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Possible Therapeutic Role Of *Ginkgo biloba* Loaded On Gold Nanoparticles Against Potassium Bromate-Induced Hepatotoxicity In Rats.

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ABSTRACT

The present study investigated the effects of *Ginkgo biloba* extract (GBE) and *Ginkgo biloba* extract loaded on gold nanoparticles (GBE AuNPs) in attenuating potassium bromate (KBrO3) induced hepatotoxicity. Rats were divided into eight groups (control, GBE, AuNPs, GBE AuNPs, $KBrO_3$, $KBrO_3$ /GBE, $KBrO_3$/AuNPS and $KBrO_3$/GBE AuNPs). $KBrO_3$ administration resulted in significant elevations in the level of serum alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (Alb), total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), creatinine, urea and uric acid. $KBrO_3$ also caused degeneration at the periphery of the hepatic lobules, accompanied by increased hepatic oxidative stress markers malondialdehyde (MDA), protein carbonyl (PC) and nitric oxide (NO) concomitant with exhausting the hepatic antioxidant molecules superoxide dismutase (SOD), catalase (CAT), glutathione-S-tranferase (GST) and reduced glutathione (GSH). Treatment with (GBE, AuNPS or GBE AuNPS) reduced the extent of liver damage induced by $KBrO_3$ as indicated by decreased ALT, AST, ALP, TP, Alb, TB, DB, creatinine, urea, uric and lipid profile levels. These treatments ameliorated also the histopathological alternations of the liver tissue induced by $KBrO_3$. In conclusion: Ginkgo biloba extract loaded on gold nanoparticles was the most efficient in attenuating $KBrO_3$ induced hepatotoxicity, and this is the first study to demonstrate this.

Keywords: *Ginkgo biloba* extract; *Ginkgo* loaded on gold nanoparticles; potassium bromate; hepatotoxicity; Oxidative stress

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INTRODUCTION

Potassium bromate ($KBrO_3$) is a water-soluble white crystalline powder used in laboratory reagents, oxidizing agents and explosives, fermented beverages, and fish paste in many countries. It is also used as neutralizing agent in cold wave hair lotions and cosmetics **(Li *et al*., 2017)**. $KBrO_3$ is often used in food industry as a food additive, especially in bread production **(Öztürk *et al*., 2020)**. This ionic compound, consisting of potassium and bromate salts, is a strong oxidizing agent, it has no medicinal importance but is added to flour as a maturing agent. Bromate was first discovered to cause tumors in rats in 1982, and subsequent studies validate its damage to the liver and other organs **(Busuyi Kolade *et al*., 2020)**.

Administration of $KBrO_3$ to rats was found to induce oxidative stress and passively impaired the antioxidant power **(Bayomy *et al*., 2016)**. Hepatocyte degeneration and necrosis, congestion and swelling of tubular cells were observed in rats treated with $KBrO_3$ **(Gheth *et al*., 2019)**. $KBrO_3$ causes primary DNA oxidative damage and increases 8-hydroxydeoxyguanosine (8-OHDG) which is the most abundant oxidized DNA lesion. It also caused structural chromosomal aberrations in bone marrow cells of rats **(Starek & Starek-Świechowicz, 2016)**.

Potassium bromate ($KBrO_3$) in various consumer items posses mild to severe toxicity to critical organs liver, and brain in the living systems. It has been categorized as a potential class II B carcinogen for humans, while it is confirmed as a carcinogen in the experimental animals attributed to its extensive oxidizing property and mutagenicity. For these harmful effects, its usage in food products is banned in many countries of the European Union, Canada, and many south American, African, and Asian countries, including India, China, and Sri Lanka, yet it is used in countries like the USA and Japan with certain limitations **(Ajarem *et al*., 2016)**. Also, it is restrictively or illegally used in many other countries.

The increasing rate of herbal medicine use is gaining approval in both the public and medical world. One of such herbal products is GBE. *Ginkgo biloba* leaf has been used in traditional Chinese medicine to treat various conditions for several years and it is one of the top selling herbs in USA. *Ginkgo biloba* (maidenhair tree) is one of the oldest herbal medicines that have been used as therapeutic agents in modern pharmacology. GBE contains flavonoids and flavone glycosides, lactone derivatives (ginkgolides), bilobalide, ascorbic acid, iron-based superoxide, 6-hydroxykinuretic acid, protocatechuic acid, sterols and vanillic acid. The major classes of active ingredients are the ginkgolides and bilobalides (also known as terpenes) and the flavonoid **(Olubunmi *et al*., 2017)**.

Ginkgo biloba reduced significantly ALT and AST of liver, reversed oxidative damage induced by mercury in liver and relieved the hepatocyte swelling and necrosis **(Şener *et al*., 2007)**.

Abdul-Hamid *et al*. (2018) reported that the GBE decreased the liver abnormalities induced by amiodarone in male albino rats. In these studies, the protective properties of GBE were associated with the active ingredients such as 6% terpenoids such as ginkgolides and bilobalides and 24% flavonoid glycosides such kaempferol, quercetin, and isorhamnetin, these active compounds are reported to prevent LPO, reduce oxidative stress and apoptosis, histopathological damages, and inhibit inflammation **(Singh *et al*., 2019; Yalçın *et al*., 2020)**.

After the advent of nanotechnology, there is a growing trend about the design, synthesis, and use of engineered nanoparticles (NPs) in different areas including medicine, cosmetics, coating, bioremediation, paints, electronics, and food industry. Recently, gold nanoparticles (AuNPs) have been regarded as promising candidates for optical sensors, imaging, drug delivery, and therapeutic applications due to their size and shape dependent physical properties and their inherent biocompatibility compared with other metallic nanoparticles **(Ibrahim *et al*., 2018)**.

Gold nanoparticles (AuNPs) are versatile tools, highly used in biomedical applications, including targeted transport of some drugs. AuNPs inorganic nanoparticles, present physicochemical properties that cannot be found in organic-inorganic hybrid nanostructures. The small diameter of AuNPs can be irreversibly bound to the cellular DNA. Kupffer cells present an increased capacity to assimilate the nanomaterials and for this reason they can be used as target-cells in case of drug delivery coupled with administration of AuNPs. Therefore, AuNPs were proposed in liver fibrosis therapy, reducing liver fibrosis. AuNPs proved their beneficial

effects in alcohol-methamphetamine-induced liver injury and acetaminophen induced hepato-renal injury in rats **(Clichicia *et al*., 2020)**.

Based on these data, the present study aimed to evaluate the possible therapeutic effect of GBE, AuNPs and GBE loaded on AuNPs against $KBrO_3$ induced hepatotoxicity in rats, and to investigate the mechanisms underlying their effects.

MATERIALS AND METHODS

Experimental animals

Forty-eight healthy male Spargue Dawely (SD) rats with 6–7 weeks old, with average weight of 95g were used for experiment. Rats were obtained from Egyptian Institute for Serological and Vaccine production, Helwan, Egypt and were housed in the animal house of the Department of Zoology, Faculty of Science, Kafr El-Sheikh University. Rats were placed in stainless steel cages containing wood-chip bedding, renewed every day. They were kept in a temperature-controlled environment with a 12 h light/dark cycle. All rats were acclimatized to the place for one week before the commencement of the experiments. All rats were provided with normal diet and water was allowed *ad libitum* during the study. The experimental protocol was carried out in accordance with the guide of the National Research Council for the Care and Use of Laboratory Animals and was approved by the local experimental animal ethics committee of the Department of Zoology, Faculty of Science, Kafr El-Sheikh University.

Animal grouping and mode of treatment

After one week of acclimatization period, animals were divided into eight groups, each consisting of six animals as follows:

1. **Control group**: Rats of this group did not receive any treatment.
2. ***Ginkgo biloba* extract (GBE) treated group:** Rats were administered GBE (100mg/kg bw) by intragastroluminal gavage (i.g.) twice weekly for 4 weeks. The chosen dose of GBE (100 mg/kg bw) was according to the previous study of **Lebda *et al*. (2018)**.
3. **Gold nanoparticles (AuNPs) treated group:** Rats were administered AuNPs (5µg Au/Animal) by i.g. twice weekly for 4 weeks. The chosen dose of AuNPs (5µg Au/Animal) was according to the previous study of **Ibrahim *et al*. (2018)**.
4. ***Ginkgo biloba* extract loaded on gold nanoparticles (GBEAuNPs) treated group:** Rats were administered GBE AuNPs (100 mg/kg bw) by i.g. twice weekly for 4 weeks. The chosen dose of GBEAuNPs (100 mg/kg bw) was according to previous studies of **Yallapragada & Velaga (2015)**.
5. **Potassium bromate ($KBrO_3$) treated group:** Rats were administered $KBrO_3$ (100mg/kg bw) by i.g. twice weekly for 4 weeks. The dose of $KBrO_3$ (100 mg/kg bw) was chosen according to previous studies of **Moubarak *et al*. (2020)**.
6. **Potassium bromate and *Ginkgo biloba* extract ($KBrO_3$ + GBE) treated group:** Rats were administered $KBrO_3$ (100 mg/kg bw) by i.g. twice weekly for 4 weeks alone, then rats were administered GBE (100 mg/kg bw) twice weekly for another 4 weeks after termination of $KBrO_3$ administration.
7. **Potassium bromate and Gold nanoparticles ($KBrO_3$ + AuNPs) treated group:** Rats were administered by i.g. $KBrO_3$ (100 mg/kg bw) twice weekly for 4 weeks alone, then rats were administered (5µg Au/Animal) twice weekly for another 4 weeks after termination of $KBrO_3$ administration.
8. **Potassium bromate and *Ginkgo biloba* extract loaded on gold nanoparticles ($KBrO_3$ + GBEAuNPs) treated group:** Rats were by i.g. administered $KBrO_3$ (100 mg/kg bw) twice weekly for 4 weeks, then rats were administered GBEAuNPs (100 mg/kg bw) twice weekly for another 4 weeks after termination of $KBrO_3$ administration.

Chemicals

Potassium bromate ($KBrO_3$) is an odorless white crystalline powder. It was obtained in powder form from El-Gomhouria Chemicals Company, (Cairo, Egypt), $KBrO_3$ was dissolved in distilled water.

Ginkgo biloba extract (GBE) was obtained from EMA Pharm Company for Pharmaceuticals and Medicinal Plants (Pharma Plaza building, Asma Fahmy Street, Nozha, Nasr City, Cairo, Egypt). GBE was dissolved in distilled water.

Gold nanoparticles (AuNPs) is a suspension of a spheroidal to rod shape. It was obtained from Nano Gate Company (25 Ibrahim Abo Elnaga-street, Abbas ElAkkad, Nasr City, Cairo, Egypt).

Formation of gold nanoparticles

1 mM solution of 100 ml chloroauric acid (0.034 g) at concentration of 10-3 M was done according to the method described by **Arulkumar and Sabesan, (2010) ; Arundoss and Ar, (2013)**.

Structural characterization of NPs, gold concentration and size

It was carried out using the method of **Ibrahim *et al*. (2018)**.

Methods

Blood sampling and liver tissue preparation

At the end of the experimental period (9 weeks) rats were fasted overnight, sacrificed 24 hrs after the last treatment and blood samples were collected in clean centrifuge glass tubes, left to clot then centrifuged at 3000 rpm for 15 min. The clear nonhemolyzed supernatant was quickly collected. In labeled Eppendorf's tubes, the sera were divided in aliquots and frozen at -20^{o}C for different biochemical analysis. Liver samples were cleaned and homogenized (10% w/v) in cold saline. The homogenate was kept at -20^{o}C in labeled Eppendorf's tubes till used for biochemical estimations. Other samples of liver tissue were stored in neutral buffered formalin (10%) for histopathological studies.

Biochemical assays

Total protein (TP) was estimated using the Biuret method of **Doumas (1975)**. Albumin (Alb) was evaluated according to the method of **Doumas *et al*. (1997)**. Total bilirubin (TB) and direct bilirubin (DB) was evaluated according to **Abd Elhalem *et al*. (2016)**. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were accomplished using the method of **Schumann and Klauke (2003)**. Alkaline phosphatase (ALP) level was measured according to the method of **Belfield and Goldberg (1970)**. Serum urea (Ur) level was determined by **Mohamed and Ashour (2019)** and creatinine (Cr) concentration were calculated according to **Slot (1965)**. Moreover, serum Total cholesterol (TC) was determined as described by **Iwata *et al*. (1990)**. Triglycerides (TG) were determined by the method of **Rojkin *et al*. (1974)**.

Estimation of antioxidant markers

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 and 30 °C according to **Misra and Fridovich (1972)**. Catalase (CAT) activity was measured using hydrogen peroxide as the substrate according to the method previously described by **Manubolu *et al*. (2014)**. Reduced glutathione (GSH) was determined according to the method of **Jollow *et al*. (1974)**. The determination of glutathione-S-tranferase (GST) activity was assayed according to the method of **Habig *et al*. (1974)**.

Estimation of oxidative stress markers

Malondialdehyde (MDA) level was assayed according to the method of **Doğru-Abbasoğlu *et al*. (1997)**. The levels of NO production were measured according to the method of **Yousef and Hussien (2015)**. The levels of PC according to the method of **Levine *et al*. (1990)**.

Histopathological examination

Liver specimens were dehydrated in ascending grades of ethyl alcohol (70 %, 90 % and 100 %), cleared in xylene and impregnated and embedded in paraffin wax. Serial sections of 4-5 micrometers thick were obtained using a rotary microtome and stained with Harris's Haematoxylin and Eosin stain for general histological examination **(Harris, 1900)**.

Statistical analysis

All statistical analyses were conducted using Graph pad prism 5.0 software (Graph pad prism software Inc., San Diego, California, USA). Results are presented as mean ± standard error of the mean (SEM) (n=6). Statistical Comparisons were made by one-way analysis of variance (ANOVA) followed by Neuman-Keuls post-hoc test **(Armitage *et al*., 2008)**. A significant difference was considered when the P value was ≤ 0.05 and any greater significance level was noted.

RESULTS

Characterization of AuNPs

Nanoparticles characteristics were monitored by UV-VIS spectrophotometer and by Transmission Electron Microscope (TEM). The identification results are presented in **Fig.(1& 2)**. For the UV-VIS data, the absorbance was detected between λ 200:800 nm **Fig.(1)** which ensures the presence of the AuNPs in the suspension **(Gowri, et al.,2013)**. On the other hand, the TEM images **Fig.(2)** have shown a majority of nearly spherical AgNPs with diameters ranging from 59.9:81.4 nm. Presently, there have been stunning effort to improve the synthesis of nanoparticles with anticipated sizes and characteristics to grow their biomedical applications, TEM is one of the reliable methods to detect AuNPs sizes **(Gowri, et al.,2013)**.

Effect of different treatments on lipid profile levels

As shown in Table (1), administration of $KBro_3$ resulted in significant increases in serum levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) as compared to normal control group. On the other hand, $KBro_3$ administration resulted in a significant reduction in serum level of high density lipoprotein cholesterol (HDL-C) as compared to normal control group. Treatment with either GBE, AuNPs or GBE/AuNPs significantly reduced serum levels of TC, TG and LDL-C and caused significant increase in HDL-C as compared to $KBro_3$ group.

Effect of different treatments on liver function tests

As shown in Table (2), administration of $KBro_3$ resulted in significant increases in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and direct bilirubin (DB) as compared to normal control group. On the other hand, $KBro_3$ administration resulted in a significant reduction in serum levels of total protein (TP) and albumin (Alb) as compared to normal control group. Treatment with either GBE, AuNPs or GBE/AuNPs significantly reduced serum levels of ALT, AST, ALP, TB, DB and caused significant increase in serum levels of TP, Alb as compared to $KBro_3$ group.

Effect of different treatments on kidney function tests

As shown in Table (3), administration of $KBro_3$ resulted in significant increases in serum levels of creatinine (Cr), urea (Ur), uric acid (UA) as compared to normal control group. Treatment with either GBE, AuNPs or GBE/AuNPs significantly reduced serum levels of Cr, Ur, UA as compared to $KBro_3$ group.

Effect of different treatments on oxidative stress markers in hepatic tissue

As shown in Table (4), administration of $KBro_3$ resulted in significant increases in hepatic tissue contents of malondialdehyde (MDA), protein carbonyl (PC), nitric oxide(NO) as compared to normal control group. On the other hand, $KBro_3$ administration resulted in a significant reduction in superoxide dismutase (SOD), catalase (CAT), glutathione-S-tranferase (GST), reduced glutathione (GSH), as compared to normal

control group. Treatment with either GBE, AuNPs or GBE/AuNPs significantly reduced tissue contents of MDA, PC and NO and caused significant increase in tissue contents of SOD, CAT, GST and GSH as compared to $KBro_3$ group.

Histological examinations

The normal hepatic lobules are the structural units of the liver; each is formed of cords of hepatocytes and blood sinusoids in-between. The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and abundant cytoplasm. The cytoplasm of such cells is granular and strongly eosinophilic. The nuclei of the hepatocytes are large with peripherally dispersed chromatin and prominent nucleoli. Hepatocytes are oriented in cords composed of a single row of cells separated from vascular sinusoids by endothelial cells. The central vein joins to the hepatic vein to carry blood out from the liver. A distinctive component of a lobule is the portal triad (portal spare), which was found running along each of the lobule's corners. The portal area, consists of five structures: a branch of the hepatic artery, a branch of the hepatic portal vein and a bile duct, as well as lymphatic vessels and a branch of the vagus nerve. **Fig.(3-A,B)** control group show architecture of hepatic lobule with normal hepatocytes, portal vein and sinusoids. **Fig.(4-A,B)** GBE group show normal sized, intact central vein (CV) and intact blood sinusoid, intact polyhedral shaped hepatocytes with centrally located nucleus, separated by blood sinusoids. **Fig.(5-A,B)** AuNPs group showed intact CV and hepatocytes arranged in cord like pattern, separated by blood sinusoids, at higher magnification of liver showing hepatic cords, separated by blood sinusoids and portal area. **Fig.(6-A,B)** GBE AuNPs group showed normal hepatic architecture with normal CV (asterisk), normal blood sinusoid and normal hepatic cord. **Fig.(7-A,B)** $KBrO_3$ group showed dilated centro-lobular blood sinusoid, degeneration at the periphery of the lobules, Congestion of the portal vein with inflammatory cells infiltration at the portal area. **Fig.(8-A,B)** KBrO3+GBE group showed mild to moderate dilation of CV and blood sinusoids beside intact hepatocytes, B higher magnification of A. **Fig.(9-A,B)** KBrO3+AuNPs group showed mild dilation of CV and blood sinusoids, beside intact hepatocytes, B higher magnification of A. **Fig.(10-A,B)** KBrO3+GBE/AuNPs group showed intact hepatocytes, radiating from normal sized CV and separated by blood sinusoids.

Table (1): lipid profiles of control and different treated rat groups.

Animal groups / Parameter	C	GBE	AuNPs	GBE AuNPs	$KBrO_3$	$KBrO_3$ + GBE	$KBrO_3$ + AuNPs	$KBrO_3$ + GBE/AuNPs
TC (mg/dl)	72.4 ±1.22	70.1 ±0.64	71.3 ±0.88	69.0 ±1.16	211 ±5.13^{a}	164.8 ±9.67^{ab}	172.0 ±9.32^{ab}	100.3 ±12.41^{b}
TG (mg/dl)	65.7 ±4.28	54.6 ±3.90	62.9 ±5.90	54.0 ±5.02	178.9 ±7.63^{a}	133.2 ±14.29^{ab}	147.7 ±7.81^{a}	84.7 ±3.15^{ab}
HDL-C (mg/dl)	27.8 ±1.42	29.1 ±1.68	27.1 ±0.96	31.2 ±1.07	14.1 ±1.47^{a}	22.0 ±0.83^{ab}	20.6 ±1.20^{ab}	27.1 ±0.96^{b}
LDL-C (mg/dl)	41.6 ±3.93	40.3 ±1.45	39.8 ±1.42	34.9 ±3.13	126.4 ±10.63^{a}	96.9 ±8.14^{a}	99.1 ±8.46^{a}	67.8 ±6.86^{b}

Results are presented as mean ±SE for 6 rats in each group.
C: Control, **GBE:** *Ginkgo biloba* extract, **AuNPs**: Gold nanoparticles, **GBE AuNPs**: *Ginkgo biloba* extract loaded on gold nanoparticles, **$KBrO_3$**: Potassium bromate.
a and b: significant as compared to control and $KBrO_3$ groups, respectively at P≤0.05.

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Table (2): liver function tests of control and different treated rat groups.

Animal groups / Parameter	C	GBE	AuNPs	GBE AuNPs	$KBrO_3$	$KBrO_3$ + GBE	$KBrO_3$ + AuNPs	$KBrO_3$ + GBE/AuNPs
ALT (U/L)	36.0 ±1.85	35.9 ±3.25	36.0 ±2.40	27.7 ±2.51	126.6 ±9.07^{a}	64.8 ±5.87^{ab}	69.2 ±6.72^{ab}	47.1 ±5.17^{ab}
AST (U/L)	51.6 ±3.136	49.7 ±1.715	51.4 ±4.513	48.5 ±1.893	193.5 ±5.60^{a}	97.3 ±1.76^{ab}	111.4 ±6.18^{ab}	79.5 ±3.32^{ab}
ALP (U/L)	143.8 ±7.16	140.7 ±3.32	142.1 ±5.88	108.8 ±12.00	247.1 ±7.43^{a}	212.8 ±6.71^{ab}	230.5 ±21.42^{ab}	194.1 ±4.55^{b}
TP (g/dl)	6.0 ±0.26	6.8 ±0.35	6.7 ±0.20	7.2 ±0.18	3.7 ±0.26^{a}	5.2 ±0.23^{a}	4.7 ±0.32^{b}	5.6 ±0.24^{b}
Alb (g/dl)	4.1 ±0.12	4.1 ±0.21	3.94 ±0.07	4.1 ±0.05	1.5 ±0.18^{a}	3.3 ±0.25^{b}	3.2 ±0.25^{b}	3.9 ±0.09^{b}
TB (mg/dl)	0.2 ±0.03	0.2 ±0.02	0.2 ±0.02	0.1 ±0.01	0.9 ±0.11^{a}	0.6 ±0.09^{ab}	0.7 ±0.05^{ab}	0.4 ±0.02^{b}
DB (mg/dl)	0.1657 ±0.012	0.1467 ±0.012	0.1543 ±0.011	0.133 ±0.001	0.41 ±0.021^{a}	0.3083 ±0.009^{ab}	0.3267 ±0.015^{a}	0.21 ±0.006^{b}

Results are presented as mean ±SE for 6 rats in each group.
C: Control, **GBE:** *Ginkgo biloba* extract, **AuNPs**: Gold nanoparticles, **GBE AuNPs**: *Ginkgo biloba* extract loaded on gold nanoparticles, **$KBrO_3$**: Potassium bromate.
a and b: significant as compared to control and $KBrO_3$ groups, respectively at P≤0.05.

Table (3): Kidney function tests of control and different treated rat groups.

Animal groups / Parameter	C	GBE	AuNPs	GBE AuNPs	$KBrO_3$	$KBrO_3$ + GBE	$KBrO_3$ + AuNPs	$KBrO_3$ + GBE/AuNPs
Cr (mg/dl)	0.7 ±0.04	0.7 ±0.05	0.7 ±0.03	0.6 ±0.04	1.4 ±0.16^{a}	0.7 ±0.04^{b}	0.7 ±0.04^{b}	0.7 ±0.06^{b}
Ur (mg/dl)	18.8 ±2.14	17.3 ±2.03	18.9 ±1.678	15.7 ±2.03	46.7 ±2.03^{a}	35.3 ±2.03^{ab}	36.0 ±2.65^{ab}	24.0 ±1.16^{ab}
UA (mg/dl)	3.2 ±0.50	2.9 ±0.39	3.1 ±0.36	2.2 ±0.34	8.8 ±0.26^{a}	7.0 ±0.38^{a}	7.9 ±0.35^{a}	4.7 ±0.32^{b}

Results are presented as mean ±SE for 6 rats in each group.
C: Control, **GBE:** *Ginkgo biloba* extract, **AuNPs**: Gold nanoparticles, **GBE AuNPs**: *Ginkgo biloba* extract loaded on gold nanoparticles, **$KBrO_3$**: Potassium bromate.
a and b: significant as compared to control and $KBrO_3$ groups, respectively at P≤0.05.

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Table (4): Oxidative stress and antioxidant markers in hepatic tissue of control and different treated rat groups.

Animal groups / Parameter	C	GBE	AuNPs	GBE AuNPs	$KBrO_3$	$KBrO_3$ + GBE	$KBrO_3$ + AuNPs	$KBrO_3$ + GBE/AuNPs
MDA (nmol/g)	1437 ±36.07	1236 ±42.75	1353 ±46.39	1128 ±38.27	2278 ±17.63^{a}	2027 ±46.97^{a}	2079 ±79.66^{a}	1701 ±136.10^{b}
PC (µmol DNPH / mg)	1.1 ±0.27	1.0 ±0.22	1.1 ±0.22	0.8 ±0.07	4.5 ±0.25^{a}	3.1 ±0.38^{a}	4.0 ±0.05^{a}	2.3 ±0.28^{ab}
NO (mg/g)	79.3 ±5.75	62.7 ±11.21	75.2 ±8.98	60.5 ±3.15	142.2 ±10.03^{a}	105.4 ±5.13^{b}	124.3 ±3.55^{a}	93.7 ±3.41^{b}
SOD (U/g)	192.6 ±7.32	198.7 ±6.96	192.0 ±6.03	214.0 ±5.57	67.03 ±4.08^{a}	145.2 ±3.08^{ab}	123.3 ±5.99^{ab}	176.9 ±7.70^{b}
CAT (U/g)	187.5 ±2.21	195.0 ±4.70	193.4 ±3.48	204.5 ±10.17	126.6 ±2.15^{a}	174.3 ±4.90^{b}	149.8 ±10.58^{a}	184.3 ±3.60^{b}
GST (U/g)	5.2 ±0.19	5.8 ±0.14	5.3 ±0.21	6.1 ±0.22	2.94 ±0.18^{a}	4.6 ±0.31^{a}	3.9 ±0.16^{b}	4.8 ±0.28^{b}
GSH (mg/g)	80.9 ±5.68	85.8 ±8.90	81.6 ±9.00	96.9 ±8.44	25.6 ±3.71^{ab}	52.0 ±4.38	47.3 ±5.76^{a}	64.8 ±5.46^{b}

Results are presented as mean ±SE for 6 rats in each group.
C: Control, **GBE:** *Ginkgo biloba* extract, **AuNPs**: Gold nanoparticles, **GBE AuNPs**: *Ginkgo biloba* extract loaded on gold nanoparticles, **$KBrO_3$**: Potassium bromate.
a and b: significant as compared to control and $KBrO_3$ groups, respectively at P≤0.05.

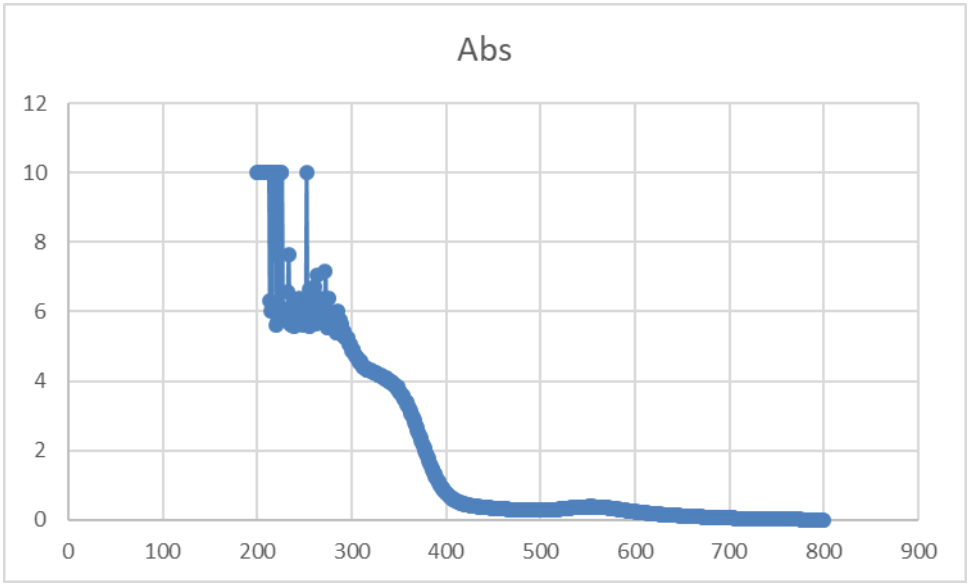

Fig.(1): AuNPs were examined by UV/VIS spectrophotometer Particles absorbance was maintained at range between 200:800 nm.

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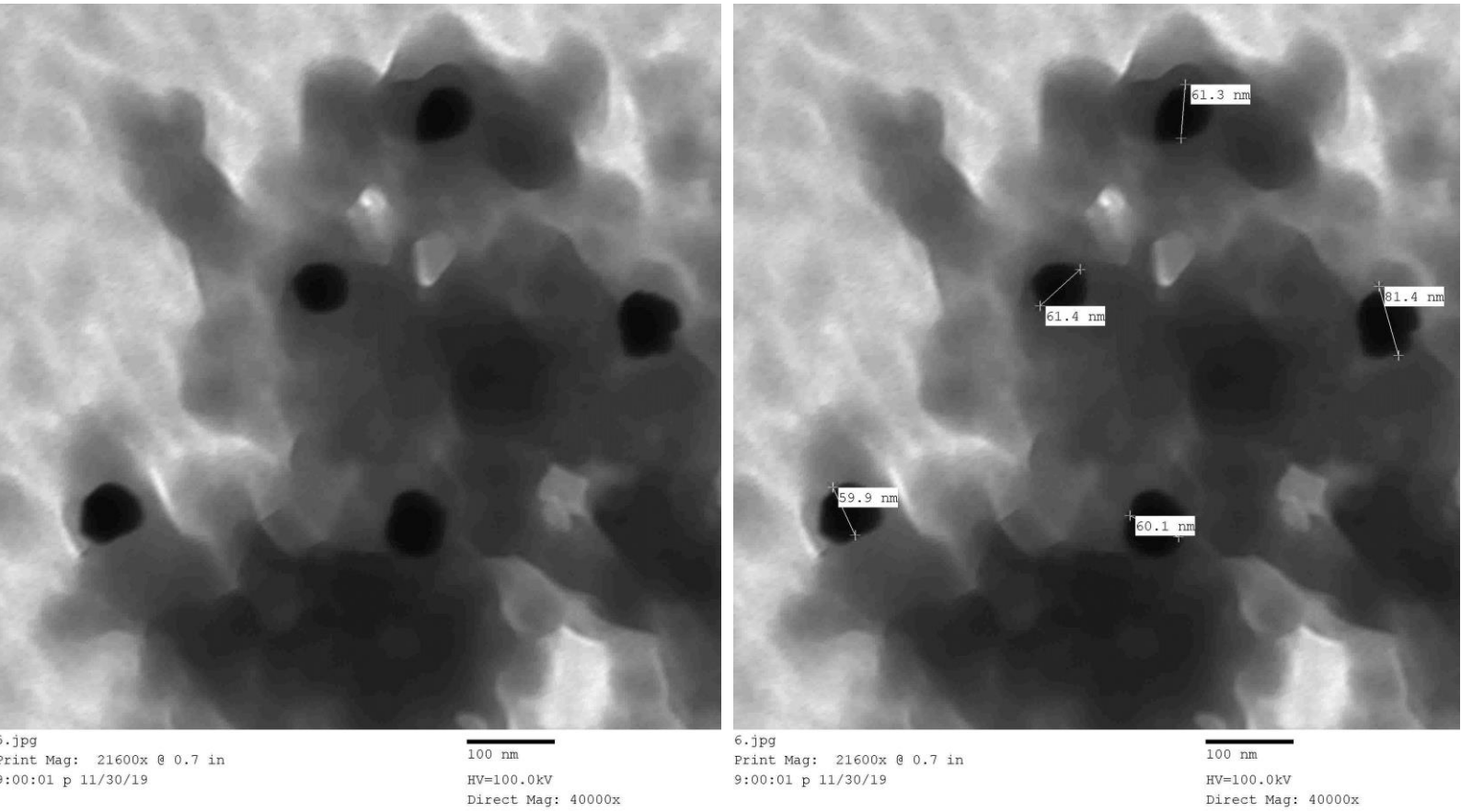

Fig.(2): Electron micrograph of AuNPs suspension on Transmission Electron Microscopy (TEM) showing majority of almost spheroidal to rod shaped gold nanoparticles with diameters ranging between 59.9:81.4 nm.

Histological examinations

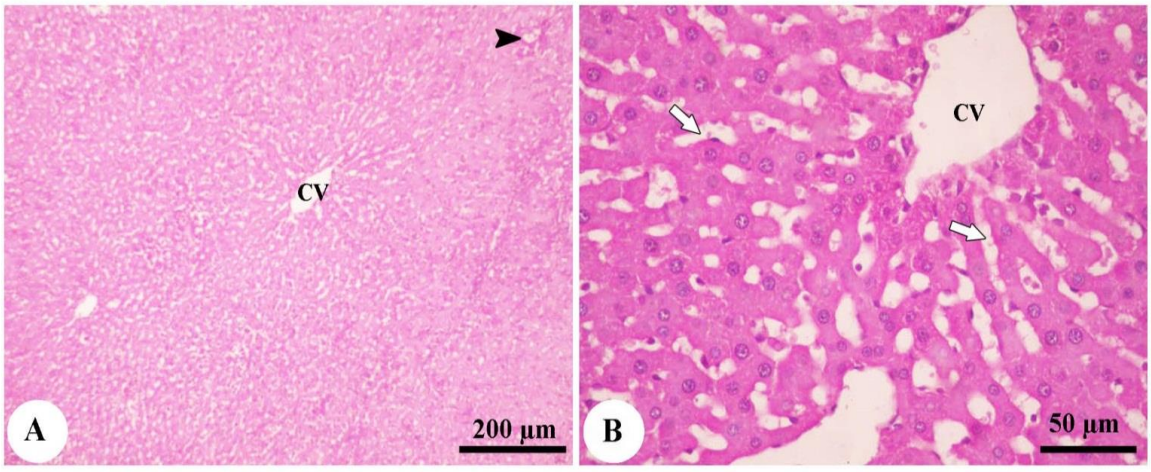

Fig (3) Photomicrograph of liver from group normal control showing; A, normal architecture of liver tissue including, central vein (CV), portal area with portal vein (arrow head), B, normal hepatic cords with normal blood sinusoid (white arrow). H&E

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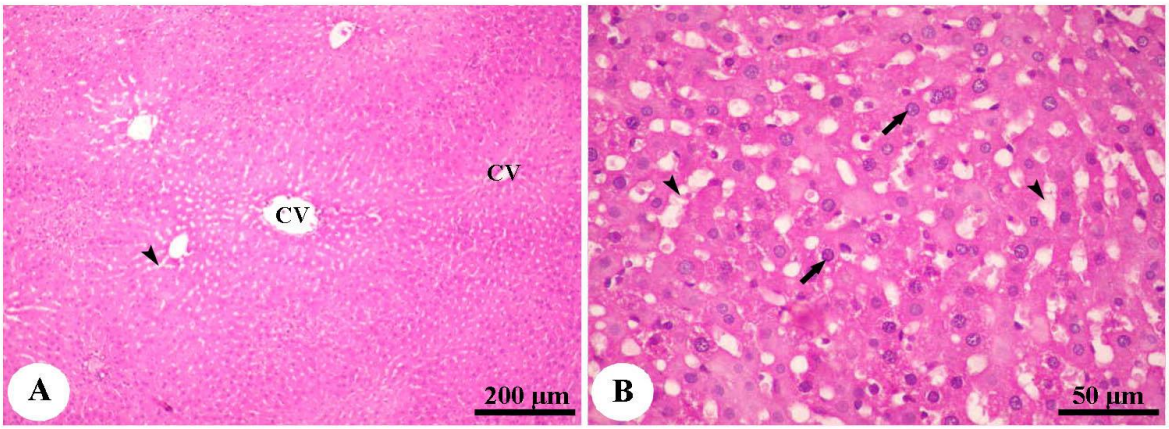

Fig (4) Photomicrograph of liver from group GBE showing; A, normal sized, intact central vein (CV) and intact blood sinusoid (arrow head). B, intact polyhedral shaped hepatocytes with centrally located nucleus (arrow) separated by blood sinusoids (arrow head). H&E.

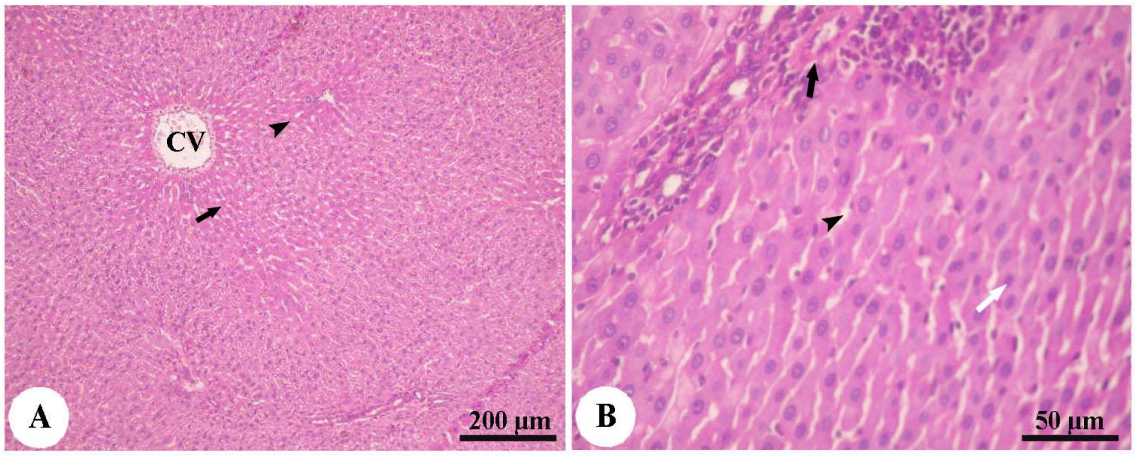

Fig (5) Photomicrograph of liver from group AuNPs showing; A, intact central vein (CV) and hepatocytes arranged in cord like pattern (arrow) separated by blood sinusoids (arrow head). B, higher magnification of liver showing hepatic cords (white arrow) separated by blood sinusoids (arrow head) and portal area (black arrow). H&E.

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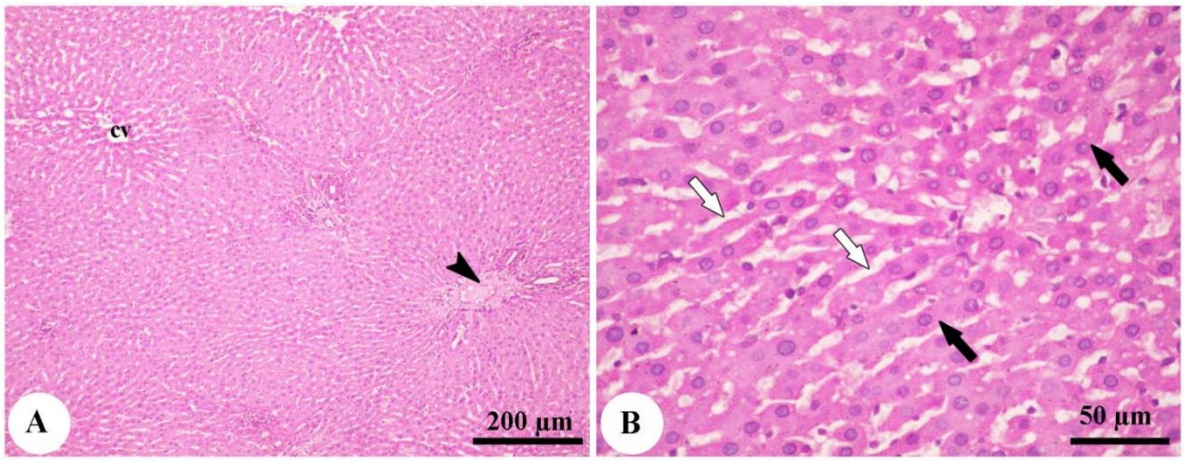

Fig (6) Photomicrograph of liver from group GBE/AuNPs showing; A, normal hepatic architecture with normal central vein (asterisk) B, normal blood sinusoid (white arrow) , normal hepatic cord (black arrow).

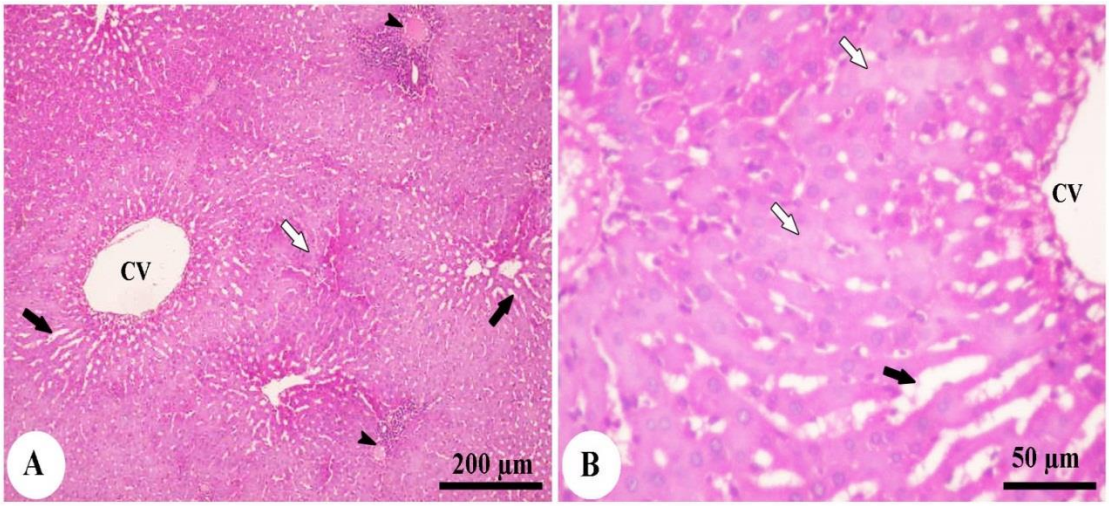

Fig (7) Photomicrograph of liver from group $KBrO_3$ showing; A, dilated centro-lobular blood sinusoid (black arrow), degeneration at the periphery of the lobules(white arrow). B, Congestion of the portal vein with inflammatory cells infiltration at the portal area (arrow head).

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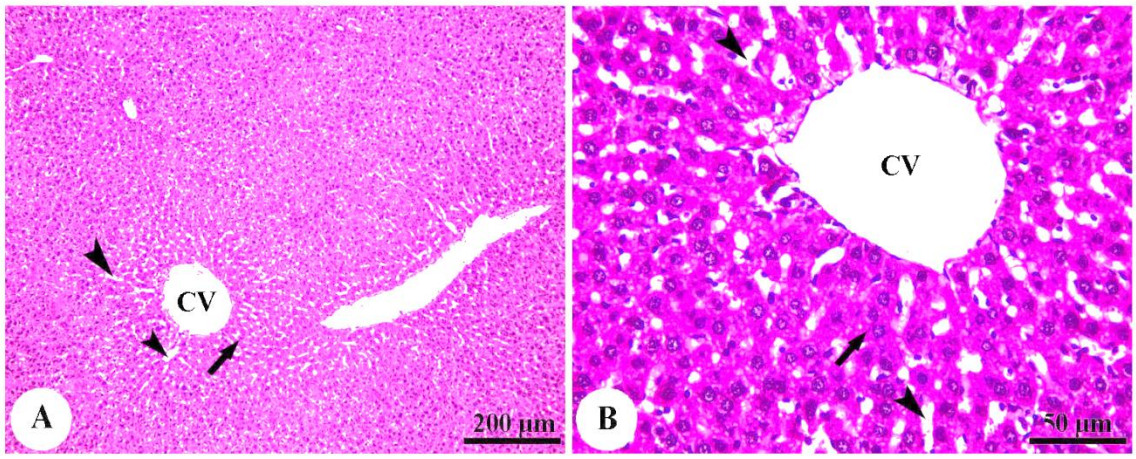

Fig (8) Photomicrograph of liver from group $KBrO_3$+GBE showing; mild to moderate dilation of central vein (CV) and blood sinusoids (arrow head) beside intact hepatocytes (arrow) B, higher magnification of A. H&E.

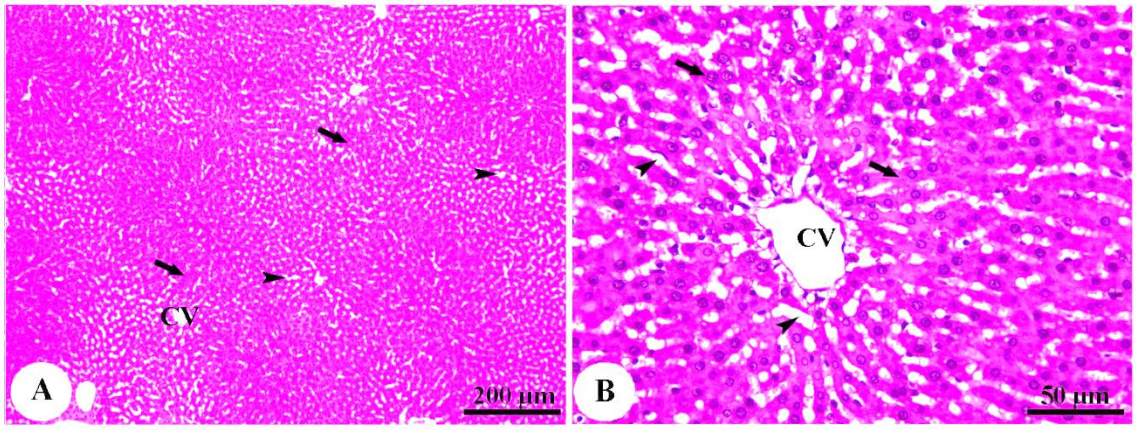

Fig (9) Photomicrograph of liver from group $KBrO_3$+ AuNPs showing A, mild dilation of central vein (CV) and blood sinusoids (arrow head) beside intact hepatocytes (arrow). B, higher magnification of A. H&E.

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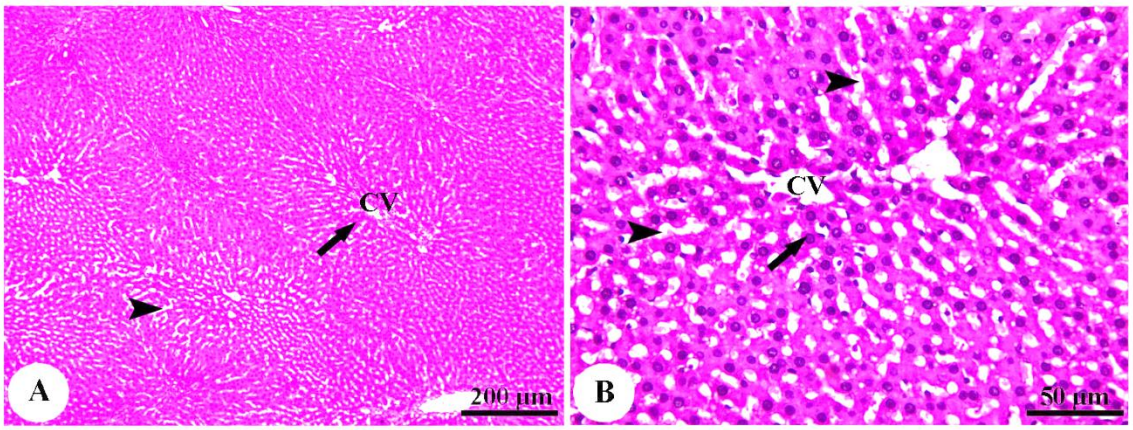

Fig (10) Photomicrograph of liver (A&B) from group $KBrO_3$+GBE AuNPs showing intact hepatocytes (arrow) radiating from normal sized central vein (CV) and separated by blood sinusoids (arrow head). H&E.

DISCUSSION

Various studies showed that $KBrO_3$ is a strong oxidizing agent that generates free radicals during xenobiotic metabolism. It perturbs the redox balance in the cells damaging the structural and functional status of the target tissues and macromolecules. Such derogatory effect, if prolonged, can cause many diseases, including cancer, depending on the dose, duration, and concurrent circumstances in the exposed organisms **(Hassan *et al*., 2020)**.

The present study investigated the potential therapeutic effects of GBE, AuNPs and GBE AuNPs on $KBrO_3$ induced hepatotoxicity.

In the current study, $KBrO_3$ administration significantly increased TC, TG and LDL-C while decreased HDL-C compared to control group. These results are in agreement with other previous studies **(Altoom *et al*., 2018; Ben Saad *et al*., 2016; Rezq, 2019)**. While treatment groups (GBE, AuNPs, GBEAuNPs) showed significant decrease in TC, TG and LDL-C, and significantly improved HDL-C. Similar results were obtained by **Dubey *et al*. (2005)** as they registered elevated levels of serum TC, TG, LDL-C and decreased HDL-C that induced by Coconut oil was used as a vehicle for cholesterol feeding, that were returned towards normal values by GBE. Also, **Wei *et al*. (2013)** reported that simvastatin elevated levels of serum TC, TG, LDL-C and decreased HDL-C but were returned towards normal values by GBE.Also, **Vinodhini *et al*. (2014)** reported elevated levels of serum TC, TG, LDL-C and decreased HDL-C that induced by isoproterenol and returned towards normal values by AuNPs.

$KBrO_3$ significantly increased serum level of ALT, AST, ALP, TB and DB while significantly decreased TP and Alb compared to control group.

ALT and AST levels increased in the current study by $KBrO_3$ as compared to control group and in agreement with **Omer *et al*. (2008) ; Oseni *et al*. (2015)**.While treatment groups showed significant improvement in ALT and AST levels. Similar results were obtained by **Chávez-Morales *et al*. (2011)** who reported that there were elevated levels of serum ALT and AST induced by carbon tetrachloride that were returned towards normal values by GBE. Also, **Parimoo *et al*. (2014)** reported elevated levels of serum ALT and AST induced by lantadenes that returned towards normal values by GBE. Also, **Vinodhini *et al*. (2014)** reported that elevated levels of serum ALT and AST induced by isoproterenol that returned towards normal values by AuNPs.

Similarly, ALP level was significantly increased by $KBrO_3$ administration compared to control group, this agrees with other previous studies **(Farombi *et al*., 2002; Oseni *et al*., 2015)**. While treatment groups showed significant improvement in ALP level. **Ding *et al*. (2005)** reported that the elevated level of serum ALP

by carbon tetrachloride was returned towards normal value by GBE. Also, **Parimoo *et al.* (2014)** reported elevated level of serum ALP induced by lantadenes that were returned towards normal values by GBE. Moreover **Abdelhalim and Moussa (2013)** reported that AuNPs decrease the level of serum ALP compared to control group.

In the current study, $KBrO_3$ significantly reduced TP and Alb that may be due to liver cell damage which resulted in reduction of TP and Alb synthesis compared to control group. This was consistent with data obtained by **Diachenko and Warner (2002) ; Omer *et al.* (2008) ; Saad *et al.* (2016) ; Stuti and D'Souza (2013)**. On the other hand, treatment groups showed significant improvement in Alb and TP levels. Similar results were obtained by other authors working on different toxic materials such as **Zhang *et al.* (2004) and Ding *et al.* (2005)** who reported that the elevated level of serum Alb and TP induced by carbon tetrachloride were returned towards normal values by GBE. In this line **Zhang *et al.* (2011)** reported that treatment by AuNPs improved the levels of serum Alb and TP compared to control group.

Elevations of total bilirubin (TB) and direct bilirubin (DB) in the current study, are indicators of liver injury, cholestasis, and hepatic dysfunction. This effect was consistent with the previous study of **Ben Saad *et al.* (2016).** While treatment groups showed significant decrease in TB and DB levels. These data are in accordance with **Ding, Yu *et al.* (2005) and Chávez-Morales, Jaramillo-Juárez *et al.* (2011)** reported elevated level of serum TB and DB induced by carbon tetrachloride were returned towards normal values by GBE. **Zhang *et al.* (2011)** reported that AuNPs improve level of serum TB and DB compared to control group.

Increased creatinine, urea, and uric acid levels resulted from an impairment in protein metabolism which accumulate in the blood, hence causing uremia, high levels of creatinine a byproduct of creatinine phosphate and an indicative of progression of renal damage. In the current study, renal function was deteriorated caused by $KBrO_3$ administration compared to control group, a result that consistent with other previous studies **Khan, Khan, *et al.* (2012a) ; Kanadi *et al.* (2019) ; Akomolafe *et al.* (2020)** . While treatment groups showed significant improvement in renal function. Similar results were obtained by **Okuyan, Izzettin *et al.* (2012)** who reported elevated level of serum creatinine, urea, and uric acid induced by Cisplatin were returned towards normal values by GBE. Also, **Chang *et al.* (2020)** reported elevated level of serum creatinine, urea, and uric acid induced by Streptozotocin were returned towards normal values by GBE. **Zhang *et al.* (2011)** reported that AuNPs improved the levels of serum creatinine, urea, and uric acid compared to control group.

Oxidative stress is caused by destructive and progressive modifications in one or more body tissues, leading to dysfunction of organs, premature aging, and sometimes diseases and death. It is a natural and fundamental process of the body, but it also involves the acceleration of destructive modifications over time, not only at the cellular level but also at the molecular level **(de Souza *et al.*, 2020)**.

In the current study, $KBrO_3$ induced hepatotoxicity have been identified to involve oxidative stress among others. The increased concentration of MDA in liver tissues of rats are suggestive of facilitated LPO resulting in tissue damage and failure of body's antioxidant defense mechanisms to hinder the formation of excessive free radicals.

In the current study, MDA was significantly increased by $KBrO_3$ administration compared to control group. This is consistent with other previous studies **(Akanji *et al.*, 2008; Bayomy *et al.*, 2016)**. While treatment groups showed significant decreased level of hepatic MDA tissue. Similar results were obtained by**Aljadaani *et al.* (2016)** who reported elevated level of hepatic MDA tissue induced by carbon tetrachloride were returned towards normal values by GBE. Also, **Yalçın *et al.* (2020)** reported elevated level of hepatic MDA tissue induced hydrogen peroxide (H_2O_2) were returned towards normal values by GBE. Also **Dkhil *et al.* (2015)** reported elevated level of hepatic MDA tissue that induced by schistosomiasis were returned towards normal values by AuNPs. Mean while, **Tian *et al.* (2019)** reported effect of GBE exopleura extract and chitosan coating against elevated level of hepatic MDA tissue.

Alternatively, PC has been found to be sensitive to antioxidant intervention. PC is the most commonly measured end product of ROS-induced protein oxidation in biological samples, and this modification is known for its deleterious effects on protein function and structure, is implicated in several diseases **(Shacter, 2000)**.

In the current study, PC was significantly increased by $KBrO_3$ compared to control group, as shown in previous studies **(Ahlborn *et al*., 2009; Ahmad *et al*., 2015; Ben Saad *et al*., 2016; Saad *et al*., 2016)**. While treatment groups significantly decreased level of hepatic PC tissue. Similar results were obtained by **Yallapragada and Velaga (2015)** reported elevated level of hepatic PC tissue induced lead were returned towards normal values by GBE. Also, **Li *et al*. (2019)** reported elevated level of hepatic PC tissue induced D-Gal-Induced Aging were returned towards normal values by GBE. **Lopez-Chaves *et al*. (2018** reported elevated level of hepatic PC tissue that induced by hydrogen peroxide were returned towards normal values by AuNPs.

In fact, increased levels of NO induce nitrosative stress. NO is known to react with superoxide radicals to form the damaging peroxynitrite, a reactive nitrogen species. In the current study, NO was significantly increased by $KBrO_3$ compared to control group, These results agree with previous reports **(Ahmad & Mahmood, 2012, 2016)**. While treatment group showed significant decrease in NO level. Similar results were obtained by **El-Boghdady (2013)** who reported elevated level of hepatic No tissue induced by adriamycin, were returned towards normal values by GBE. Also, **Al Kury *et al*. (2020)** reported elevated level of hepatic No tissue induced by methotrexate were returned towards normal values by GBE and **Dkhil *et al*. (2015)** reported elevated level of hepatic NO tissue that induced by schistosomiasis, were returned towards normal values by AuNPs.

However, antioxidant enzymes play important role in detoxification of oxidative damages and constitute a mutually supportive team of defense against ROS. The oxidative stress is produced as a result of an imbalance between reactive oxygen species and antioxidant defense system. SOD, CAT, GST and GSH represents an armory of antioxidants produced by the body to neutralize or 'mop up' free radicals that can harm the cells and hence defend it against oxidative stress. The ability of the body to produce these antioxidants is controlled by genetic makeup and influenced by exposure to environmental factors such as diet and chemicals **(Dwivedi & Sarkar, 2010)**.

Potassium bromate ($KBrO_3$) results in significant reduction in the levels and activities of non-enzymatic and enzymatic antioxidant molecules including reduced SOD, CAT, GST and GSH in the liver and many other organs **(Tsuchiya *et al*., 2018)**. The involvement of ROS such as H_2O_2, hydroxyl radicals (OH^-) and superoxide anion (O^{2-}) in $KBrO_3$ induced hepatotoxicity has been reported thereby culminating in oxidative stress, which is one of the important mechanisms for several pathological conditions including hepatic injury, tissue wasting, neoplastic transformation, and tumor generation **(Adewale *et al*., 2019)**.

In the current study, SOD was significantly decreased by $KBrO_3$ compared to control group, This is consistent with other previous studies **(Sahin *et al*., 2012; Ahmad *et al*., 2015;Adewale *et al*., 2019; Mohamed & Ashour, 2019)**.While treatment groups showed significant improvement, as shown in previous studies. Similar results were obtained by **Chávez-Morales *et al*. (2011)** who reported decrease level of hepatic SOD tissue induced carbon tetrachloride were returned towards normal values by GBE. Also, **Wahby *et al*. (2017)** reported decreased level of hepatic SOD tissue induced by Bisphenol were returned towards normal values by GBE and **Vinodhini *et al*. (2014)** reported decreased level of hepatic SOD tissue induced by isoproterenol were returned towards normal values by AuNPs. while **Arulkumar and Sabesan (2010)** reported decreased level of hepatic SOD tissue induced by restrain stress were returned towards normal values by GBEAuNPs.

In the current study, CAT was significantly decreased by $KBrO_3$ compared to control group. Previous studies confirmed this **(Sahin *et al*., 2012; Ahmad *et al*., 2015;Adewale *et al*., 2019)** .While treatment groups showed significant improvement in CAT levels. Similar results were obtained by **Chávez-Morales *et al*. (2011)** reported decreased levels of hepatic CAT tissue induced by carbon tetrachloride, were returned towards normal values by GBE. Also, **Wahby *et al*. (2017)** reported decreased levels of hepatic CAT tissue induced by Bisphenol, were returned towards normal values by GBE and **Vinodhini *et al*. (2014)** reported decreased levels of hepatic CAT tissue induced by isoproterenol were returned towards normal values by AuNPs. **Arulkumar and Sabesan (2010)** reported decreased levels of hepatic CAT tissue induced by restrain stress, were returned towards normal values by GBEAuNPs.

In the current study, GSH was significantly decreased by $KBrO_3$ compared to control group, in consistent with previous reports **(Adewale *et al*., 2019; Ahmad *et al*., 2015; Mohamed & Ashour, 2019)**. While treatment groups showed significant improvement in GSH levels. Similar results were obtained by **Abd El-Maksoud *et al*. (2019)** who reported decreased level of hepatic GSH tissue induced by silver nanoparticles were

returned towards normal values by GBE. Also, **Al Kury *et al*. (2020)** reported decreased level of hepatic GSH tissue induced methotrexate were returned towards normal values by GBE. Also, **Yalçın *et al*. (2020)** reported decrease level of hepatic GSH tissue induced by H_2O_2 were returned towards normal values by GBE. While **Vinodhini *et al*. (2014)** reported decrease level of hepatic GSH tissue induced by isoproterenol were returned towards normal values by AuNPs and **Arulkumar and Sabesan, (2010)** reported decreased level of hepatic GSH tissue induced by restrain stress were returned towards normal values by GBEAuNPs.

In the current study, GST activity was significantly decreased by $KBrO_3$ compared to control group, other previous studies support this finding **(Murata *et al*., 2001; Arulkumar and Sabesan, 2010; Khan, Khan, *et al*., 2012b;Ahmad *et al*., 2015)**.While treatment groups showed significant improvement in GST levels. Similar results were obtained by **Wahby *et al*. (2017)** who reported decreased level of hepatic GST tissue induced by Bisphenol were returned towards normal values by GBE. Also, **Al Kury *et al*. (2020)** reported decreased level of hepatic GST tissue induced methotrexate were returned towards normal values by GBE. Moreover **Abdelhafidh *et al*. (2018)** reported that AuNPs improved the level of hepatic GST tissue compared to control group and **Arulkumar and Sabesan (2010)** reported decreased level of hepatic GST tissue induced by restrain stress were returned towards normal values by GBEAuNPs.

In the current study, histological examination showed inflammation and necrosis to liver caused by $KBrO_3$ compared to control group, as supported by other previous studies **(Omer *et al*., 2008; Oyewo *et al*., 2017;Gheth *et al*., 2019)**. While treatment groups exhibited improved histological structure towards normal. Similar results were obtained by **Olubunmi *et al*. (2017)** reported improvement effect of GBE against cadmium. Also, **Abdul-Hamid *et al*. (2018)** reported improvement effect of GBE against amiodarone.

CONCLUSION

The results show that treatment groups ameliorated the hepatotoxic effect produced by $KBrO_3$. This observation indicated the treatment group could be a potential therapeutic agent in the treatment of toxic effects of KBrO3. GBEAuNPs treated group had more effect as compared to GBE treated group and AuNPs treated group.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] Abd El-Maksoud, E.M., et al. Environmental Science and Pollution Research,Ginkgo biloba mitigates silver nanoparticles-induced hepatotoxicity in Wistar rats via improvement of mitochondrial biogenesis and antioxidant status. (2019), *26*(25), 25844-25854.

[2] Abd Elhalem, S., et al. Egypt. J. Exp. Biol. Zool,Short term toxicity of food additive azo dye, sunset yellow (E110), at low doses, in male Sprague-Dawley rats. (2016), *12*, 13-21.

[3] Abdelhafidh, K., et al. Biomarkers,Triangular gold nanoparticles modify shell characteristics and increase antioxidant enzyme activities in the clam Ruditapes decussatus. (2018), *23*(6), 580-588.

[4] Abdelhalim, M.A.K., andMoussa, S.A.A. Saudi journal of biological sciences,The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: In vivo. (2013), *20*(2), 177-181.

[5] Abdul-Hamid, M., et al. Beni-Suef University Journal of Basic and Applied Sciences,The protective effect of grape seed and Ginkgo biloba against hepatotoxicity induced by the antidysrhythmic drug “amiodarone” in male albino rats. (2018), *7*(2), 223-230.

[6] Abuelgasim, A., et al. American journal of food technology,Serrobiochemical effects of potassium bromate on Wistar albino rats. (2008), *3*(5), 303-309.

[7] Adewale, O.O., et al. Notulae Scientia Biologicae,Curcumin Alleviates Potassium Bromate-Induced Hepatic Damage by Repressing CRP Induction through TNF-Î±and IL-1Î² and by Suppressing Oxidative Stress. (2019), *11*(4), 337-344.

[8] Ahlborn, G.J., et al. Food and chemical toxicology,Early alterations in protein and gene expression in rat kidney following bromate exposure. (2009), *47*(6), 1154-1160.

[9] Ahmad, M.K., et al. PloS one,Chemoprotective effect of taurine on potassium bromate-induced DNA damage, DNA-protein cross-linking and oxidative stress in rat intestine. (2015), *10*(3), e0119137.

[10] Ahmad, M.K., andMahmood, R. Chemosphere,Oral administration of potassium bromate, a major water disinfection by-product, induces oxidative stress and impairs the antioxidant power of rat blood. (2012), *87*(7), 750-756.
[11] Ahmad, M.K., andMahmood, R. Environmental toxicology,Protective effect of taurine against potassium bromate-induced hemoglobin oxidation, oxidative stress, and impairment of antioxidant defense system in blood. (2016), *31*(3), 304-313.
[12] Ajarem, J., et al. Behavioral and Brain Functions,Oral administration of potassium bromate induces neurobehavioral changes, alters cerebral neurotransmitters level and impairs brain tissue of swiss mice. (2016), *12*(1), 14.
[13] Akanji, M., et al. African Journal of Biomedical Research,Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. (2008), *11*(1).
[14] Akomolafe, S.F., et al. Biological Trace Element Research,Curcumin Improves Biomolecules Associated with Renal Function and Attenuates Oxidative Injury and Histopathological Changes in Potassium-Induced Toxicity in Rats' Kidney. (2020), 1-8.
[15] Al Kury, L.T., et al. Molecules,Ginkgo biloba Extract Protects against Methotrexate-Induced Hepatotoxicity: A Computational and Pharmacological Approach. (2020), *25*(11), 2540.
[16] Ali, B.H., et al. American journal of translational research,Potassium bromate-induced kidney damage in rats and the effect of gum acacia thereon. (2018), *10*(1), 126.
[17] Aljadaani, B.S., et al. World J Pharm Sci,Effect of Ginkgo biloba and Commiphora opobalsamum extracts on liver fibrosis and kidney injury induced by carbon tetra chloride in experimental models. (2016), *4*(2), 148-152.
[18] Altoom, N.G., et al. Saudi journal of biological sciences,Deleterious effects of potassium bromate administration on renal and hepatic tissues of Swiss mice. (2018), *25*(2), 278-284.
[19] Armitage, P., et al. *Statistical methods in medical research* (2008).). John Wiley & Sons.
[20] Arulkumar, S., andSabesan, M. International Journal of Research in Pharmaceutical Sciences,Behavior and Biochemical changes of nanoginkgoba (Ginkgo biloba gold nano-particles) on restraint stress-induced male albino mice. (2010), *1*(4), 533-538.
[21] Arundoss, T., andAr, S.,MICE MODEL OF PARKINSON'S DISEASE. (2013).
[22] Bayomy, N.A., et al. The Anatomical Record,Effect of potassium bromate on the liver of adult male albino rat and a possible protective role of vitamin C: histological, immunohistochemical, and biochemical study. (2016), *299*(9), 1256-1269.
[23] Belfield, A., andGoldberg, D. Biochemical medicine,Hydrolysis of adenosine monophosphates by acid phosphatases as measured by a continuous spectrophotometric assay. (1970), *4*(2), 135-148.
[24] Ben Saad, H., et al. Environmental toxicology,Altered hepatic m RNA expression of immune response-associated DNA damage in mice liver induced by potassium bromate: Protective role of vanillin. (2016), *31*(12), 1796-1807.
[25] Bozzola, J.J., andRussell, L.D. *Electron microscopy: principles and techniques for biologists* (1999).). Jones & Bartlett Learning.
[26] Busuyi Kolade, A., et al.,The protective effects of Telfairia occidentalis on potassium bromate induced hepatotoxicity in adult Wistar rats. (2020).
[27] Chang, T.-T., et al. Journal of ethnopharmacology,Nrf-2 mediated heme oxygenase-1 activation contributes to the anti-inflammatory and renal protective effects of Ginkgo biloba extract in diabetic nephropathy. (2020), 113474.
[28] Chávez-Morales, R., et al. Human & experimental toxicology,Protective effect of Ginkgo biloba extract on liver damage by a single dose of CCl 4 in male rats. (2011), *30*(3), 209-216.
[29] Clichicia, S., et al. Materials Science and Engineering: C,Hepatoprotective effects of silymarin coated gold nanoparticles in experimental cholestasis. (2020), 111117.
[30] de Souza, G.A., et al. Planta medica,Effects of Ginkgo biloba on diseases related to oxidative stress. (2020), *86*(06), 376-386.
[31] Diachenko, G.W., andWarner, C.R. (2002). Potassium bromate in bakery products: Food technology, toxicological concerns, and analytical methodology. In. ACS Publications.
[32] Ding, J., et al. Liver International,Ginkgo biloba extract alleviates liver fibrosis induced by CCl4 in rats. (2005), *25*(6), 1224-1232.
[33] Dkhil, M.A., et al. International journal of nanomedicine,Antioxidant and hepatoprotective role of gold nanoparticles against murine hepatic schistosomiasis. (2015), *10*, 7467.
[34] Dodd, D.E., et al. Environmental Toxicology and Pharmacology,Subchronic toxicity evaluation of potassium bromate in Fischer 344 rats. (2013), *36*(3), 1227-1234.

[35] Doğru-Abbasoğlu, S., et al. Mechanisms of ageing and development,Lipid peroxidation and antioxidant enzymes in livers and brains of aged rats. (1997), *98*(2), 177-180.
[36] Doumas, B. Clin. Chem,Colorimetric determination of total protein in serum or plasma. (1975), *21*(8), 1159-1166.
[37] Doumas, B.T., et al. Clinica chimica acta,Albumin standards and the measurement of serum albumin with bromcresol green. (1997), *258*(1), 21-30.
[38] Dubey, A.K., et al. IJPT,Hypolipidemic activity of Ginkgo biloba extract, EGb 761 in hypercholesterolemic wistar rats. (2005), *4*(1), 9-12.
[39] Dwivedi, J., andSarkar, P.D. Int J Appl Biol Pharm Technol,Oxidative stress with homocysteine, lipoprotein (a) and lipid profile in diabetic nephropathy. (2010), *1*, 840-846.
[40] El-Boghdady, N.A.,Increased cardiac endothelin-1 and nitric oxide in adriamycin-induced acute cardiotoxicity: protective effect of Ginkgo biloba extract. (2013).
[41] El Mesallamy, H.O., et al. Cancer cell international,The chemopreventive effect of Ginkgo biloba and Silybum marianum extracts on hepatocarcinogenesis in rats. (2011), *11*(1), 38.
[42] Elgendy, H.A., andBayomy, N.A. European Journal of anatomy,Effect of rosmarinic acid on potassium bromate induced renal cortical oxidative stress and apoptosis in adult male albino rat. (2020), *24*(2), 89-98.
[43] Farombi, E.O., et al. Pharmacological research,Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromate (KBrO3) in rats. (2002), *45*(1), 63-68.
[44] Gheth, E.M., et al. Asian Journal of Pharmaceutical Research and Development,Histopathological Effects of Potassium Bromate on Liver Male Rat's and Possible Protective Role of Ruta chalepensis L.(Rutacae) Oil Extract. (2019), *7*(2), 93-97.
[45] Guo, W.-y., et al. Journal of Medicinal Plants Research,The protective effects of ginkgo leaf extract on CCl4-induced liver injury in mice. (2011), *5*(11), 2361-2364.
[46] Habig, W.H., et al. Journal of Biological chemistry,Glutathione S-transferases the first enzymatic step in mercapturic acid formation. (1974), *249*(22), 7130-7139.
[47] Hamed, S.S., et al. Frontiers in Physiology,The protective properties of the strawberry (Fragaria ananassa) against carbon tetrachloride-induced hepatotoxicity in rats mediated by anti-apoptotic and upregulation of antioxidant genes expression effects. (2016), *7*, 325.
[48] Harris, H. Histochemical Technique, by Methuen and Co. Ltd,After Bruce Casselman WC (1959). (1900).
[49] Hashim, I., et al. Journal of Nuclear Technology in Applied Science,Protective effect of Ginkgo biloba extract against oxidative stress induced by gamma-irradiation in rats. (2013), *1*(1), 61-74.
[50] Hassan, I., et al. BioMed Research International,The Alleviative Effect of Vitamin B2 on Potassium Bromate-Induced Hepatotoxicity in Male Rats. (2020), *2020*.
[51] Hirata, B.K., et al. Frontiers in endocrinology,Potential anti-obesogenic effects of ginkgo biloba observed in epididymal white adipose tissue of obese rats. (2019), *10*, 284.
[52] Ibrahim, K.E., et al. Molecules,Histopathology of the liver, kidney, and spleen of mice exposed to gold nanoparticles. (2018), *23*(8), 1848.
[53] Iwata, K., et al. Journal of Nutritional Science and Vitaminology,Effects of Spirulina platensis on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. (1990), *36*(2), 165-171.
[54] Jollow, D., et al. Pharmacology,Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. (1974), *11*(3), 151-169.
[55] Kanadi, M., et al. Asian Journal of Biochemistry, Genetics and Molecular Biology,Dose-dependent chemopreventive effect of methanol extract of Carica papaya seed on potassium bromate-induced nephrotoxicity in rats. (2019), 1-12.
[56] Keles, M.S., et al. Clinical and experimental medicine,Protective effects of N-acetylcysteine and Ginkgo biloba extract on ischaemia-reperfusion-induced hepatic DNA damage in rats. (2008), *8*(4), 193-198.
[57] Khan, R.A., et al. BMC complementary and alternative medicine,Protective effects of rutin against potassium bromate induced nephrotoxicity in rats. (2012a), *12*(1), 204.
[58] Khan, R.A., et al. Lipids in health and Disease,Protective effects of Sonchus asper against KBrO 3 induced lipid peroxidation in rats. (2012b), *11*(1), 164.
[59] Khan, R.A., et al. African Journal of Pharmacy and Pharmacology,Alteration of renal function by potassium bromate (KBrO3): Protective effects of Launaea procumbens. (2012), *6*(19), 1400-1404.
[60] Kurokawa, Y., et al. Environmental health perspectives,Toxicity and carcinogenicity of potassium bromate--a new renal carcinogen. (1990), *87*, 309-335.

[61] Lalier, L., et al. Apoptosis,Bax activation and mitochondrial insertion during apoptosis. (2007), *12*(5), 887-896.

[62] Lebda, M.A., et al. Life sciences,Potential role of α-lipoic acid and Ginkgo biloba against silver nanoparticles-induced neuronal apoptosis and blood-brain barrier impairments in rats. (2018), *212*, 251-260.

[63] Levine, R.L., et al. (1990). [49] Determination of carbonyl content in oxidatively modified proteins. In *Methods in enzymology* (Vol. 186, pp. 464-478). Elsevier.

[64] Li, J., et al. Food & function,Protective effects of fraction 4a of polysaccharides isolated from Lycium barbarum against KBrO 3-induced renal damage in rats. (2017), *8*(7), 2566-2572.

[65] Li, J., et al. Evidence-Based Complementary and Alternative Medicine,Effect of Ginkgo biloba Extract EGb761 on Hippocampal Neuronal Injury and Carbonyl Stress of D-Gal-Induced Aging Rats. (2019), *2019*.

[66] Lopez-Chaves, C., et al. Nanomedicine: Nanotechnology, Biology and Medicine,Gold nanoparticles: distribution, bioaccumulation and toxicity. In vitro and in vivo studies. (2018), *14*(1), 1-12.

[67] Manubolu, M., et al. Journal of ethnopharmacology,Protective effect of Actiniopteris radiata (Sw.) Link. against CCl4 induced oxidative stress in albino rats. (2014), *153*(3), 744-752.

[68] Mercer, E.H., andBirbeck, M.S. *Electron microscopy: a handbook for biologists* (1972).).

[69] Misra, H.P., andFridovich, I. Journal of Biological chemistry,The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. (1972), *247*(10), 3170-3175.

[70] Mohamed, N.E., andAshour, S.E. The Journal of Basic and Applied Zoology,Influence of ethanolic extract of strawberry leaves for abrogating bromate hazards in male rats. (2019), *80*(1), 19.

[71] Moreno, S.R.F., et al. Brazilian Archives of Biology and Technology,Ultrastructural analysis of kidney, liver and duodenum isolated from treated rats with Ginkgo Biloba extract and effects of this medicinal plant on the biodistribution of the padiopharmaceutical sodium pertechnetate. (2008), *51*(SPE), 185-190.

[72] Moubarak, H.S., et al. Journal of Radiation Research and Applied Sciences,Carcinogenic effect of potassium bromate on tongue of adult male albino rats. (2020), *13*(1), 121-131.

[73] Murata, M., et al. Chemical research in toxicology,Requirement of glutathione and cysteine in guanine-specific oxidation of DNA by carcinogenic potassium bromate. (2001), *14*(6), 678-685.

[74] Okuyan, B., et al. European Journal of Biology,The effects of Ginkgo biloba on nephrotoxicity induced by cisplatin-based chemotherapy protocols in rats. (2012), *71*(2), 103-111.

[75] Olubunmi, O.P., et al. International Journal of Clinical and Developmental Anatomy,Gingko biloba Extract Ameliorates Cadmium-Induced Hepatotoxicity in Experimental Animals. (2017), *3*(4), 16.

[76] Omer, R., et al. American journal of food technology,Effect of potassium bromate on liver and blood constituents of wistar albino rats. (2008), *3*(5), 310-314.

[77] Ono, M., et al. Shock,Increased susceptibility to liver injury after hemorrhagic shock in rats chronically fed ethanol: Role of nuclear factor-κB, interleukin-6, and granulocyte colony-stimulating factor. (2004), *21*(6), 519-525.

[78] Oseni, O., et al. Journal of Advances in Medicine and Medical Research,Potassium bromate induced renal toxicity in Wistar albino rats: Effects of aqueous extract of nutmeg (Myristica fragrans Houtt). (2015), 1547-1556.

[79] Oyewo, O., et al. Journal of Morphological Sciences,Hepatotoxic effect of potassium bromate on the liver of wistar rats. (2017), *30*(2), 0-0.

[80] Öztürk, G., et al. Environmental Science and Pollution Research,Dose–response analysis of potassium bromate–induced toxicity in Allium cepa L. meristematic cells. (2020), 1-10.

[81] Parimoo, H.A., et al. Toxicon,Hepatoprotective effect of Ginkgo biloba leaf extract on lantadenes-induced hepatotoxicity in guinea pigs. (2014), *81*, 1-12.

[82] Rezq, A.A. مجلة دراسات وبحوث التربية النوعية,Potential Protective and Ameliorate Effects of Sesame Oil and Jojoba Oil Against Potassium Bromate (Kbro3)-Induced Oxidative Stress in Rats. (2019), *1*(1).

[83] Rojkin, M., et al. Bioquímica del Atlántico,Proteínas totales del suero. (1974), *63*, 1931-1954.

[84] Saad, H.B., et al. Environmental Science and Pollution Research,Biological properties of Alsidium corallinum and its potential protective effects against damage caused by potassium bromate in the mouse liver. (2016), *23*(4), 3809-3823.

[85] Sahin, O., et al. Scientia horticulturae,Influence of chloride and bromate interaction on oxidative stress in carrot plants. (2012), *137*, 81-86.

[86] Schumann, G., andKlauke, R. Clinica chimica acta,New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. (2003), *327*(1-2), 69-79.
[87] Şener, G., et al. Food and chemical toxicology,Ginkgo biloba extract protects against mercury (II)-induced oxidative tissue damage in rats. (2007), *45*(4), 543-550.
[88] Servais, H., et al. Apoptosis,Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. (2008), *13*(1), 11-32.
[89] Shacter, E. Drug metabolism reviews,Quantification and significance of protein oxidation in biological samples. (2000), *32*(3-4), 307-326.
[90] Shirasaki, T., et al. Hepatology,Impaired interferon signaling in chronic hepatitis C patients with advanced fibrosis via the transforming growth factor beta signaling pathway. (2014), *60*(5), 1519-1530.
[91] Singh, N.P., et al. Experimental cell research,A simple technique for quantitation of low levels of DNA damage in individual cells. (1988), *175*(1), 184-191.
[92] Singh, S.K., et al. Neurotherapeutics,Neuroprotective and antioxidant effect of Ginkgo biloba extract against AD and other neurological disorders. (2019), 1-9.
[93] Slot, C. Scandinavian journal of clinical and laboratory investigation,Plasma creatinine determination a new and specific Jaffe reaction method. (1965), *17*(4), 381-387.
[94] Starek, A., andStarek-Świechowicz, B.,Toxicological properties of potassium bromate. (2016).
[95] Stuti, M., andD'Souza, D. Bioscan,Effects of potassium bromate on the kidney and haematological parameters of swiss albino mice. (2013), *8*(3), 1011-1014.
[96] Tang, Y., et al. Oncotarget,Protective effect of Ginkgo biloba leaves extract, EGb761, on myocardium injury in ischemia reperfusion rats via regulation of TLR-4/NF-κB signaling pathway. (2017), *8*(49), 86671.
[97] Tian, F., et al. Journal of the Science of Food and Agriculture,Effect of Ginkgo biloba seed exopleura extract and chitosan coating on the postharvest quality of ginkgo seed. (2019), *99*(6), 3124-3133.
[98] Tsuchiya, T., et al. Journal of toxicologic pathology,Mechanisms of oxidative stress-induced in vivo mutagenicity by potassium bromate and nitrofurantoin. (2018).
[99] Vinodhini, A., et al. Colloids and Surfaces B: Biointerfaces,Cardioprotective potential of biobased gold nanoparticles. (2014), *117*, 480-486.
[100] Wahby, M.M., et al. Egyptian journal of basic and applied sciences,Mitigating potential of Ginkgo biloba extract and melatonin against hepatic and nephrotoxicity induced by Bisphenol A in male rats. (2017), *4*(4), 350-357.
[101] Wang, Y., et al. Drug design, development and therapy,Ginkgo biloba extract mitigates liver fibrosis and apoptosis by regulating p38 MAPK, NF-κB/IκBα, and Bcl-2/Bax signaling. (2015), *9*, 6303.
[102] Wei, J.M., et al. Archives of Medical Science: AMS,Ginkgo suppresses atherosclerosis through downregulating the expression of connexin 43 in rabbits. (2013), *9*(2), 340.
[103] Yalçın, E., et al. Environmental Science and Pollution Research,In vivo protective effects of Ginkgo biloba L. leaf extract against hydrogen peroxide toxicity: cytogenetic and biochemical evaluation. (2020), *27*(3), 3156-3164.
[104] Yallapragada, P.R., andVelaga, M.K. Journal of environmental pathology, toxicology and oncology,Effect of Ginkgo biloba extract on lead-induced oxidative stress in different regions of rat brain. (2015), *34*(2).
[105] Yousef, M.I., andHussien, H.M. Food and chemical toxicology,Cisplatin-induced renal toxicity via tumor necrosis factor-α, interleukin 6, tumor suppressor P53, DNA damage, xanthine oxidase, histological changes, oxidative stress and nitric oxide in rats: protective effect of ginseng. (2015), *78*, 17-25.
[106] Zhang, C., et al. The American Journal of Chinese Medicine,The effect of Ginkgo biloba extract (EGb 761) on hepatic sinusoidal endothelial cells and hepatic microcirculation in CCl 4 rats. (2004), *32*(01), 21-31.
[107] Zhang, X.-D., et al. International journal of nanomedicine,Size-dependent in vivo toxicity of PEG-coated gold nanoparticles. (2011), *6*, 2071.