

Radical Scavenging Activities of Albumin-Copper Complex against Bromobenzene Induced Hepatotoxicity

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Abstract

This study was performed to examine that copper-albumin complex can restore oxidative damage and cytotoxicity in induced by Bromobenzene (BB) in liver. Rats were divided into three groups. Group I served as control received corn oil only. Group II exposed to BB at a dose of 300 mg/Kg by weight, twice/week, orally, dissolved in corn oil for one month. Group III received BB as previous dose plus copper albumin- Serum total protein and albumin showed significant reduction while the activities of ALT and AST enzymes significantly increased in BB-treated group when compared to control. The antioxidant enzymes activities were found to be decreased in BB treated rats in contrast nitric oxide and lipid peroxidation levels were increased in comparison with normal control. Histopathology revealed that BB had a hepatotoxic effects represented by hepatitis and fatty degeneration. Nile blue positive stain showed lipofuscin and/or ceroid granules. Necrotic nuclei with disintegrated DNA stained with acridine orange. The treated rats with copper-albumin complex reversed the most of parameters to normal control.

Keywords: Bromobenzene; Copper-albumin complex; Nitric oxide; Lipid peroxidation; Glutathione-S-Transferase

Introduction

The environment is exposed to a variety of chemicals and toxicants that pose a serious damage of health in the living beings. Bromobenzene (C_6H_5Br) is a xenobiotic; that exists in the environment during its output in the industries or during its usage in different fields [1]. BB is a toxic substance, which is a colorless stinging liquid, with a special aromatic odor [2]. It is devised by combining of bromide with benzene in the existence of iron powder. BB is not only utilized in the manufacture of different drugs and chemicals [3], an additive for motor oils, for organic synthesis, but also as crystallizing solvents [4].

The subjection to BB may be through the ingestion, dermal and occupational contact that is pursued by its metabolism in the liver by three stages. The phase I of biotransformation conducted by cytochrome p450 monooxygenases and give highly electrophilic compound; BB 3, 4-epoxides [5], while phase II the reactive epoxides of BB adjoin with GSH, which catalysed by GST, thus, with high doses of BB; reducing hepatic GSH and subsequently prevent the protection against ROS. This goes to secondary hazards, as increasing the lipid peroxidation, decreasing ATP, local inflammation, mitochondrial dysfunction and amending the intracellular calcium levels, and impairing calcium homeostasis that element virulent damage to the mobile phone and its organelles [6].

The presence of sufficient quantities of antioxidant in the body scavenges the reactive oxygen species. But in heavy doses, acute or chronic liability to the toxicant could overcome the antioxidant defence mechanism in the liver [1]. BB-treatment resulted in a maximal enhanced production of nitric oxide products, activation of pro-inflammatory marker; COX-2 and caspase-3 [7]. More than 20 liver proteins were noted to be increased in the production glutamylcysteine synthetase, which is essential in glutathione biosynthesis. The secondary metabolites of BB which is forged during its metabolism in the liver are highly nephrotoxic [8].

Many researchers plotted the protective effects against BB; prior administration of Aqueous Extract of *Phyllanthus fraternus* (AEPF) that could prevent the mitochondrial dysfunction in the liver and the kidney [6]. Black seed oil inflated the hepato-renal protection against BB [9], and the nephroprotective effect of the *Cassia fistula* fruit extract was confirmed by Kalantari et al. [2]. Renal oxidative stress caused by BB could be prevented by withaferin A, an active compound of *Withania somnifera* [10].

Latterly, in that location are numerous examples of societal systems with high affinity towards biomolecules, including nucleic acids and proteins, and presenting real potential to be developed into therapeutic agents [11]. Many compounds show unique properties, passing on great opportunities to design new pharmacologically active molecules, such as adjustable ligand kinetics and redox activity and multiple of geometries and coordination numbers presented by metal ions, leading to high structural diversity [12]. The synergy of the serum albumin and its ability to bound of a large array of mononuclear and polynuclear Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Pt^{2+} complexes with aromatic ligands has been explained [13].

Many copper-albumin complex are exhibited biological activity includes anti-inflammatory activity, it was concluded that some proactive copper complexes usually have cardinally forceful power than their originator compounds without copper. Copper complexes were checked and aptly applied in the cure of several conditions of inflammation. Copper-albumin chelating complex, which holds an egg albumin and copper as one of the copper peptides can be used as antiinflammatory and antioxidant agent.

And besides it has an extremely adequate toward hepatotoxicity [14]. Copper complexes can affect along the cells biochemically and change the metabolism, especially highly effective copper dependent enzymes, such as Superoxide Dismutase (SOD). It is likewise admitted that copper containing vitamins or biochemically active organic compounds such as amino acids and peptides in combinations is used to catch rid of superoxide radical [15]. Therefore, the present work aimed to assess the healing activity of copper albumin complex against BB induced oxidative damage and hepato-toxicity.

Materials and Methods

Animals

30 male Sprague Dawley rats, (4-8 weeks old), weighted 120-150 gms were used in this experiment. They were obtained from the animal house, faculty of Medicine, Assiut University. The animals were accustomed to the environment for a week prior to the experiment. Animals were kept under controlled environment at room temperature and a 12 hrs/12 hrs light/night time. Standard commercial pellets for feeding, water ad libitum and other animal health conditions during all time courses of the experiments were provided.

Chemicals

Bromobenzene (BB) was purchased from Sigma Chemical Co. (St. Louis, USA). It was dissolved in corn oil as a vehicle. The copperalbumin complex was obtained from Prof. Dr. Ahmed Yassein Nassar-Professor of Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt as Patent Cooperation Treaty (PCT) in the international Bureau of World Intellectual Property Organization (WIPO), Geneva, Switzerland World Organization (SWO) 2008 028497.

Experimental design

Rats were divided into three groups (10 rats each). The first group (GI) served as control received corn oil only. The second group (GII) exposed to BB at a dose of 300 mg/Kg b.w, twice/week, orally, dissolved in corn oil for one month. The third group (GIII) received BB as previous doses plus copper albumin complex at a dose of 400 u/kg b.w, twice/ week, orally in corn oil for one month.

Sampling

At the conclusion of the experiment, blood samples were compiled from the retro orbital plexus under light ether anaesthesia and collected in clean test tubes without anticoagulant for serum preparation. Serum was prepared after centrifugation of the blood samples at 3000 rpm for 10 minutes and stored at -20° C for determination of biochemical parameters. Eventually, the rats were sacrificed under chloroform anaesthesia and tissue specimens from liver were removed after careful post mortem examination and secured at 10% neutral buffered formalin for histopathological examination. Other liver tissues were submitted for determination of biochemical parameters.

Liver homogenate preparation

1 gm of liver tissues was washed with cold saline and dried. Each of these tissues was separately transferred to a glass homogenizer containing 9 mL of 10 mm cold Phosphate Buffer Saline (PBS-pH 7.4). The tissues were homogenized using an electrical homogenizer (Remi 8000 RPM). The unbroken cells and cell debris were removed by centrifugation at 3000 RPM for 10 minutes by using Remi C 24 refrigerated centrifuge (-4° C). The obtained supernatant was applied for the biochemical estimations.

The biochemical examination

The biochemical tests determined total protein content and Albumin levels, according to Young [16], Nitric Oxide [17], Thiol concentration [18], AST and ALT activities according to Henery [19], Glutathione (GSH) [20], Superoxide Dismutase (SOD) [21], Glutathione-S-Transferease (GST) [22], Ceruloplasmin [23] and Lipid peroxide [24].

Histopathological examination

Liver was collected from rats and fixed at 10% neutral buffered formalin. The fixed samples were dehydrated in ascending grades of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Paraffin sections at $5 \,\mu$ m in thickness were cut and stained with the following histological stains:-

Haematoxylin and Eosin (H&E): For general histological examination [25].

Periodic Acid Schiff (PAS): Technique for manifestation of neutral mucopolysaccharides [26].

Nile blue stain: For detection of lipofuscin pigments [27].

Acridine orange stain: For revelation of necrotic hepatocytes according to [28]. Stained sections were examined by OLYMPUS BX51 fluorescence microscope and the photos were taken by OLYMPUS DP72 camera adapted into the microscope.

Statistical analysis

All data were expressed as mean \pm Standard Deviation (SD). Statistical significance was performed by ANOVA followed by Bonferroni's test (p<0.001) was considered to be significant.

Results

Biochemical analysis

Serum total protein (g/dL): Table 1, shows the animals in GII had a dramatic reduction in serum total proteins that was highly significantly reduced than normal control animals in GI (p<0.001). Intoxicated animals co-treated with copper-albumin complex in GIII revealed that it was near normal controls. Serum albumin level (g/dL): Table 1 shows the BB intoxicated animals GII that it was significantly lower than control (p<0.001). BB intoxicated animals co-treated with copper-albumin complex in GIII showed restored to the normal levels.

No. of Groups	GI	GII	GIII
	n=10	n=10	n=10
Total protein (g/dL)	8.17 ± 0.098	4.86 ± 0.088	7.79 ± 0.056

Ρ		***	N.S
Albumin (g/dL)	4.49 ± 0.018	2.31± 0.026	4.18 ± 0.029
Ρ		***	N.S

Table 1: The effect of (BB) on total protein and albumin with or without administration of copper-albumin complex in experimental and control rats. Each value represents as Mean \pm Standard Error (M \pm SE), P: probability for significance has been performed as the following: ***p<0.001 for: all of 10 rats. Comparisons were made with G1.

Table 2 shows the activities of ALT and AST enzymes in serum of the animals. The activities of these enzymes were significantly (p<0.001) increased in BB-treated group when compared to the normal control group indicating injury to the liver. In contrast, treated with copper-albumin complex exhibited significantly decreased levels of these enzymes reaching the average normal control levels, indicating that it counteracted the hepatotoxic effect of BB.

No. of Groups	GI n=10	GII n=10	GIII n=10
ALT (U/L)	27.5 ± 0.036	52.25 ± 0.043	29.5 ± 0.054
Р		* * *	N.S
AST (U/L)	24.45 ± 0.15	65.08 ± 0.088	32.5 ± 0.092
Р		***	*

Table 2: The effect of (BB) on Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) with or without administration of copper-albumin complex in experimental and control rats. Each value represents as Mean \pm Standard Error (M \pm SE), P: probability for significance has been performed as the following: *p<0.05; ***p<0.001 for: all of 10 rats. Comparisons were made with G1.

Oxidative status in serum and liver

Board 3 and 4 showed the effect of BB and copper-albumin complex on the layers of the antioxidant enzymes glutathione-s-transferase, glutathione, and tile in both serum and liver; serum SOD and ceruloplasmin. The levels were found to be decreased in BB treated rats when compared with normal control. The treated rats with copperalbumin complex reversed the antioxidant levels reached to normal control. In BB intoxicated group, nitric oxide and lipid peroxidation in both serum and liver were found to be higher than the control group. Their levels were reduced to normal on action with copper-albumin complex (Table 3).

No. of Groups	GI n=10	GII n=10	GIII n=10
Thiol (nmol/mL)	5.348 ± 0.013	1.785 ± 0.028	3.721 ± 0.03
Р		***	*
Nitric oxide (µmol/mL)	210.4 ± 0.12	258.7 ± 0.11	205.6 ± 0.11
Ρ		***	N.S

SOD (U/mL)	6.5 ± 0.51	3.6 ± 0.46	8.4 ± 0.42
Р		***	***
GSH (nmoL/mL)	43.51 ± 0.31	31.12 ± 0.19	43.57 ± 0.23
Р		***	N.S
GST (nmoL/min/mL)	340.5 ± 0.08	215.3 ± 0.09	336.2 ± 0.05
Р		***	N.S
Ceruloplasmin (mg/dL)	63.28 ± 0.21	37.06 ± 0.13	71.31 ± 0.11
Ρ		***	*

Table 3: Effect of BB on oxidative status, Glutathione (GSH), nitric oxide, Glutathione-s-Transferase (GST), Superoxide Dismutase (SOD), thiol and ceruloplasmin concentration with or without administration of copper- albumin complex in serum of experimental and control rats. Each value represents as Mean \pm standard error (M \pm SE), P: probability for significance has been performed as the following: *p<0.05; ***p<0.001 for: all of 10 rats. Comparisons were made with G1.

Histopathological examination

The histological assessment of liver of the control rats showed that, the parenchyma of the liver was consisted of several non-defined hepatic lobules (Figure 1A). Each hepatic lobule was consisted of branching and anatomizing two cells thick hepatic plates extended from a central vein. The hepatic sinusoids were lined with endothelial and phagocytic kupffer cells (Figures 2A and 3A). Hepatocytes were seemed as polyhedral cells with vacuolated acidophilic cytoplasm contained PAS positive glycogen granules and vesicular rounded central nuclei (Figure 4A). The portal areas were dispersed through the liver parenchyma. The portal triads were manifested as triangular areas of loose connective tissue containing a branch of portal vein, hepatic artery and bile ductile (Figure 1A).

The histopathological evaluation of liver tissues of BB intoxicated rats showed that BB had a hepatotoxic effects represented by hepatitis, fatty degeneration, fatty change (steatosis), hepatic necrosis and nuclear changes. Hepatitis was characterized by leukocyte infiltration and congestion in the blood vessels (Figures 1B and 3B), and fatty degeneration and fatty change (steatosis) (Figure 1B). Steatosis represented as two types, the first single was the microvesicular steatosis, which indicated with the presence of several small intracytoplasmic fat droplets (vacuoles) in the hepatocytes. The moment was the marovesicular statues which represented by large intracytoplasmic lipid vacuole filling most of the cytoplasm of the hepatocyte (Figures 2B and 3C).

The nuclear alterations in the hepatocytes were included: pyknosis, karyorrhexis and karyolysis, irregular contour, imagination, shrinkage, loss of chromatin, its serenity and the absence of the nucleus (Figure 3C). The hepatic plates were disorganized, degenerated and the hepatocytes had ill-defined cell bounders (Figures 2B and 3B). There was depletion in the PAS positive glycogen granules in the hepatocytes of BB intoxicated rats (Figure 4B). Staining of the hepatic tissues with

Page 3 of 10

Page 4 of 10

the histochemical stain; Nile blue showed that the hepatocytes of BB intoxicated rats had numerous Nile blue positive (dark blue) lipofuscin and/or ceroid granules (Figure 5B). Paraffin sections in the liver of BB-treated rats stained with acridine orange stain and examined by

fluorescence microscope showed numerous necrotic hepatocytes. Necrotic nuclei (disintegrated DNA) stained with acridine orange were appeared as orange spots under fluorescence microscope (Figure 6B).



Figure 1: Photomicrographs of paraffin sections in the rat liver stained by hematoxylin and eosin stain. A: Showing the liver of the control group with normal histological architecture of the Hepatic Lobules (HL) and Portal Areas (PA). B: Showing the hepatotoxic effect of the Bromobenzene; disarranged Hepatic Lobules (HL) and fatty change or steatosis of hepatocytes (F). C: Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity as indicated by restoration of normal histological architecture of the Hepatic Lobules (HL) and Portal Area (PA). Original magnification, 40X, scale bar=500 µm.



Figure 2: Photomicrographs of paraffin sections in the rat liver stained by hematoxylin and eosin illustrating the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. A: the control group, showing the normal histological structure of the hepatic lobule; hepatic plates radiating from the Central Vein (CV) and formed of polyhedral hepatocytes (arrow). B: Bromobenzene-treated group, showing the hepatotoxic effect of the Bromobenzene; Central Vein (CV), surround by nearly Healthy Hepatocytes (H), disarranged Hepatic Plates (HP), Fatty degeneration (F), macrovesicular steatosis (arrow) and coalesce fat Changed hepatocytes (C). C: Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity as indicated by restoration of normal histological architecture of the hepatic lobules which formed of hepatic plates radiating from the Central Vein (CV) and acidophilic polyhedral hepatocytes (arrow). Original magnification, 200X, scale bar=100 µm.

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Figure 3: Photomicrographs of paraffin sections in the rat liver stained by hematoxylin and eosin illustrating the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. A: the control group, showing the Central Vein (CV) and the acidophilic polyhedral Hepatocytes (H). B and C: Bromobenzene-treated group, showing the hepatotoxic effect of the Bromobenzene. B: showing the Congested Central Vein (CCV), Necrotic Hepatocytes (NH), and congested Portal Vein (PV) in the Portal Area (PA) and Coagulative Necrotic hepatocytes in the periphery of the hepatic lobule (CN). C: Showing the microvesicular steatosis (arrow), Macrovesicular Steatosis (MVS) with nuclear alteration as double Nuclei (N), Karyorrhexis (K) and karyolysis (arrow head) (D): Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. Note the Central Vein (CV) and the acidophilic polyhedral Hepatocytes (H). Original magnification, (A and B) 200X, scale bar=100 µm, (C and D) 400X, scale bar= 50 µm.

The histopathological assessment of liver tissues of copper complex treated rats revealed that, copper complex had an ameliorative effect on the BB induced hepatotoxicity. This protective effect was indicated by restoration of normal histological and histochemical structure of the hepatic tissue (Figures 1C and 2C). Mild leukocytes infiltration and congestion could be observed in the liver of copper complex plus BB-treated rats (Figures 1C and 2C). Fatty change hepatocytes were retained to nearly normal state and appeared as polyhedral cells with

vacuolated acidophilic cytoplasm and vesicular rounded centrally located nuclei (Figure 3D). The hepatocytes became full of PAS positive glycogen granules (Figure 4C) and had no or few Nile blue positive lipofuscin granules (Figure 5C). Paraffin sections in the liver of copper complex-BB treated rats stained with acridine orange stain and examined by fluorescence microscope showed that most hepatocytes with healthy nuclei and few with necrotic ones (Figure 6C). Citation: Abdel-Magiud DS, Nassar AY, Shatat AR, Mohamed AS (2019) Radical Scavenging Activities of Albumin-Copper Complex against Bromobenzene Induced Hepatotoxicity. J Clin Toxicol 9: 405.



Figure 4: Photomicrographs of paraffin sections in the rat liver stained by PAS and Hx illustrating the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. A: the control group, showing the Polyhedral Hepatocytes (H) with rounded centrally located Nucleus (N) and full of PAS positive glycogen granules (arrow). B: Bromobenzene-treated group, showing Hepatocytes (H) with depletion of PAS positive glycogen granules (arrow) and with eccentric Nucleus (N). C: Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. Note the Hepatocytes (H) full of PAS positive glycogen granules (arrow). Original magnification, (A-C) 400X, scale bar=50 µm.



Figure 5: Photomicrographs of paraffin sections in the rat liver stained with Nile blue stain illustrating the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. A: the control group, showing the Central Vein (CV) and the Hepatocytes (H) without Nile blue positive lipofuscin granules. B: Bromobenzene-treated group, showing Hepatocytes (H) with numerous Nile blue positive lipofuscin granules (arrow) C: Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. Note the Central Vein (CV) and the Hepatocytes (H) with no or few Nile blue positive lipofuscin granules. Original magnification, (A-C) 100X, scale bar=200 µm.

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Figure 6: Photomicrograph of paraffin sections in the rat liver stained with acridine orange stain illustrating the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. A: the control group, showing the Hepatocytes (H) with healthy nuclei (arrow). B: Bromobenzene-treated group showing Hepatocytes (H) with necrotic and damaged nuclei (arrow) C: Showing the ameliorative effect of copper complex on the Bromobenzene induced hepatotoxicity. Note that most Hepatocytes (H) with healthy nuclei (arrow) and few with necrotic ones (arrow head). The necrotic nuclei (DNA) stained with acridine orange were appeared as orange spots under fluorescence microscope. Original magnification, (A-C) 400X, scale bar=50 µm.

Discussion and Conclusion

In the current study, hepatotoxicity induced by BB exposure revealed that serum total proteins and albumin levels were significantly reduced than control animals. While the activities of ALT and AST enzymes in serum of the animals of BB-treated group showed a significant increase when compared to the control. These results were confirmed by NTP, (1985) after subchronic gavage study of BB in rats. El-Sharaky et al. [7] showed that the excretory and synthetic functions of the liver were impaired after oral ingestion of BB in male rat, which represented with significant reduction in serum total proteins and significant elevation of AST and ALT compared to manipulate. And besides a modest increase in ALP and ALT were observed occasionally at shorter durations of BB exposure in male rats dosed at 300 or 400 mg kg-1/day [29]. This suggests an injury, impaired functions and damage of liver as a consequence of oral ingestion of BB. The mechanism by which BB induces liver damage is not clearly understood, however, the formation of reactive metabolites and oxidative stress are implicated.

Oxidative stress can result either from low levels of antioxidants and/or from an increased production of reactive species [30]. Our results suggested that induction of oxidative stress is perhaps one of the serious mechanisms by which BB exerts their cellular action. In this study, we specifically examined the effects of BB treatment on the oxidative indices in the serum and hepatic tissues. A substantial reduction in the strata of the antioxidant enzymes, including glutathione-s-transferase, glutathione, and tile in both serum and liver; serum SOD and ceruloplasmin was obtained. A concomitant increase in nitrous oxide and lipid peroxidation level following chronic exposure to BB were noticed in the current survey. At that place is an important in free radical induced disruption to biological systems due to xenobiotics contact. Many of xenobiotics have been exposed to have the strength to form free radicals in the body [31]. The liver has a diversity of redox systems, among which GSH is essential. It was significant to examine the GSH level in liver together with the activities of GP_x, GR, GST, SOD, tiles and ceruloplasmin since they may efficiently retrieve toxic free radicals and be partly giving a defence against lipid peroxidation due to xenobiotics effect. Glutathione S-Transferases (GSTs), a group of cytosolic several functional enzymes, are detoxifying compounds that exist in all aerobic organisms. They helped the conjugation of glutathione with a multiple of reactive electrophilic compounds, thereby equalizing their active electrophilic sites and changing over the mother compound more water soluble. In addition to catalytic properties, the GSTs can also bind covalently/noncovalently to a multiple of hydrophobic compounds [32]. These are the effective elements in redox-mediated processes in the cell phone, as glutathione, these other LMW this also engaging in disulfide bonds with proteins and deliver a wide ability of regulation of the metabolism and also take on many of biophysical properties, as well (i.e., pKa and redox potential) [33]. Thiol groups showed reaction with electrophiles and oxidants and have high reactivity with metals [34,35], making them multilateral in the biological function they can do, but also potential Achilles' heels for alterations that destroy the normal biology [36]. These are groups of mercaptan that include a sulfhydryl functional group. Biothiols (or biologically-derived files) are the most essential antioxidants that keep cells from any source of oxidative damage [37]. Ceruloplasmin has a ferroxidase efficacy, the altered activity of coagulation, angiogenesis, stagnation of biogenetic amines and protection against oxidative stress [38]. Due to its ferroxidase function, the ceruloplasmin connects to iron metabolism, since stimulate the oxidation of ferrous to ferric iron. This function presented ceruloplasmin has antioxidant role, bringing down the oxidative damage through the inhibition of Fenton reaction, which uses up the ferrous iron to form the Reactive Oxygen Species (ROS) [39].

Antioxidant enzymes and free radical scavengers such as GSH, GST and SOD are essential for keeping the liver from the toxicity of free radicals induced by xenobiotics [1]. And then the antioxidant enzymes such as GST and SOD are important in the detoxification process of xenobiotics. Hepatic glutathione bind with free radicals and block lipid peroxidation. The addition of GSH level gives a protection toward the chemical toxicity [40]. BB intoxication resulted in a substantial reduction in GSH levels in serum and liver, this may be ascribable to its highest use. The decreased levels of GSH in the liver after BB exposure may have ensued from the activity of GP_x in reducing lipid hydroperoxide to stable non-radical lipid alcohols or GSH is directly used as an antioxidant in terminating free radical reaction induced by BB. Alternatively, GSH conjugation to the BB epoxides or its metabolite *in vivo* could be a major pathway of detoxification of BB and GSH reduction [41].

Oxygen metabolism by-products and ROS are reactions due to the presence of unpaired valence shell electrons. Our findings indicated that rats treated with BB exhibited a great enhanced elevation of NO and reduction of the antioxidant enzymes; GPx and SOD in liver tissues. These are in accordance with the study of Heijne et al. [42] which indicates the down regulation of the GP_x gene. The production of highly reactive metabolites from BB is responsible for the elevation of NO and depletion of GSH, in addition, the dimension of the intracellular protective mechanism against ROS leads to oxidative damage and chemical alteration of biologically active macromolecules [43]. NO• is responsible, together with other ROS, to induce cytotoxicity and cytostasis. Many articles on NO⁻⁻ and H₂O₂⁻caused oxidative stress have cited similarities between the two chemicals in production, enzymatic their chemical interference with macromolecules and inducing cytotoxicity [44]. NO• is an inorganic free radical gas emitted from L-arginine by a group of isoenzymes called NO synthases [45]. It is well known that NO• has either antioxidant or pro-oxidant properties. Endogenous NO[•] has a dual function in tissues and cells, where it is an essential physiological signalling molecule mediating various cell processes, but also induces cytotoxic and mutagenic effects when grown in surplus. NO• reacts rapidly with superoxide anion to form peroxynitrite, which may be cytotoxic by itself or easily changed to the highly reactive and toxic hydroxyl radical and nitrogen dioxide. Peroxynitrite is a lot more reactive than NO• or superoxide, which will cause variable chemical reactions in biological systems, including nitration of tyrosine residues of proteins, triggering of lipid peroxidation, inactivation of aconitases, inhibition of mitochondrial electron transport and oxidation of biological thiol compounds [46]. NO• could also be directly oxidized to NO which induces DNA damage. In summation, the reaction of N. with H₂O₂ has been proven to produce potentially cytotoxic singlet oxygen [47].

The process of lipid peroxidation is one of oxidative transformation of polyunsaturated fatty acids to compounds called MDA, which is the most examined, biologically relevant, free radical reaction [48]. Lipid peroxides due to their high cytotoxicity and inhibition to the antioxidant enzymes, are argued to play as a tumour initiator and a cocarcinogenic agents [49]. On the other hand, it is recorded that lipid hydroperoxides broken to give reactive aldehydes, such as MDA and 4hydroxynoneal. MDA is a well-famous mutagen that binds with deoxyguanosine to constitute a major endogenous adduct presented in the DNA of liver [50]. In this study, enhancing in lipid peroxidation was observed in BB-treated rats, this attributed to the elevation production of ROS. Ace of the important causes of BB induced liver injury is lipid peroxidation that mediated by the BB free radical derivatives [4]. Lipid peroxidation is a complex procedure that interrupts the cell construction and role. Peroxidation of membrane lipids stimulates the loss of membrane integrity; membrane bind

enzyme activity and cell lies [51]. In the current survey, the stage of lipid peroxidation was increased in the liver tissues of BB-treated rats indicating occurrence of oxidative stress.

The histopathological investigation of BB treated-rat's revealed hepatitis, fatty degeneration, fatty change (steatosis), hepatic necrosis and nuclear changes. Hepatitis was indicated with leukocytes infiltration and congestion in the blood vessels. The nuclear alterations in the hepatocytes were included: pyknosis, karyorrhexis and karyolysis, irregular contour, imagination, shrinkage, loss of chromatin, its serenity and the absence of the nucleus. A depletion glycogen granules in the hepatocytes in the PAS positive and the histochemical stain; Nile blue the hepatocytes of BB intoxicated rats had numerous Nile blue positive lipofuscin and/or ceroid granules These results were correlated with US EPA, [52] they recorded that a centrilobular inflammation and cytomegaly in male rats at BB doses \geq 200 mg/kg⁻¹ per day, and liver necrosis at BB doses \geq 400 mg/kg⁻¹ per day. Liver injured with BB showed portal loss of hepatic lobular architecture, ballooning of hepatocytes, deformed cord arrangement and disturbed sinusoids. The hepatocytes showed a marked level of hydraulic changes, massive necrosis and marked number of chronic inflammatory cells [53]. After 5 days of BB exposure, most rats of the 300 and 400 mg/kg⁻¹ /day had centrilobular inflammation, including necrotic hepatocytes in some areas and anisokaryocytosis of hepatocytes surrounding the central vein. Multifocal areas of centrilobular granulomatous containing giant cells and focal mineralization were recorded in granulomatous areas [29]. Furthermore, our results of paraffin sections stained with acridine orange stain which examined DNA integrity and chromatin fragmentation under fluorescence microscope showed numerous necrotic hepatocytes. Necrotic nuclei (DNA) stained with acridine orange were appeared as orange spots under a fluorescence microscope, which indicted DNA fragmentation, these findings also indicated that an increase of apoptotic cell death in liver cells (massive DNA degradation) [54]. Our results of oxidative stress parameters indicated that BB-treatment resulted in impairment in liver redox systems which scavenge toxic free radicals and protect against lipid peroxidation which induced DNA damage and cytotoxicity.

The present survey was done to examine the hypothesis that copperalbumin complex can ameliorate against BB induced oxidative damage and hepatic-cytotoxicity in the rat liver. In this respect, our results revealed that liver metabolites (total proteins and albumin) and liver enzyme activities (ALT and AST enzymes) in serum of intoxicated rats treated with copper-albumin complex restored to near normal level. Furthermore, the observed decrease in antioxidant enzymes (GP_x, GR, GST, SOD, tiles and ceruloplasmin) in BB intoxicated rats is conversed to normal levels and also NO and lipid peroxidation levels were significantly decreased after copper-albumin complex treatment. The histopathological assessment of the liver tissues of copper complex treated rats showed that, copper complex had an ameliorative effect on the BB induced hepatotoxicity. This protective effect was indicated by restoration of normal histological and histochemical structure of the hepatic tissue. Mild leukocyte infiltration, congestion and fatty change hepatocytes were retained to nearly normal state. The hepatocytes became full of PAS positive glycogen granules and had no or few Nile blue positive lipofuscin granules. Acridine orange stain sections exhibited most of hepatocytes were healthy nuclei and few with necrotic ones. Former subjects have confirmed such activity for copper nicotinate and copper glycinate complex in rats as anti-inflammatory agents [55] and also in gastric ulcer remedy [14]. This ameliorative effect albumin copper complex may be attributed to chemical attraction of a new copper complex to have effective antioxidant/ radical scavenging activities [5]. Among the cationic ligands, copper earns particular importance because as transition metal, it is very hard to generate ROS after a reaction with O. Free Cu (II) ions can react with hydrogen peroxide (H₂O₂) resulting in the formation of the harmful hydroxyl radical via the Fenton reaction. Bind to proteins, copper is greatly less susceptible to occupy in the Fenton reaction. In connection of metals bound to albumin, hydroxyl radicals released from Fenton reaction are generally directed to the protein sparing more important objectives. Antioxidant and radical scavenging activities of the copper (II) complex were evaluated by variable in vitro assays [5]. In plasma, most of the copper is bound to caeruloplasmin, but a high dimension of the metal ion may present bounded to albumin [56]. Protein binding to Cu (II) ions has been proven to prevent ROS-generating reactions. The first four amino acids of the Nterminus of albumin, Asp-Ala-His-Lys (DAHK), constitute a tightbinding site for Cu (II) ions. Many assays have documented the antioxidant property of albumin by binding of copper using the fuzzinduced Low-Density Lipoprotein (LDL) oxidation assay [57-59]. DAHK inhibited LDL lipid peroxidation and DAHK/C complex revealed a superoxide dismutase-like activity by significantly prohibit the production of ROS [58]. Moreover, HAS and the tetra peptide occupying its N-terminus (DAHK) were found to inhibit neuronal death in murine cortical cell cultures exposed to oxidative damage caused by H₂O₂ and by a mixture of copper and ascorbic acid [60].

In conclusion, BB is highly hepatotoxic through its issue of the antioxidant defence mechanism in a topic that the ions produce NO associated with free radicals accumulation and lipid peroxidation. Free radicals interact with different portions of the tissues and subsequently cause liver injury and dysfunction. Still, copper-albumin treated BB rats were shown variable revert to normal. Cop involved in quenching of reactive oxygen species. Its usefulness in these several systems is often attributed to its unparalleled ability to cycle between the Cu (I) and Cu (II) oxidation states. Therefore, it can behave as an electron donor or acceptor as redox cycling occurs.

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Page 10 of 10

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