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Evaluation of Cytotoxicity and Acute Oral Toxicity of Two Anthraquinones

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Abstract

Bioactive compounds have been a significant therapeutic agent for decades, but the use of bio-active natural metabolites in traditional medicines and the discovery of drugs are still active. Anthraquinones are one of the secondary metabolites that are responsible for colour, fragrance and flavour to the plant; also used as colouring agents in the food and textile industries. The present aim of the study was to evaluate the cytotoxicity of Quinizarine (QNZ) and 2-Methyl Anthraquinone (MAQ) in C8-B4 (microglia cell lines, Mouse), SH-SY-5Y (Neuroblastoma cell lines, Human). As well, the study extended to determine the acute toxicity of these compounds on rat Sprague Dawley. Inhibitory concentration (IC_{50}) was determined for both compounds as well as acute toxicity of MAQ was determined in Sprague Dawley (SD) rat. The IC_{50} values of MAQ and QNZ were 88.66 µg/ml, 55.33 µg/ml on C8-B4 and 88.68 µg/ml, 108.89 µg/ml on SH-SY-5Y, respectively. The acute toxicity study of fixed doses was carried out in female SD rats for both compounds. The LD_{50} of QNZ and MAQ were found to be greater than 5000 mg/kg and less than 300 mg/kg, respectively. The haematological investigation did not show any significant variation changes among treated rats. The following biochemical parameters were investigated to include Kidney Function Tests (KFTs), Liver Function Tests (LFTs), lipid profile, electrolytes and Random Blood Glucose (RBG) were found no significant changes in the QNZ treated group as compared with the sham group but elevation of Na^+, creatinine and SGOT levels in MAQ treated animals. Gross necropsy showed non-significant alteration upon QNZ administration but Lung inflammation was found in the MAQ group. The histopathological finding suggested no significant alteration in tissue histology in QNZ group but infiltration of neutrophils, lymphocytes and macrophages in MAQ treated group. A high dose of both compounds for Single oral administration did not produce any significant alteration in morphological and behavioural parameters. The histopathological finding also supports the safety of both compounds in SD Female rats.

Keywords: Acute Toxicity, C8-B4, Quinizarine, SH-SY-5Y, 2-methyl Anthraquinone

1. Introduction

Bioactive compounds have been a significant therapeutic agent for decades, but the use of bioactive natural metabolites in traditional medicines and the discovery of drugs are still active. Anthraquinones are one of the secondary metabolites that are responsible for colour, fragrance and flavour to the plant, therefore it is as colouring agents in the food and textile industries. Quinizarine (QNZ) and 2-Methyl Anthraquinone (MAQ; Tectoquinone) are two phenolic compounds having a basic structure of 9, 10-anthracenedione with formula $C_{14}H_8O_2$, which are

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abundantly present in a plant of *Rubia* family. Previously, QNZ and MAQ were isolated from *Cassia tora* seeds[1] and *Clausena heptaphylla*[2], respectively. Currently, authors are isolated above two compounds from the roots of *Rubia cordifolia* Linn. Anthraquinones are found in all parts of the plants, animals, bacteria, insects and other sources[3]. Anthraquinones are having significant biological uses such as laxatives, antioxidants, fungicides, antibacterial, antifungal, anticancer and other biological actions[4–5]. Anthraquinones are also used in the food processing industry, textile dying, cosmetics and pharmaceuticals[6–8]. The dust particles of teak contain MAQ, upon exposure in working places may lead to sino-nasal cancer, which is more in Europe[9]. Additionally, Alzheimer's Disease (AD) is a complicated neurodegenerative disorder leads to cause amnesia; few synthetic compounds are currently available for treating AD such as tacrine, rivastigmine, donepezil and galantamine, are usually approved. Though, these drugs having side effects (such as fatigue, insomnia, gastrointestinal disorders, weight loss and hepatotoxicity) and also a problem with bioavailability[10]. Because of all these side effects, there has remained a lot of interest in the therapeutic potential of natural products. To overcome the bioavailability and side effects of synthetic drugs for treating AD, there is an imperative requirement to identify the superior bioactive molecule. As per the old toxicity demonstrated through *in vitro, in vivo* studies on cell lines and animals, respectively, we conducted MTT assay on C8-B4 (microglia cell lines, Mouse), SH-SY-5Y (Neuroblastoma cell lines, Human), 14 days single dose oral toxicity of MAQ and QNZ.

2. Material and Methods

2.1 Materials

Quinizarine (CAS number: 81-64-1) and 2-Methyl Anthraquinone (CAS number: 84-54-8) were procured from Sigma Aldrich, USA. Hydroxy Propyl Methyl Cellulose (K100 Grade; HPMC), Paraffin wax, haematoxylin-eosin stain and glycerine used were of analytical grade. Diagnostic kits for biochemical estimations were procured from Beacon Pvt. Ltd, India. Other materials used in the study were of commercial grade and procured from the local market. All other reagents were of analytical grade unless otherwise stated.

2.2 Cell Culture

The human neuroblastoma SH-SY-5Y and the mouse microglia C8B4 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, United States). The cells were held at 37°C in a humidified atmosphere of 5% CO_2. The Cells were grown with 10% fetal bovine serum, 1% non-essential amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 µg/mL) and 1% amphotericin (250 U/mL) in Dulbecco's Revised Eagles Medium (Gibco Invitrogen, Paisley, UK). The cells were enzymatically transferred with 0.25% trypsin- 1mM EDTA and subcultured with a density of 2.2 x 10^4 cells/cm^2 on 75 cm^2 plastic flasks. The medium of culture has been replaced every 2 days. Microscopic examination confirmed the cell confluence (80%). In order to avoid cell differentiation, treatments were given 12 hrs following seeding[11].

2.3 Cell Viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[12] is a colourimetric method for determining cell metabolic activity. The Nicotinamide Adenine Dinucleotide Phosphate (NADPH) dependent cellular oxidoreductase enzymes present in the cell, convert the tetrazolium dye of MTT to its insoluble formazan with a purple colour. The SH-SY-5Y and C8B4 cells were grown at a density of 10000 cells/well in a 96-well microtiter plate. After 24 hrs of normal cell growth, of MAQ and QNZ were added (10, 20, 40, 60, 80, 100 µg/ml) then incubated for 48 hrs and then treated with MTT solution (0.5 mg/ml PBS) for 4 hrs at 37°C until intracellular purple formazan crystals are visible under a microscope. In each well, 100 µL of Dimethyl Sulfoxide (DMSO) was added to dissolve the formalin crystals formed in the wells and gently mixed at room temperature on an orbital shaker for an hour. Depending on the volume of the cell culture, DMSO volume was adjusted. The color is observed after 1 hr incubation, which is measured under Elisa plate reader at 570nm. Three times the whole experiment was repeated.

2.4 Experimental Animals

Twelve weeks old female SD rats, weighing 200-250 g, were used and the protocol (1529/PO/Re/11/CPCSEA./CHIPS/IAEC7/PRO-05/2019-20) was approved by the Institutional Animal Ethics Committee (IAEC), Chebrolu Hanumaiah Institute of Pharmaceutical Sciences (CHIPS), Guntur, A.P, India. All the experimental procedure was done as per the guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and New Delhi. The animals were procured Mahaveera Enterprises, Hyderabad and quarantined under the conditions of room temperature 22 ± 3°C with relative humidity 50 ± 10 % as well as Lighting sequence being 12 hours light and dark. Single-dose oral acute toxicity was carried out as per Organization for Economic Cooperation and Development (OECD) guideline No- 423[13].

2.5 Acute Oral Toxicity Study

Female SD rats were randomly divided into 9 groups, each group consisting of 3 animals. The grouping of animals was as follows:
Group 1: Sham
Group 2: QNZ (5 mg/kg.)
Group 3: QNZ (50 mg/kg.)
Group 4: QNZ (300 mg/kg.)
Group 5: QNZ (2000 mg/kg.)
Group 6: QNZ (5000 mg/kg.)
Group 7: MAQ (5 mg/kg.)
Group 8: MAQ (50 mg/kg.)
Group 9: MAQ (300 mg/kg.)
Group 9: MAQ (2000 mg/kg.)

The animal treatment for acute toxicity was in an ascending manner after conformation of survival of previous groups. The fixed doses of both compounds were prepared individually by adding two drops of tween 80 as a wetting agent, dissolved in 0.5% Hydroxy Propyl Methyl Cellulose (HPMC) and made up to the required volume. Both compounds were administered through oral route at different dosing levels after conformation of survivals of one group by another and animals were observed for 14 days for any sign of mortality or toxicity. Animals were observed individually after dosing for the first 30 minutes, with special attention up to 4hrs and continued the observation for the first 24 hours, daily thereafter, for a total of 14 days.

2.6 Body Weight

The animal weights were taken individually before administration of compounds; and once a week up to 14days. The body weight changes in animals were recorded.

2.7 Haematological and Biochemical Investigation

At the end of protocol, animals were subjected to anaesthesia under light diethyl ether anaesthesia and blood samples were collected from retro-orbital plexus into polypropylene Eppendorf tubes containing 20 µL Potassium salt of Ethylene Diamine Tetra Acetic Acid (K_2-EDTA) for analyzing various haematological parameters such as Red Blood Cell (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), White Blood Cell (WBC), Platelet (PLT), Mean Platelet Volume (MPV) and Plateletcrit (PCT). Plasma was separated from the blood for biochemical investigation. The following biochemical parameters were investigated such as Kidney Function Tests such as blood urea, serum creatinine, total protein, Liver Function Tests such as Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), lipid profile such as Serum Triglycerides (TG), Serum total cholesterol, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL). Electrolytes such as Na^+, K^+ and Random Blood Glucose (RBG) were also analyzed. Analysis of all plasma samples was performed on semi-automatic chemistry and coagulation analyzer (Vector Biotek Pvt. Ltd, India).

2.8 Gross Necropsy and Histopathological Study

After blood sampling, animals were euthanized and various organs were isolated for a gross necropsy. The following organs such as liver, kidney, heart, brain, spleen, eye, ovaries, stomach, lung, thymus, thigh bone were subjected for gross necropsy and wet weight of organs was recorded. Three animals from the highest dose of both compounds and vehicle-treated group were subjected to histopathological studies. Kidney, heart, lung, brain and liver were chosen for histopathological studies for MAQ, QNZ and vehicle treated groups, Representative 4mm thick bits were taken from the specimens; they were fixed in 4% formalin. Specimen were Dehydrated through ascending graded alcohol and then cleared in xylol. Specimen were Impregnated and embedded in the paraffin wax, 3-4 µ sections were taken on the slides by cutting the blocks with a microtome. After rehydration and clearing, sections were stained with hematoxylin and eosin. Then after dehydration and clearing, sections were mounted with Dibutylphthalate Polystyrene Xylene (DPX). All the stained sections were subjected to histopathological studies examined under optical microscopy using an Olympus microscope. Coding was given to the stained sections and the blinded study was carried out by the pathologist.

2.9 Statistical Analysis

Results were represented as mean± standard error of the mean (S.E.M). The statistical significance between the means of various groups was analyzed by unpaired two-tailed students t-test using graph pad prism statistical software (version 8.0). Data was considered statistically significant when $p< 0.05$.

3. Results

3.1 Cytotoxicity

The cytotoxicity assay was carried out for MAQ and QNZ to assess cell viability. The safety of compounds was assessed by examining the cell morphology and survival of C4B4 and SH-SY-5Y cell lines. The IC_{50} values of MAQ and QNZ were 88.66 µg/ml, 55.33 µg/ml on C8-B4 and 88.68 µg/ml, 108.89 µg/ml on SH-SY-5Y, respectively (Figures 1 and 2).

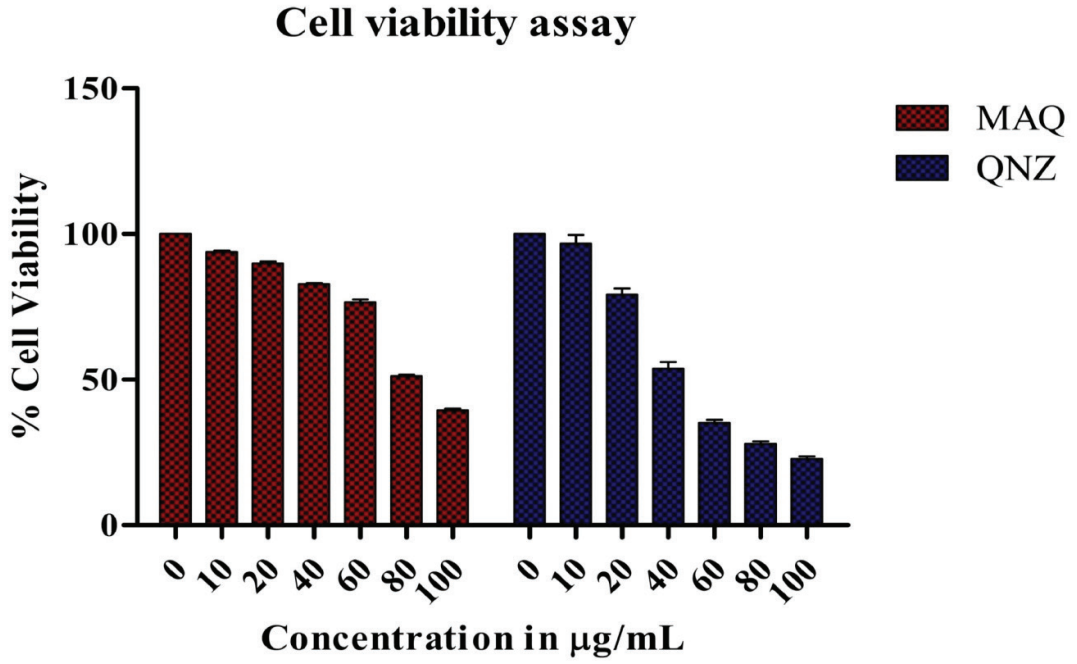


Figure 1. Effect of MAQ and QNZ on *Mus musculus* microglial (C8B4) cell line.

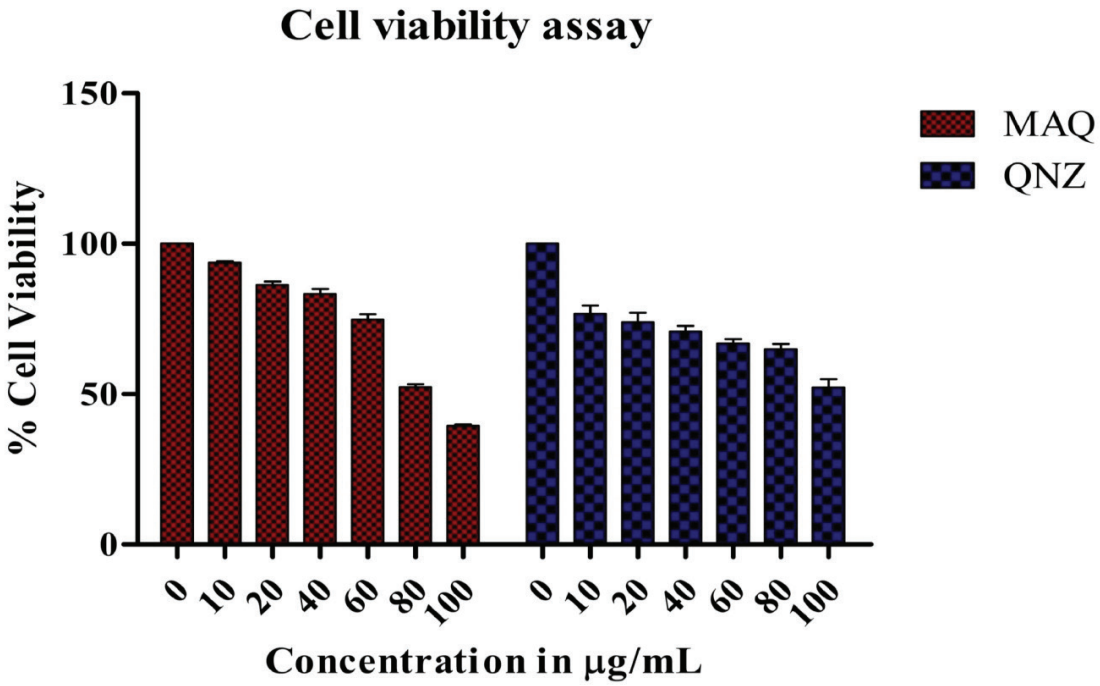


Figure 2. Effect of MAQ and QNZ on Human neuroblastoma (SH-SY-5Y) cell line.

3.2 Acute oral Toxicity Study

Acute oral toxicity (single exposure; fixed dose) of MAQ and QNZ was done as per the OECD guideline no 423[13] on animals and reported accordingly. After oral administration of both compounds at various fixed doses, the animals showed no alteration in behavioural parameters.

3.3 Change of Body Weight and Food-water Study

The body weights were examined weekly for all treated rats and compared with sham group. There is no significant body weight changes were observed in MAQ and QNZ treated groups while compared with vehicle-treated group i.e. sham. Hence, there is no effect of both compounds on

body weights and shown in Figure 3. Similarly, the food and water intake of MAQ and QNZ treated groups are normal as compared with sham (data not shown); therefore, there is no significant changes were observed on water and food consumption also.

3.4 Morphological Changes

There is no abnormal appearance was observed concerning to the alertness, grooming, touch response, torch response, pain response, tremors, righting reflex, pinna reflex, corneal reflex, writhing, pupils, urination, salivation, skin colour, lacrimation, hyperactivity, food intake, temperature and eye color. The MAQ and QNZ treated groups doesn't show any changes in position, walk and response to handling. In addition, there was an absence of any sign of tonic-clonic actions, motor stereotypic behaviours such as excessive grooming, recurring circling or any bizarre behaviour like backwards walking and self deformity. There is no significant changes in motor activities, as well as grip strength, were observed in all treated groups. Shortness of breath and nose bleeding was observed in the high fixed doses of MAQ. Whereas, in the QNZ treated group was found diarrohea on the first day of drug exposure when compared with sham group.

3.5 Haematological and Biochemical Examination

Blood samples were collected from all treated and sham group animals were subjected to analysis of various haematological and biochemical parameters. As demonstrated in Tables 1 and 2, all the haematological and biochemical parameters were compared with the sham group. The haematological parameters such as RBC, HGB, HCT, MCV, MCH, WBC, PLT, MPV and PCT were observed with no significant deviation compared to the sham group as per the normal referring ranges. The biochemical parameters are subjected to analyze such as Kidney Function Tests (such as blood urea, serum creatinine, total protein) Liver Function Tests (such as SGPT, SGOT) lipid profile (such as Serum TG, Serum total cholesterol, HDL, LDL) electrolytes (such as Na+, K+) and RBG indicated no significant deviation was observed with the sham group as per the normal referring ranges. All animals were euthanized and subjected for organ weights. Whereas, high dose of both compounds and vehicle treated animals were subjected for histopathological studies.

3.6 Macroscopic Gross Study

The macroscopic assessment showed normal architecture without any sign of inflammation. As shown in Figure 4, all treated groups were observed with no alteration of organ weights compared with sham group indicating the absence of inflammation, atrophy, hypertrophy or oedema except in MAQ treated group. The MAQ treated group animal lungs were found with pulmonary embolism and presence of inflammation. It was depicted as safe after administration of QNZ in various organs such as Liver, kidney, heart, brain, spleen, eye, ovaries, stomach, lung, thymus and thigh bone. The gross necropsy revealed no organ macroscopic lesions or anomalies in animals treated with QNZ but inflammation was found in MAQ treated animals.

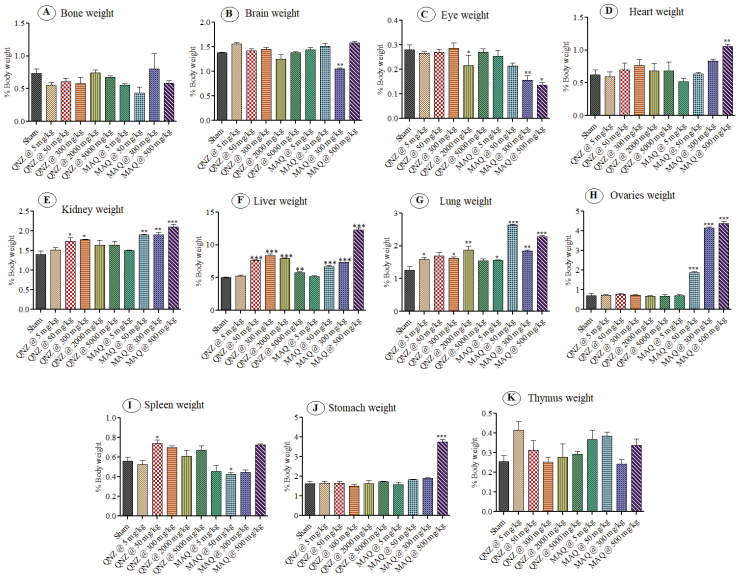


Figure 4. Effect of MAQ, QNZ and vehicle treated groups on organ weights of rats. Excised organ weights from the study groups were represented as (a) Bone; (b) Brain; (c) Eye; (d) Heart; (e) Kidney; (f) Liver; (g) Lung; (h) Ovaries; (i) Spleen; (j) Stomach; (k) Thymus.

Table 1. Effect of different doses of MAQ, QNZ and vehicle (Sham) treated groups on hematological parameters. The results were represented as mean± S.E.M. The statistical significance between the means of various groups was analyzed by unpaired two-tailed students t-test and statistically significant when $p< 0.05$.

Parameters	Ref Ranges	Sham	QNZ @ 5 mg/kg	QNZ @ 50 mg/kg	QNZ @ 300 mg/kg	QNZ @ 2000 mg/kg	QNZ @ 5000 mg/kg	MAQ @ 5 mg/kg	MAQ @ 50 mg/kg	MAQ @ 300 mg/kg
RBC	6.39-8.01 X 10^{12}/L	5.7 ± 0.15	5.7 ± 0.54	6.6 ± 0.27	5.5 ± 0.44	6.5 ± 0.59	6.2 ± 0.67	6.4 ± 0.26	7.2 ± 0.14	6.6 ± 0.12
HGB	12.9-15.4 g/dL	12.3 ± 0.31	12.33 ± 0.23	14 ± 1.04	13.93 ± 0.23	13.1 ± 0.20	11.6 ± 0.49	12.2 ± 0.31	13 ± 0.26	13.3 ± 0.08
HCT	40-46 %	45.7 ± 1.17	45.6 ± 0.86	51.8 ± 3.85	51.5 ± 0.86	48.7 ± 0.75	42.9 ± 1.8	45.38 ± 1.17	39.81 ± 0.21	40.42 ± 0.78
MCV	55.21-64.80 fL	79 ± 2.77	80.9 ± 7.65	78.1 ± 5.13	95.0 ± 8.59	76.10 ± 8.50	69.5 ± 5.18	71.2 ± 4.4	54.33 ± 0.98	59.76 ± 1.26
MCH	18.70-21.20 pg	21.3 ± 0.74	21.8 ± 2.06	21.12 ± 1.38	25.69 ± 2.32	20.5 ± 2.29	18.7 ± 1.40	19.26 ± 1.20	17.88 ± 0.01	20.06 ± 0.51
WBC	2.58-7.34 X 10^{9}/L	6.23 ± 1.0	8.6 ± 0.86	6.23 ± 0.77	6.3 ± 0.43	8.96 ± 0.95	4.53 ± 0.70	8.83 ± 0.12	6.8 ± 0.1	9.23 ± 0.31
PLT	923-1580 X 10^{9}/L	923.3 ± 81.94	830 ± 32	956 ± 39.8	807.6 ± 44.5	897.6 ± 45.4	622.3 ± 101.05	894.3 ± 43.05	1151.7 ± 23.4	920.33 ± 10.17
MPV	6.70-8.10 fL	4.33 ± 0.80	4.5 ± 0.411	5.2 ± 1.10	5.6 ± 0.1	5.1 ± 1.39	6.4 ± 1.07	6.5 ± 0.28	7.1 ±v 0.08	7.16 ± 0.21
PCT	0.108-0.282 %	0.393 ± 0.052	0.383 ± 0.02	0.501 ± 0.08	0.456 ± 0.01	0.453 ± 0.10	0.382 ± 0.01	0.58 ± 0.05	0.82 ± 0.03	0.68 ± 0.008

Table 2. EEffect of different doses of MAQ, QNZ and vehicle (Sham) treated groups on Biochemical parameters. The results were represented as mean± S.E.M. The statistical significance between the means of various groups was analyzed by unpaired two-tailed students t-test and statistically significant when $p< 0.05$.

Parameters	Sham	QNZ @ 5 mg/kg	QNZ @ 50 mg/kg	QNZ @ 300 mg/kg	QNZ @ 2000 mg/kg	QNZ @ 5000 mg/kg	MAQ @ 5 mg/kg	MAQ @ 50 mg/kg	MAQ @ 300 mg/kg
Creatinine (mg/dL)	1.18 ± 0.14	1.76 ± 0.32	1.53 ± 0.04	1.33 ± 0.04	1.10 ± 0.03	1.16 ± 0.06	0.84 ± 0.083	1.75 ± 0.05	2.135 ± 0.09
Glucose (mg/dL)	132.82 ± 7.08	100.83 ± 2.20	132.86 ± 8.37	120.69 ± 1.79	102.16 ± 2.94	114.04 ± 2.09	92.4 ± 3.20	111.41 ± 0.40	98.48 ± 0.30
Triglycerides (mg/dL)	37.67 ± 0.60	27.39 ± 0.40	55.10 ± 25.85	43.23 ± 7.15	63.90 ± 24.46	36.53 ± 2.30	55 ± 4.04	48.33 ± 0.88	23.16 ± 0.11
Cholesterol (mg/dL)	19.83 ± 1.10	22.43± 0.52	25.74 ± 1.64	31.88 ± 0.92	24.61 ± 1.09	19.04 ± 0.87	28.66 ± 1.45	27.1 ± 0.34	15.9 ± 0.46
HDL (mg/dL)	8 ± 0.36	8.36 ± 0.32	7.83 ± 0.89	7.13 ± 0.33	4.2 ± 0.50	4.36 ± 0.42	66.66 ± 10.17	98.59 ± 0.67	51.85 ± 0.08
LDL (mg/dL)	4.63 ± 0.08	10.76 ± 0.69	5 ± 0.92	5.93 ± 0.71	5.96 ± 1.97	4.47 ± 0.39	13 ± 4.63	1.51 ± 0.67	1.79 ± 0.67
SGOT (U/L)	93.61 ± 2.14	91.74 ± 2.83	94.34 ± 1.85	102.94 ± 7.96	108.15 ± 5.49	101.18 ± 1.65	45.33 ± 3.75	105.65 ± 1.01	250.25 ± 0.89
SGPT (U/L)	48.11 v 1.47	37.18 ± 2.56	38.37 ± 0.45	53.88 ± 4.44	55.81 ± 3.65	47.81 ± 1.92	43.26 ± 2.86	66.8 ± 1.50	76.55 ± 0.49
Total Protein (g/dL)	5.95 ± 0.11	5.7 ± 0.18	5.52 ± 0.24	5.77 ± 0.26	6.03 ± 0.09	5.91 ± 0.03	5.76 ± 0.55	6.605 ± 0.02	7.53 ± 0.05
Urea	16.33 ± 0.88	30 ± 8.96	15.33 ± 2.90	16.33 ± 0.88	16.66 ± 1.20	16 ± 1.15	24.66 ± 1.76	22.5 ± 0.28	76.5 ± 0.28
Na^{+} (mmol/L)	149.1 ± 2.17	146.8 ± 2.25	146.98 ± 4.3	145.13 ± 0.966	144.87 ± 2.22	143.66 ± 2.98	135.33 ± 2.33	135.15 ± 0.20	159 ± 13.85
K^{+} (mmol/L)	4.69 ± 0.41	4.64 ± 0.33	4.94 ± 0.10	4.61 ± 0.06	4.48 ± 0.105	5.02 ± 0.21	4.04 ± 0.09	4.245 ± 0.03	4.08 ± 0.04

3.7 Histopathological Studies

As depicted in Figure 5, the brain of QNZ treated group rats were found with normal cerebral tissue having astrocytes, oligodendrocytes, neurons and the cerebellar tissue showed normal molecular layer, Purkinje cells, granular layer. The ventricles showed normal ependymal cell lining, normal choroid plexus, found similar structures of cerebral, cerebellar and ventricles as that of sham group rat sections, whereas, the MAQ treated group shown mild gliosis in cerebral cortex and hippo campus regions of brain. The liver sections of QNZ treated rats were showed normal hepatocytes in hexagonal architecture, sinusoids and central vein of QNZ treated group was similar to that of the sham group. MAQ treated group shown focal mild cytoplasmic vacuolations and interface hepatitis in individual hepatocytes. The portal triad showed mild periportal inflammation; central vein and sinusoids shows mild dilatation and congestion. The kidney sections are similar to that of sham group but in MAQ treated group interstitium of kidney shown mild inflammatory infiltrates. The heart sections of QNZ treated rats showed normal cardiac muscle bundles, pericardial adipose tissue with blood vessels and found similar to that of sham group rats. MAQ treated section studied from the heart shows myocardium with myocytes shows mild edema. Stroma shows focal inflammatory infiltrates. The blood vessels show congestion. The spleen sections of QNZ treated rats showed a thin capsule, normal red pulp with congested sinusoids and white pulp with lymphoid follicles. The MAQ treated section from lung shows varying sizes of alveoli lined by pneumocytes. Bronchi show normal, peribronchiole region shows inflammatory infiltrates. Interstitium shows dense infiltration of lymphocytes, neutrophils and macrophages. Blood vessels show congestion.

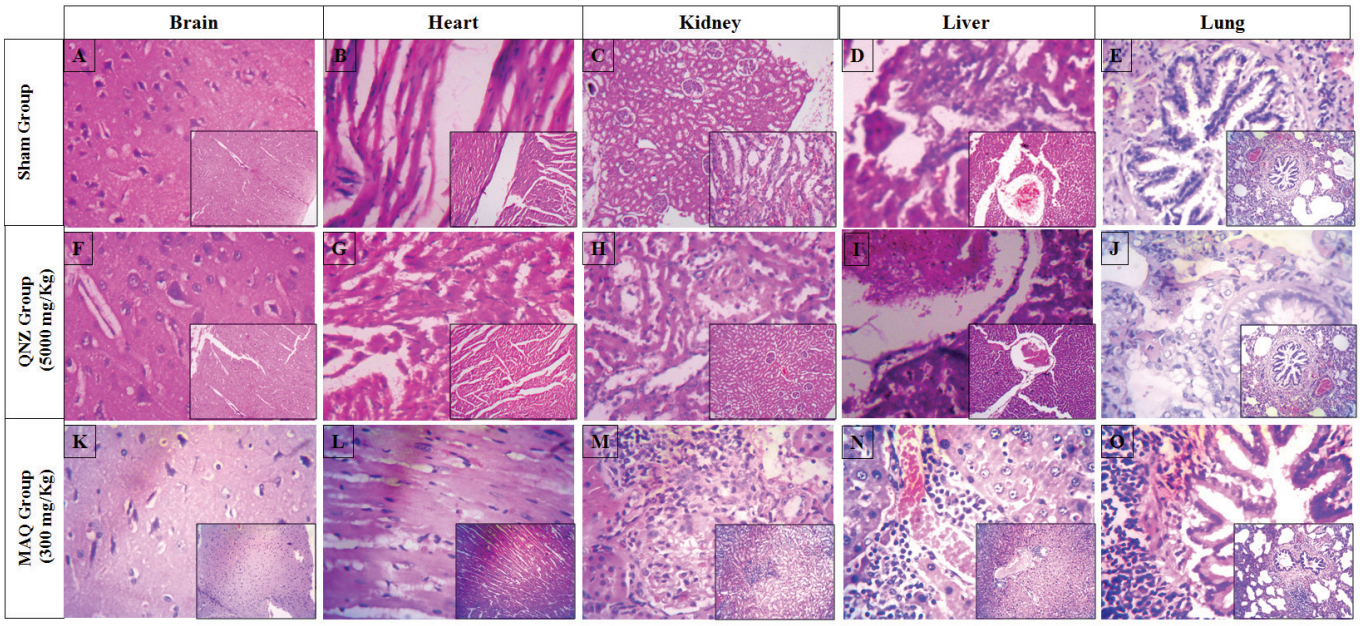


Figure 5. Histopathological study of QNZ (5000 mg/kg), MAQ (300 mg/kg) and vehicle (Sham) treated groups. Figure represents histology of various tissues such as brain, heart, kidney, liver and Lung (A-E: sham group; F-J: QNZ group; K-O: MAQ group).

4. Discussion

MAQ and QNZ are the phenolic compounds present in various Rubia family plants. Estimable usage of anthraquinones was observed in textile dying and food processing industry at present. Anthraquinones are the potential molecules for treating constipation as well as neurodegenerative disorders. Therefore, it is necessary to evaluate the toxicological status of anthraquinones for their safe usage in humans. In order, MAQ and QNZ are needed to investigate the cytotoxicity on mouse microglia (C8B4) and human neuroblastoma (SH-SY-5Y) cell lines. The IC50 values of MAQ and QNZ were 88.66 µg/ml, 55.33 µg/ml on C8-B4 and 88.68 µg/ml, 108.89 µg/ml on SH-SY-5Y, respectively. Therefore, the above said molecules are safe and don't produce toxicity. Initially, the body weights, food,

water intake of animals were analyzed and it was clearly indicated that there were no changes made with MAQ and QNZ treatment. During the study of animals, no significant general appearances changes observed in alertness, tremors, pinna reflex, pupil size, urination, salivation, skin colour, hyperactivity and pain response except diarrhoea. Diarrohea caused by colonic motility change leading to increased large intestine transit and colonic absorption. The secretion modification resulted in accumulation of fluid exactly on the day of administration[14–15]. Whereas, in the MAQ treated rat lung shows inflammatory infilrates in the region of peribronchiole and dense infiltration of neutrophils, lymphocytes and macrophages in the interstitium region of lung. The blood vessels of lung were founds to be congested. Due to this reasons the LD_{50} of the MAQ compound found less than 300mg/kg and animals were died[16–17]. For the isolated organs, we performed the gross necropsy, no significant change in weights, color and texture of organs was found. Along with that, there were no abnormalities like inflammation, oedema, atrophy and any damage of organs were found for QNZ treated animals but in MAQ treated animals had lung inflammation. Therefore, QNZ treated group doesn't elicit any deformity on various organs except in MAQ treated animals. One of the major contributing systems that were affecting toxicity of the drug is a hemopoietic system, which is required for blood cells formation; hence, it is obligatory to analyze the toxicity of QNZ by examining the Complete Blood Picture (CBP). In this point of view, various haematological parameters like RBC, HGB, HCT, MCV, MCH, WBC, PLT, MPV and PCT were estimated and the resulted values of both compounds were within the reference values as compared to the sham group. The biochemical estimation of lipid profile such as Serum TG, Serum total cholesterol, HDL, LDL, electrolytes such as Na^+, K^+ and RBG was measured and found that no significant changes in the plasma cholesterol, triglycerides, HDL and LDL levels treated with QNZ as compared with sham except MAQ treated animals, in which Na^+ levels were found high than normal values. The liver, kidney are the dynamic sites for biotransformation and elimination of drugs. Consequently, the following tests were conducted for assessing the function of both organs like SGOT, SGPT, creatinine and electrolytes of blood. There were no considerable changes discovered with QNZ treated group by comparing the sham group. Whereas, in the MAQ treated group animals the SGOT and creatinine levels were found high due to damage of lung and kidney, respectively; which was clearly demonstrated by histopathology studies. The acute toxicity for roots of RC in ethanolic extract showed more than 3000mg/kg in Wistar rats[18], whereas, alcoholic and aqueous extracts were showed more than 2000mg/kg in albino mice[19]. This states that both compounds were doesn't affect biochemical estimations thereby regulating the homeostasis of the body. It is also demonstrated that both compounds were not involved in any toxicity on various organs of the body except MAQ.

Brain isolated from both compounds treated rats explained that the cerebral tissue with oligodendrocytes, neurons were normal, while, cerebellar tissue also found normal molecular and granular layers. Along with that, the ventricles had normal choroid plexus; this specified that no signs of abnormalities were distinguished with the QNZ treatment. The cardiac tissue histology illustrates that no pathological changes were scrutinized. Neither hypertrophy nor inflammation on the layers of heart was observed, thus, which is safer on cardiac actions. Histology studies of liver demonstrate that normal hexagonal hepatocytes with central vein were observed. Therefore, QNZ treated animals don't show any abnormalities on hepatocytes. Further, The MAQ treated section from lung shows varying sizes of alveoli lined by pneumocytes and infiltration of lymphocytes, neutrophils and macrophages in the interstitium region of lung but no significant changes found in the in the lung of QNZ treated animals. Similarly, the renal histological studies indicate that normal architecture of cortex, medulla and pelvicalyceal system. Hence, there were no renal impairments discovered with both compounds.

5. Conclusion

The cytotoxicity and acute toxicity studies are important for translational research and determination of safety. The IC_{50} values of MAQ and QNZ were 88.66, 55.33 on C8-B4 and 88.68, 108.89 µg/ml on SH-SY-5Y, respectively. The LD_{50} of MAQ and QNZ was found to be less than 300mg/kg and greater than 5000 mg/kg (Category 5 or unclassified 5000 mg/kg), respectively. No major toxicity associated with haematological and biochemical parameters were produced by both compounds. The QNZ doesn't show any significant change in body weight of animals in acute toxicity. Whereas, MAQ treated animals were shown lung inflammation, hence the LD_{50} was found below 300 mg/kg b.wt. The results of this study demonstrated that there is no adverse impact or health-related acute toxicity after administration of both compounds. There is a need to evaluate the repeated oral dose toxicity (28 days) for both MAQ and QNZ.

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7. References

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