brain (cerebellum) and liver Caspase 3 Immunohistochemical Examinations

Paraffinized brain (cerebellum) and liver sections were deparaffinized and rehydrated through xylene and alcohol for immunohistochemical assessment of Caspase 3. Antigen unmasking was performed by incubating the sections for 20 min in citrate buffer (Thermo Fisher Scientific, Fremont, CA; pH 6.0) at the boiling point then cooled. Sections were then incubated overnight at 4 °C either with the rabbit polyclonal anti-Caspase 3 (1:200; Thermo Fisher Scientific) primary antibody. After washing with PBS, the slides were incubated for 30 min at 37 °C with the biotinylated secondary antibody then with the Vector Elite ABC kit (Elite reagent Avidin DH and biotinylated horseradish peroxidase H reagents; Vector Laboratories Inc., Burlingame, C A). After another wash with PBS, the antibody–biotin–avidin–peroxidase complex was developed using diaminobenzidinetetrahydrochloride (DAB Substrate K it, Vector Laboratories I nc.). Sections were counterstained with hematoxylin, dehydrated, and cleared in xylene then cover slipped, where the reaction appeared as a brown cytoplasmic reaction (400×).

Methodology of image morphometry for inspection of the slides

Images of histological sections were analyzed by Image J software to measure the optical density of the apoptotic area [47].

3.12- Histopathological examination of cerebellum and liver tissues

For histopathological examination, a liver and cerebellum tissue was dissected and tissue samples were fixed in 10% neutral formalin for 24 h. Then samples were processed using a graded ethanol series and embedded in paraffin. Paraffin sections were cut into 6µm-thick slices and stained with hematoxylin and eosin for light microscopic examination.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer Multiple Comparison Test. Probability values of less than 0.05 were considered statistically significant. Whereas the graphs were drawn using a prism computer program (GraphPad software Inc. V5, San Diego, CA).

RESULTS

Characterization of QNPs:

Figure (A&B), showed morphology and size of QNPs using SEM photographs. QNPs exhibited particles uniformity in size, less crystallinity, and absence of larger particles (Figure A). As depicted in the image, the particles possessed uniform shape. The size of all particles was found to be less than 200 nm (Figure B).

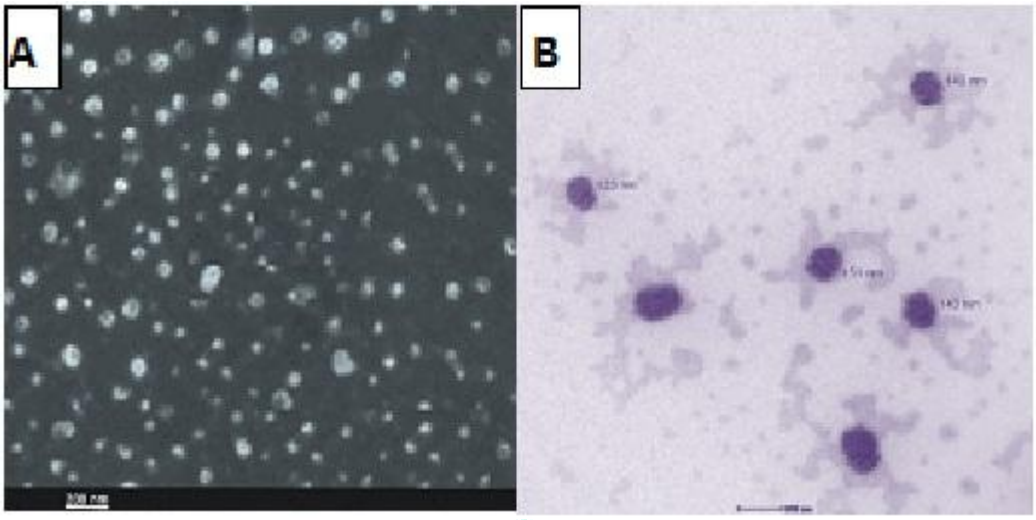

Figure (A&B): Photomicrographs & investigation of morphology and size of QNPs using SEM photographs.

Effect of QNPs on TZ induced behavioral alteration; open field test:

Figure (1), illustrated the parameters related to the open field test. Noteworthy, the latency time (Figure 1A) increased significantly to 3 folds in TZ group as compared to control group, whereas QNPs alone and as treatment (QNPs+TZ group) induced significant decreases in the time (63% and 59%, respectively) as compared to TZ group. On the other hand the ambulation frequency (Figure 1B) decreased significantly by 45% in TZ group as compared to control group. While the ambulation frequencies were augmented in QNPs and QNPs +TZ groups (nearly to 2 folds) as compared to TZ group. In addition, TZ decreased the grooming frequency (Figure 1C) by 62% as compared to control group, whilst the grooming frequencies were incremented in QNPs and QNPs +TZ groups (nearly to 2 folds) as compared to TZ group. Also the rearing frequency (Figure 1D) was decreased significantly by 26% as compared to control group, but it was increased significantly in QNPs +TZ group by 27% as compared to TZ group.

Figure 1

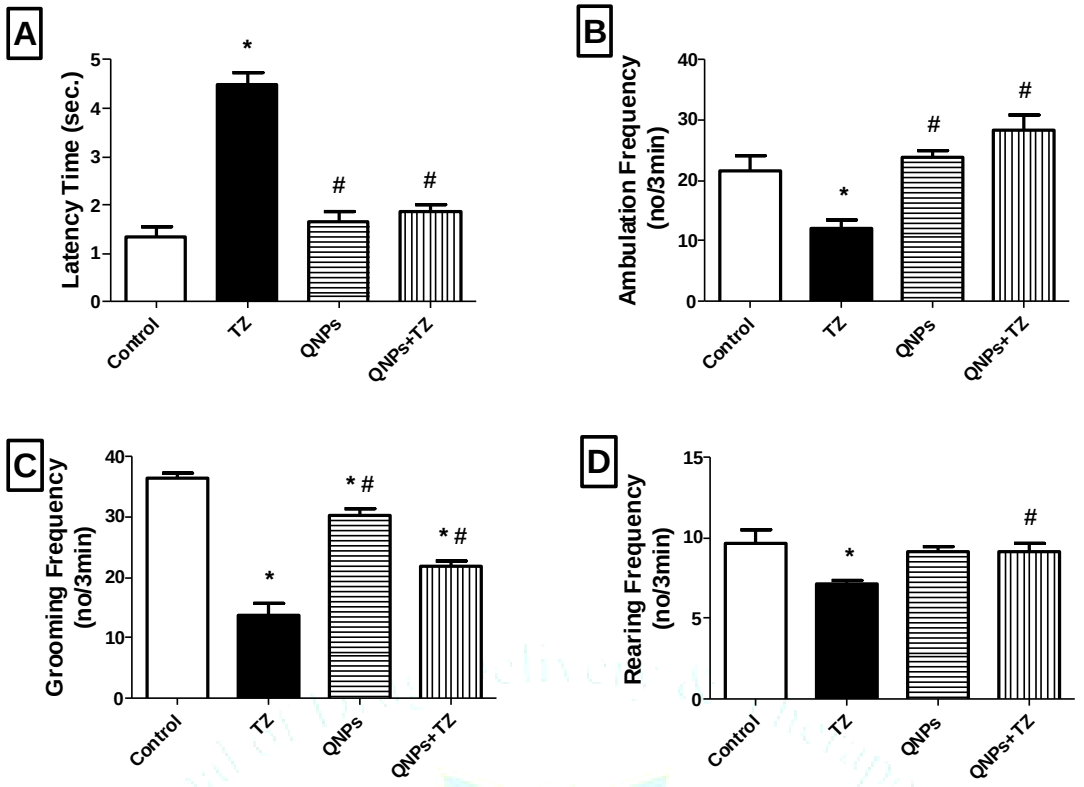

Figure 1: Effects of QNPs (5mg) on latency [A] , ambulation frequency [B], grooming frequency [C], and rearing frequency [D] of open field behavioral test in TZ (50 mg/kg) induced brain injury in rats.

Data represents mean (n = 8-10) ±SEM, * # P<0.05 relative to Control & TZ groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.

Evaluation of Body weight, blood parameters (Hb%, RBCs, and HCT %) and serum glucose level

The obtained data in table (1) revealed that, administration of QNPs alone reduced the body weight significantly compared to TZ group by 16%. Also the obtained results showed that, administration of QNPs + TZ decreased the body weight significantly compared to TZ group by 14%.

Table (1) investigated that TZ significantly reduced Hb%, RBCs, and HCT% by 24%, 24% and 26%, respectively, compared to control group. Besides TZ induced increment in serum glucose level by 41% as compared to control group. In contrary, administration of QNPs alone almost didn't change Hb%, RBCs, and HCT% as compared to control group, but as compared to TZ group QNPs increased these parameters by 33%, 33% and 32% , respectively. As well QNPs reduced the serum glucose level compared to TZ group by 65%. Noteworthy, administration of QNPs + TZ revealed significant increased in Hb%, and RBCs as compared to TZ group by 28% and 26%, respectively. Moreover administration of QNPs + TZ reduced the serum glucose level compared to TZ group by 15%.

Table (1) Ameliorative effects of quercetin nanoparticles against tartrazine toxicity on the body weight and blood parameters (Hb%, RBCs, and HCT %) and serum glucose level:

Parameters	Body weight (g)		Hematology			Glucose (mg/dl)
	Initial	Final	Hb%	RBCs (10^6/mm^3)	HCT (%)	
Control	195.5 ±1.50	262.0± 2.17	14.81 ±0.35	5.10 ±0.32	46.00 ±0.53	96.00 ±0.38
TZ (50 mg/kg)	196.5 ±2.18	310.5 ±2.83*	11.20 ±0.41*	3.90±0.20*	34.00±0.41*	135.00± 0.69*
QNPs (5mg/kg)	194.5 ± 2.15	261.0 ± 2.42#	14.84± 0.18#	5.20 ± 0.24#	45.00 ±0.52#	94.00 ± 0.47#
QNPs + TZ	197.0 ± 2.45	266.0 ± 1.83#	14.37 ±0.27#	4.90 ± 0.19#	35.00 ±0.63	115.00 ±0.58#

Values are expressed as mean ± S.E. *#*p <0.05* relative to control & TZ groups, respectively. TZ= tartrazine, QNPs = quercetin nanoparticles.

Effect of QNPs on TZ induced toxicity on AST, ALT and ALP levels:

Table (2) illustrated that administration of QNPs alone reduced levels of AST, ALT and ALP as compared to TZ group by 42%, 53% and 67%, respectively. As well administration of QNPs + TZ revealed significant decreased in AST, ALT and ALP levels as compared to TZ group by 38%, 50% and 63%, respectively.

Table (2) Ameliorative effects of quercetin nanoparticles against tartrazine toxicity on AST, ALT and ALP levels in rats.

Parameters	AST (U/mL)	ALT (U/mL)	ALP (U/mL)
Control	38.57 ± 2.83	58.17 ± 4.21	50.08 ± 5.23
TZ (50 mg/kg)	63.42 ± 2.61*	121.0 ± 4.18*	148.63 ± 5.31*
QNPs (5mg/kg)	36.92 ± 2.44 #	56.41 ± 3.96 #	49.73 ± 5.18 #
QNPs + TZ	39.14 ± 2.38 #	60.39 ± 4.17 #	54.81 ± 4.92 #

Values are expressed as mean ± S.E. *#p <0.05 relative to control & TZ groups, respectively. TZ= tartrazine, QNPs = quercetin nanoparticles.

Effect of quercetin nanoparticles against Tartrazine-induced lipid profile markers

Data in table (3) showed that administration of QNPs alone reduced levels of serum total cholesterol, triglyceride and LDL-C as compared to TZ group by 35%, 55% and 38%, respectively. On other hand, QNPs administration, as compared to TZ administration, raised HDL-C level by 67%. Besides, administration of QNPs + TZ revealed significant decreased in levels of serum total cholesterol, triglyceride and LDL-C as compared to TZ group by 34%, 49% and 27%, respectively. In addition, administration of QNPs + TZ increased HDL-C level by 35% as compared to TZ alone.

Table 3: Ameliorative effects of quercetin nanoparticles against tartrazine affected lipid profile in serum rat.

Parameters	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-Cholesterol (mg/dL)	LDL- Cholesterol (mg/dL)
Control	83.80 ± 2.76	68.9 ± 2.84	32.56 ± 0.80	41.24 ± 0.82
TZ (50 mg/kg)	128.36 ± 2.44*	141.0 ± 1.84*	21.41 ± 0.51*	61.18 ± 0.59*
QNPs (5mg/kg)	83.44 ± 2.31#	63.9 ± 1.84#	35.81 ± 0.62#	38.22 ± 0.61#
QNPs + TZ	85.27 ± 2.23#	71.4 ± 1.84#	28.91 ± 0.43#	44.71 ± 0.68#

Values are expressed as mean ± S.E. *#p <0.05 relative to control & TZ groups, respectively. TZ= tartrazine, QNPs = quercetin nanoparticles.

Evaluation of ROS, MDA, GPx, PC, and MPO in liver tissue

Table (4) investigated that TZ significantly augmented ROS, MDA, PC and MPO by 24%, 4 folds, 26%, and 39%, respectively, compared to control group. Besides TZ reduced GPx by 40% as compared to control group. In contrary, administration of QNPs alone almost didn't change ROS, MDA, PC, and GPx as compared to control group, but as compared to TZ group QNPs decreased ROS, MDA, PC and MPO by 17%, 77%, 16%, and 60%, respectively. As well QNPs increased activity of GPx compared to TZ group by 68%. Noteworthy, administration of QNPs + TZ revealed significant declined in ROS, MDA, PC and MPO by 18%, 75%, 23%, and 52%, respectively, as compared to TZ group. Furthermore administration of QNPs + TZ increased activity of GPx compared to TZ group by 69%.

Table 4: Ameliorative effects of quercetin nanoparticles against tartrazine affected reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl (PC) contents as well as GPx and MPO activities in rat liver

Parameters	ROS (µmol /g tissue)	MDA (nmol/g tissue)	GPx (IU/g tissue)	PC (nmol /mg)	MPO (U/g tissue)
Control	17.10 ± 0.21	9.84 ± 0.08	23.52 ± 0.61	25.0 ± 0.61	67.3 ± 0.614
TZ (50 mg/kg)	21.28 ± 0.44*	41.00 ± 0.09*	14.21 ± 0.31*	31.4 ± 0.55*	93.2 ± 1.4*
QNPs (5mg/kg)	17.65 ± 0.21#	9.43 ± 0.09#	23.90 ± 0.61#	24.30 ± 0.51#	37.4 ± 1.82*#
QNPs + TZ	17.32 ± 0.82#	10.25 ± 0.10#	24.05 ± 0.21#	26.3 ± 0.71#	45.0 ± 4.11*#

Values are expressed as mean ± S.E. *#p <0.05 relative to control & TZ groups, respectively. TZ= tartrazine, QNPs = quercetin nanoparticles.

Evaluation of ROS, MDA, GPx, PC, MPO, and AchE in cerebellum tissue

Table (5) disclosed that TZ significantly augmented ROS, MDA, PC and MPO by 57%, 5 folds, 33%, and 79%, respectively, compared to control group. Additionally TZ reduced GPx and AChE by 47%, and 18%, respectively, as compared to control group. In contrary, administration of QNPs alone almost didn't change ROS, MDA, GPx, PC, MPO, and AChE as compared to control group, but as compared to TZ group QNPs decreased ROS, MDA, PC and MPO by 37%, 80%, 25%, and 28%, respectively. As well QNPs increased activities of GPx and AChE compared to TZ group by 85%, and 5%, respectively. Moreover, administration of QNPs + TZ exposed significant declined in ROS, MDA, PC and MPO by 36%, 77%, 21%, and 29%, respectively, as compared to TZ group. Furthermore, administration of QNPs + TZ increased activities of GPx and AChE compared to TZ group by 76% and 20%, respectively.

Table 5: Ameliorative effects of quercetin nanoparticles against tartrazine affected reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl (PC) contents as well as GPx, MPO, and acetylcholine esterase (AChE) activities in rat cerebellum.

Parameters	ROS (µmol / g tissue)	MDA (nmol/g tissue	GPx (IU/g tissue)	PC (nmol /mg)	MPO (U/ g tissue)	AChE (U/g tissue)
Control	5.22± 0.17	8.61 ± 0.21	16.42 ± 0.51	19.09 ± 0.43	42.1 ± 2.94	628.43 ± 2.45
TZ (50 mg/kg)	8.21 ± 0.43*	40.2 ± 0.16*	8.63 ± 0.44*	25.32 ± 0.15*	75.4 ±3.25*	513.20 ± 4.21*
QNPs (5mg/kg)	5.19 ± 0.52#	8.2 ±0.13#	16.00 ±0.68#	19.00 ± 0.32#	54.7 ±4.09#	538.19 ± 4.62#
QNPs + TZ	5.28 ±0.36#	9.43 ± 0.19#	16.38 ±0.39#	20.03 ± 0.62#	53.6 ±3.63#	613.28 ± 3.19#

Values are expressed as mean ± S.E. *#p <0.05 relative to control & TZ groups, respectively. TZ= tartrazine, QNPs = quercetin nanoparticles.

Effect on GABA, 5-HT, NE and DA contents

Figure (2) showed that GABA content (Figure 2A) was increased significantly nearly to 2 folds as compared with control group, at the same time GABA contents were declined in QNPs and QNPs+TZ groups by 30% and 15%, respectively, as compared to TZ group. On the other hand, 5-HT contents (Figure 2B) were decreased significantly by 16% in TZ, and QNPs+TZ groups as well as 21% in QNPs as compared to control group. Conversely, NE (Figure 2C) and DA (Figure 2D) contents were increased significantly by 44% and 32%, respectively, as compared with TZ group.

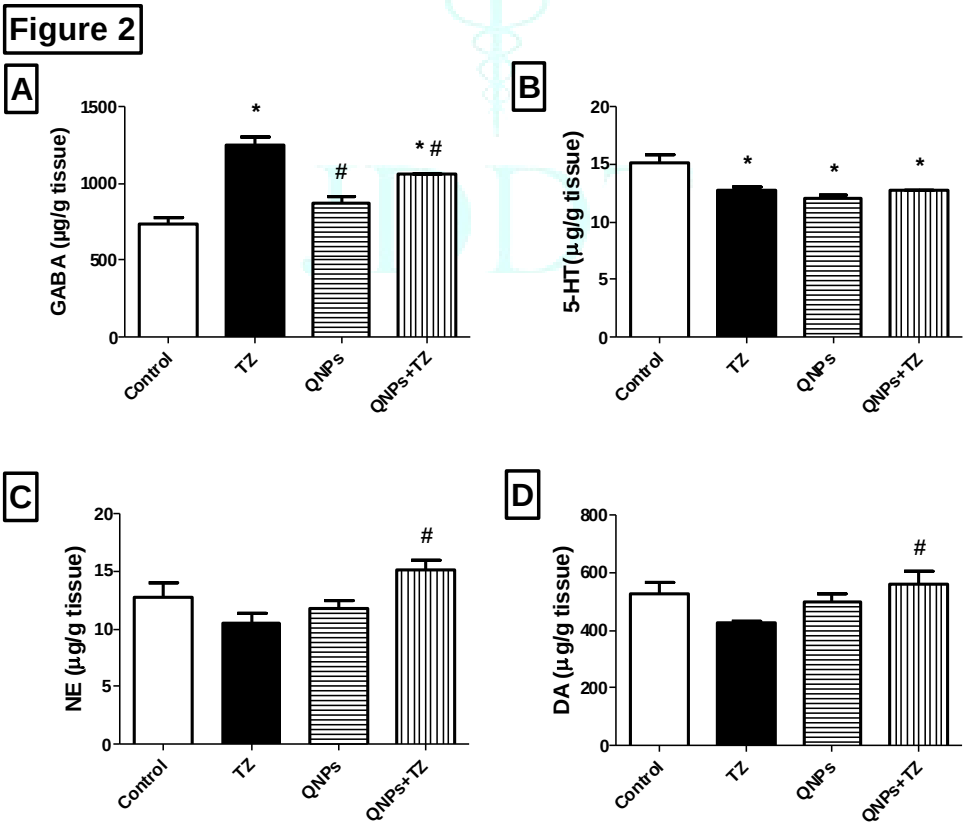

Figure 2: Effects of QNPs (5mg) on cerebellum ɤ-Amino butyric acid (GABA) [A] , Serotonin (5-HT) [B], Nor-epinephrine (NE) [C], and Dopamine (DA) [D] of open field behavioral test in TZ (50 mg/kg) induced brain injury in rats.

Data represents mean (n = 8-10) ±SEM, *# P<0.05 relative to Control & TZ groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.

Immunohistopathological examination of liver and cerebellum tissues

By using caspase 3 antidote stain for cerebellum, figure (3A) illustrated that TZ induced apoptosis by significant increasing in expression caspase 3 as compared to control group. Noteworthy, figure (3) revealed that QNPs or QNPs+TZ improved the apoptosis significantly as compared to TZ group.

Likewise, figure (3B) illustrated that each of QNPs or QNPs+TZ improved the apoptosis significantly in liver tissues as compared to TZ group.

Figure 3

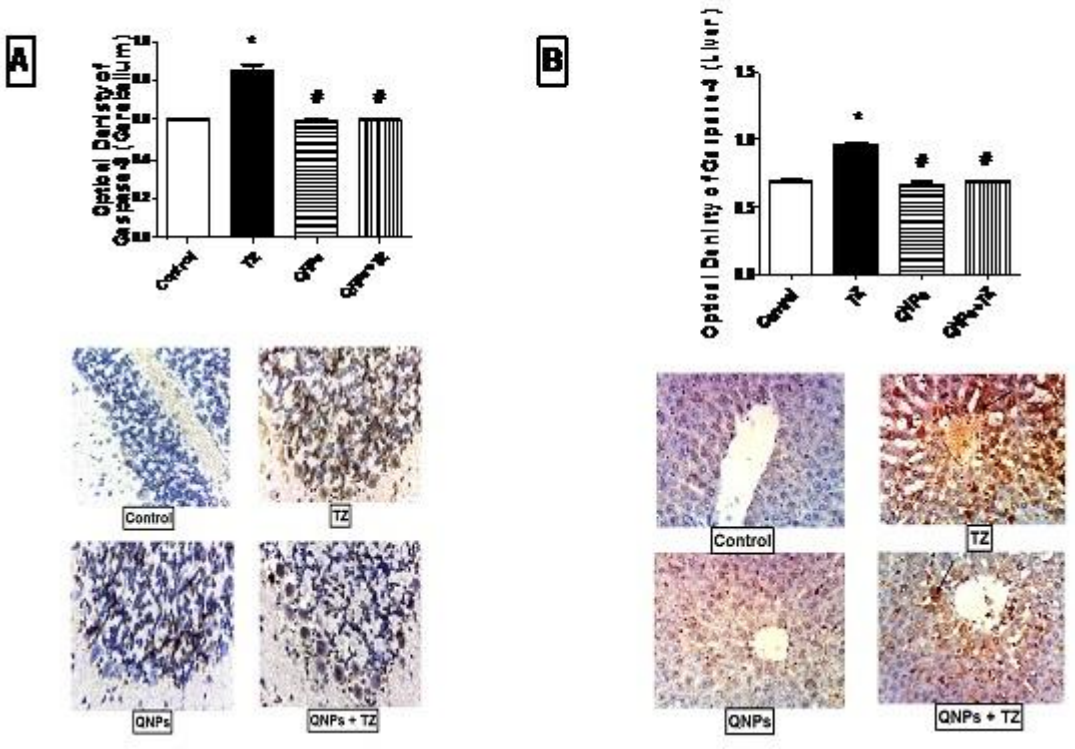

Figure 3: Effects of QNPs (5mg) on optical density of caspase 3 expresssion in cerebellum (A) and liver (B) tissues in TZ (50 mg/kg) induced brain-liver injuries in rats.

Data represents mean (n = 15-20) ±SEM, * # P<0.05 relative to Control & TZ groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test.

Histopatholgical examination of liver and cerebellum tissues

By using H&E stain, figure (4A) illustrated that TZ induced necrosis and pyknosis of purkinje cells in cerebellum tissues. On the other hand QNPs or QNPs+TZ made marked improvement in cerebellum tissues as compared to TZ group.

Similarly, figure (4B) showed that liver tissues after administration of TZ, there are induction of Kupffer cells activation, congestion of central vein and sinusoids, and focal hepatic necrosis associated with inflammatory cells infiltration. Alternatively, QNPs or QNPs+TZ made marked improvement in cerebellum tissues as compared to TZ group.

Figure 4

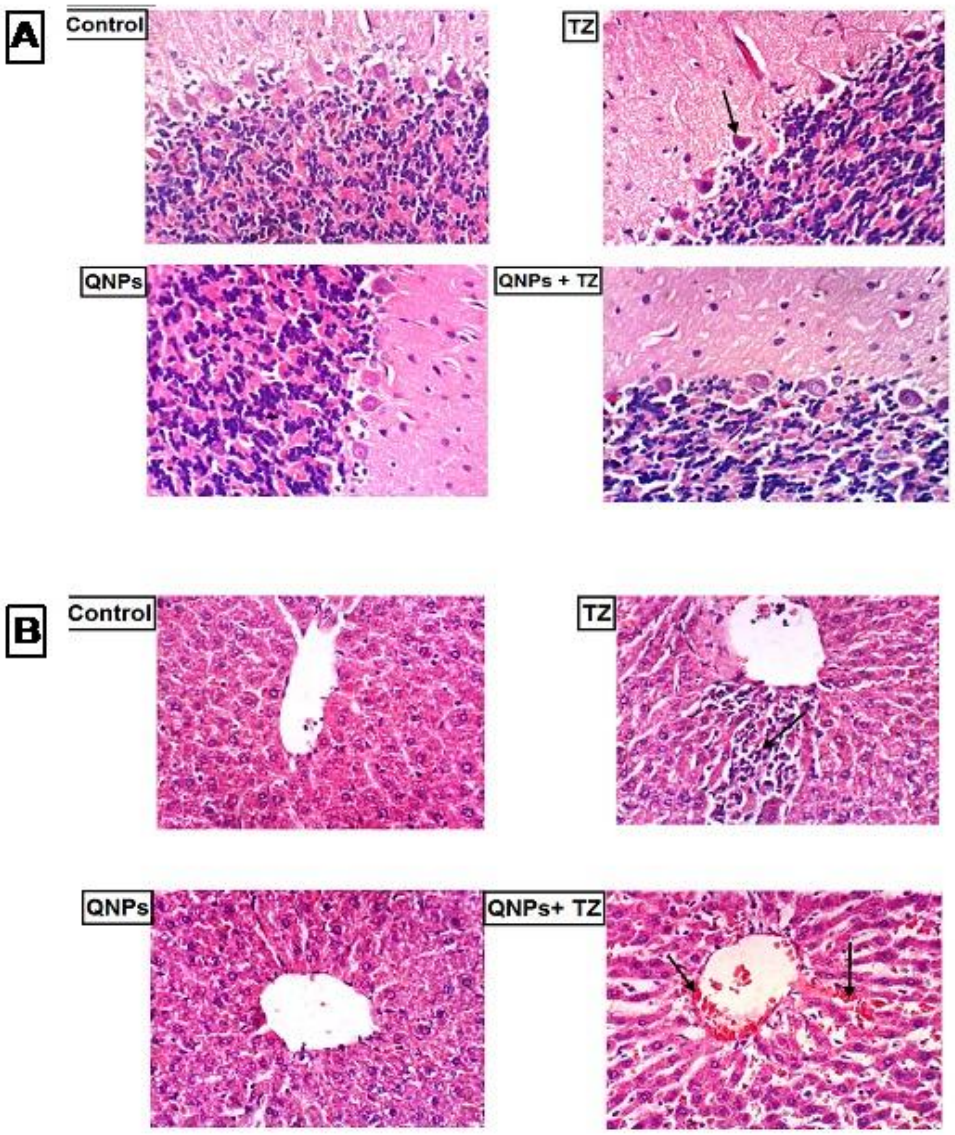

Figure 4: Effects of QNPs (5mg) on histopathological changes in cerebellum (A) and liver (B) tissues in TZ (50 mg/kg) induced brain-liver injuries in rats. Photomicrographs of rat cerebellum and liver sections stained with H&E (X400).

DISCUSSION

Food additives, considered as xenobiotic, have a significant effect in currently abundant and nourishing food sources, and allow people to be pleased. Really, they may have several metabolites, such as monosodium glutamate and nitrous compounds that are found to be carcinogens [48].

Noteworthy, the current study revealed that administration of chosen dose of TZ caused marked raise in latency time, as well decrease in ambulation frequency, grooming frequency, and rearing frequency of open field behavioral test. These indicated anxiety- and depression-like behaviors in rats and these data were in conflict with the previous study [49]. We suggested that altered apoptotic gene expressions or damage related to cerebellum were involved. On the other hand, treatment with QNPs improved these TZ deleterious effects as it has anti-apoptotic effect [50].

In this, TZ caused a significant increase in the body weight compared to normal control group. Indeed, an increase in the body weight over 20% above the mean body weight is considered as obesity [51]. Similar results have also been reported [52, 53]. Meanwhile, using of QNPs as treatment improved the body weight.

Additionally, TZ declined Hb%, RBCs count and HCT% significantly as in previous studies [54, 55]. These changes may be due to the prevention of red blood cell synthesis via inhibition of erythropoisis in the bone marrow [56] as well as increase fragility and progressive destruction of RBCs due to binding of free radicals produced by TZ [57]. Conversely, QNPs administration recovered blood markers and might be due to antioxidant activity of QNPs [58].

In fact, TZ treatment affected liver function tests. These results may be attributed to hepatocellular damage caused by the toxic effect of TZ which was indicated

by histopathological examination. Alternatively, there were current recovers in AST and ALT levels and ALP activity in QNPs + TZ group which may be due to improvement in liver tissue as appear by histopathological examination.

Herein, TZ induced harm regarding to lipid profile as another study [59]. On the other hand, QNPs improved the lipid profile. **Igarashi and Ohmuma, (1995)** [60] stated that, quercetin decreased serum total cholesterol level in rats through increasing its fecal excretion. In addition, quercetin reduces de novo synthesis of fatty acids and consequently cholesterol biosynthesis and lipoproteins formation [61].

Herein, ROS, MDA and PC levels and MPO activity increased significantly in liver and brain tissues accompanied with a significant decrease in the activity of GPx after administration of TZ, as other studies [62, 63]. In addition, the measurement of PC has some advantages as the relative early formation and the relative stability of carbonylated proteins [64]. In contrast QNPs showed valuable effects on liver and brain oxidation by enhancing antioxidant enzyme activity and decreasing pro-oxidant effect [25].

Regarding to AChE activity, TZ administration caused significant inhibition in its activity in the brain tissue. While, QNPs showed improvement in AChE activity. Moreover, TZ administration led to decrease in 5-HT level in cerebellum which may be due to free radicals that generated when azo dyes metabolized by intestinal bacteria. These free radicals inhibited formation of adenosine triphosphate ATP that cause reduces synthesis or re-uptake of neurotransmitter in the presynaptic cell [65]. Additionally, elevated GABA level by TZ may be due to inhibition of the voltage-dependent calcium channels, whereas treatment with QNPs improved the GABA level in cerebellum.

CONCLUSION

These protective effects of QNPs against TZ induced hepato- and neuro-toxicities may be due to antioxidant, anti-inflammatory and anti-apoptotic effects.

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