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Original Research Article

Hepatoprotective Effects of Kombucha Tea in Carbon Tetrachloride-Induced Hepatotoxicity: An *In Vivo* and Molecular Docking Studies

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ABSTRACT

Kombucha is a fermented tea produced by the fermentation of tea with a starter culture known as Symbiotic colony of yeast and bacteria "SCOBY". This study aimed to determine the best conditions of tea fermentation and to evaluate the antioxidant and hepatoprotective effects in CCl_4-induced hepatotoxicity in rats. This study was performed using CCl_4-induced liver injury in rat model. The fermented black tea (BTE) and green tea (GTE) extracts at dose level of 100 mg/kg b.wt were administered orally prior to CCl_4 administration. The antioxidant activity was assessed by the determination of reduced glutathione (GSH) level, while the hepatoprotective effect was assessed by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), along with histopathological examination. HPLC investigation was performed for selected polyphenolic compounds and caffeine alkaloid. We found that fermentation of black tea for three weeks gave best results as indicated by the chemical analysis and by studying of the hepatoprotective activity. *In vivo* antioxidant and hepatoprotective effects revealed significant decrease in AST, ALT and increase in GSH levels. A molecular docking study was performed regarding the polyphenolic compounds and caffeine, and the results showed a conformational fitting in the active site of human myeloperoxidase [hMPO] (ID: 3ZS0). The results of the current study could be of benefit for the development of functional drink with significant prophylactic effects, particularly in Egypt, which showed high prevalence of liver diseases.

Keywords: Antioxidant activity, Hepatoprotective Effect, CCl_4, Kombucha, Molecular docking.

Introduction

Globally, liver diseases were estimated to cause around two million deaths per year due to viral hepatitis, hepatocellullar carcinoma, and cirrhosis.[1] In Egypt, the liver disease incidence increased mainly due to bilharziasis, viral infection with hepatitis C, and hepatocellullar carcinoma which causes progressive liver damage.[2] Due to COVID-19 pandemic, there was a difficulty of transportation between the world states,[3] and a lot of need to manufacture effective and safe natural products locally.[4] This led to search for a beneficial product having antioxidant and hepatoprotective effects like Kombucha.[5] Worldwide, Kombucha is a traditional fermented tea beverage resulting from the essential process which is the fermentation of tea by mushroom known as "tea fungus" or "*Manchurian* tea".[6] It is widely spread from China to Russia and to the rest of the world. Recently, kombucha is a valuable product and powerful beverage which exerts beneficial effects on human health as antioxidant, antdiabetic,[7] hepatoprotective,[8] anticancer,[9] antimicrobial,[10] due to the release of many bioactive compounds resulting from fermentation process.

Various biological effects of black and green tea extracts have been related to their content of polyphenols, such as (-)-epicatechin, (+)-catechin, (+)-gallocatechin, (-)- gallocatechin gallate, (-)-epicatechin gallate (ECG), (-)-epigallocatechin, and (-)- epigallocatechin gallate (EGCG). In addition, flavonols: quercetin, kaempferol and phenolic acids: chlorogenic, gallic, caffeic acids, carotenes, caffeine and minerals were present.[11] These compounds scavenge free radicals, and their use has been proposed as an adjuvant therapy for liver diseases. These biologically active substances have potent antioxidant properties and exert protective effects.[12]

The term 'Tea" {*Camellia sinensis* (L.) Kuntze} is common for woody flowering plants belonging to family Theaceae. The family contains 28 genera with about 520 species.[13] Worldwide, tea is obtainable from two main varieties: *Camellia sinensis* var. *Sinensis* and var. *Assamica*. It acts as prophylactic agent and as a healthy beverage, probably due to its therapeutic constituents.[11] Molecular docking study has proved the biological activities by the fitting of the compounds in the active sites of the enzyme which exhibited significant biological effects.

Due to the high incidence of liver diseases in Egypt, the development and introduction of kombucha tea into the Egyptian market was found to be necessary as drink with high antioxidant activity and hepatoprotective effects. The molecular docking has been performed to assist the understanding of the activity of the main polyphenolic compounds and caffeine. Moreover, to explore their binding mode with the active site of myeloperoxidase (ID: 3ZS0).

Materials and Methods

Chemicals and reagents

Camellia sinensis, black and green tea leaves were of the commercial type available in local market. It was obtained from Lipton, Unilever Co., Egypt in April, 2020. Ethanol, carbon tetrachloride and methanol

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are of analytical grade and were purchased from El-Gomhorea Co. (Cairo, Egypt). Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), reduced glutathione (GSH) were purchased from Bio-diagnostic Company (Doki, Egypt). The starter culture was kindly supplied by Dr. Magdy Saad Saleh, Genetic and Breeding Department, Sugar Crops Research Institute, Agricultural Research Center, Egypt. The authentic reference samples such as caffeine, catechin, gallocatechin, epigallocatechin and epicatechin were purchased from Sigma-Aldrich Chemical Company (St. Louis.MO. USA). Acetonitrile, methanol, and ethyl acetate of HPLC grade were purchased from Sigma-Aldrich and El-Gomhorea Co., and distilled water and water for HPLC (pyrogen free) were obtained from El-Fath for Pharmaceutical and cosmetics industries (Eipco Co.).

Preparation of kombucha tea extract

Kombucha tea was prepared according to the reported methods of Hyun *et al*,[14] and Semantee *et al*,[15] with modification. Briefly, 1500 grams of sucrose was added to 20 liters of boiled water (previously boiled for 5-10min. to expel chlorine) in a sterile glass jar with continuous stirring to dissolve the sugar. Later, 100 grams of tea were added to the sweetened water, with continuous agitation and boiling for 5 min. The mixture was set aside for 15 minutes followed by filtration. After cooling, kombucha was added to the starter culture (SCOBY) and maintained at room temperature (25-27^{o}C) and protected from direct sunlight. The jar was covered with a porous or breathable cover (good airflow of O_2 to achieve the fermentation process) and the cover was secured with a thick rubber band. The added starter culture was obtained from previous fermented kombucha culture. For the best results, fermentation was continued for 21 days (three weeks) under controlled condition.[16] The selection of three-weeks fermentation was based on its higher values of total phenol and antioxidant activity using DPPH assay (unpublished data) of the fermentation process than that of two weeks.

HPLC analysis of kombucha tea extract

Samples preparation

To prepare stock solutions, 10 mg of each reference standard such as caffeine, catechin, epicatechin, epigallocatechin and gallocatechin was dissolved in 10 ml methanol separately and sonicate for 5 min. A serial dilution of each reference standard was prepared at range of 5-40 µg/mL to construct standard calibration curve. Fermented Kombucha tea extracts (100 mg each) of GTE and BTE, were separately dissolved in distilled water (10 ml). The tea extracts were subjected to HPLC to identify and quantify the main polyphenols and alkaloid (caffeine).

Chromatographic conditions

The chromatographic analysis was performed using Shimadzu HPLC (LC-20AT series, Shimadzu, Japan) with multiple wavelength detector (SPD-20AD Model) controlled with LC solution version 1.2 (Shimadzu, Kyoto, Japan) for data acquisition and manipulation. Separation was achieved using Prontosil ODS C18 (5 µm, 15 cm × 4.5 mm I.D.) column, UV detection at 254 nm and the column oven was thermo stated at 30^{o}C. The mobile phase was composed of water/ acetonitrile/methanol/ethyl acetate/glacial acetic acid (89:6:1:3:1).[17] The mobile phase flow rate started at 0.7 ml /min and was maintained for 30 minutes. Linearity of the method was established by duplicate injections of solutions. Calibration curves were constructed, and the regression equations were computed to get the linear range of each substance.

Determination of free radical scavenging activity (DPPH) and total phenol content

The total phenol content (TPC) was assessed by Folin-Ciocalteu reagent, using gallic acid as a standard (Sigma-Aldrich Co., St Louis, MO, USA) and the free radical scavenging activity was determined using DPPH and compared to standard ascorbic acid according to the method reported by Semantee *et al*.[15]

Biological evaluation

The antioxidant and hepatoprotective activities of Kombucha tea extract were evaluated using CCl_4-induced liver injury model following methods of Saba *et al*,[18] and Oyagbemi & Odetola.[19]

Animals

Male Wister rats, weighing between 180-200g were obtained from the animal house of the Faculty of Pharmacy, October 6 University. The animals were allowed to acclimatization under laboratory condition for 48 hrs. before starting the experiments. The animals were housed in propylene cages and exposed to 10-12 hrs. of daylight under standard conditions of temperature (25^{o}C), humidity (50%) and maintained on a standard diet and water *ad libitum*. All procedures were reviewed and approved by the Ethical Committee for experimentation with laboratory animals, College of Pharmacy, Cairo University, registration number (MP 1187); following the European Economic Community regulations revised guidelines (86/609/EEC).

Experimental protocol

In this study, forty Wister male rats were divided into four groups with ten animals in each group. Experimentally, the treatment and the design of the groups were carried as follows, group **I (**normal group): received an oral single daily dose of 0.9% normal saline (10 ml/ kg b.wt) for 9 days; group **II** (intoxicated group): received an intraperitoneal dose of CCl_4 [(1.25 ml/ kg b.wt) on day 8 and 9 in vegetable oil at a ratio (1:1)] to induce hepatic damage; group (**III**): received an oral single daily dose of fermented **GTE** (100 mg/ kg b.wt) for 7 days followed by the administration of CCl_4 after 30 minutes (1.25 mL/kg b.wt) on days 8 and 9; group **(IV)**: received an oral single daily dose of fermented **BTE** (100 mg /kg b.wt) for 7 days followed by the administration of CCl_4 after 30 minutes (1.25 mL/kg b.wt) on days 8 and 9.

Blood sampling

On 10th day, the blood samples were collected from retro-orbital venous plexus. The serum samples were obtained by centrifugation (3000 rpm for 15 min.) and kept at - 80^{o}C for assay of liver enzymes activities as well as reduced GSH level. Rats were then scarified by cervical dislocation under light anesthesia. The livers were separated, washed and kept in 10% formalin solution for further histopathological study.

Biochemical analysis

Serum levels of ALT and AST were measured using commercial kits according to the method reported by Reitman and Frankel.[20] The reduced glutathione (GSH) in liver was assayed according to Ellman's method.[21]

Histopathological analysis

Liver sections were taken from blocked liver in paraffin, at 8-10 microns in thickness with microtone and stained with Hematoxylin & Eosin dye (H & E). The images were magnified by light microscope and captured digitally. The morphological, cellular, and vascular changes were observed and evaluated. These changes include sinusoidal or central vein dilatation, steatosis of liver cells, and inflammation in the liver-stained sections.

Molecular docking study

Molecular docking study was carried out using the Molecular Operating Environment (MOE) version 2014.09, Chemical Computing Group (CCG) Montreal Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory.

Target compounds optimization

The target compounds were constructed into a 3D model using the builder interface of the MOE program. After checking their structures and the formal charges on atoms by 2D depiction, the following steps were carried out:

- The targeted compounds were subjected to a conformational examination.
- All conformers were subjected to energy minimization and all were performed with MOE until a (Root-Mean-Square Deviation) RMSD gradient < 2.0Kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated.

- The obtained database was then saved as MDB (Molecular Data Base) file to be used in the docking calculations.

Optimization of the enzyme's active sites

The X-ray crystallographic structures of human myeloperoxidase enzyme (PDB ID: 3ZS0) contains modified iron protoporphyrin IX (ID: 3ZS0) was obtained from Protein data bank through the internet (http://www.rcsb.org). The protein structure was loaded into MOE software using the "Load PDB File" panel. Structural issues with protein were corrected using the "Structure Preparation" panel, adding hydrogens and correcting structural issues in the protein. Partial charges and hydrogen bond optimization were conducted with the force field "MMFF94x" and the "Protonate 3D" panel, respectively. The chains A and D were deleted, and the remaining chains B and C were prepared for docking studies by:

- The hydrogen atoms were added to the system with their standard geometry.
- The atoms connection and type were checked for any errors with automatic correction.
- The selection of the receptor and its atoms potential were fixed.
- MOE Alpha Site Finder was used for the active site search in the enzyme structure using all default items. Dummy atoms were created from the obtained alpha Spheres.

Docking of the target molecules to human Myeloperoxidase enzyme (Code: 3ZS0) active sites

Docking of the selected conformer in database of the target compounds was carried out using MOE-Dock software. The following methodology was generally applied:

- The enzyme active site file was loaded, and the Dock tool was initiated. The program specifications were adjusted to: dummy atoms as the docking site; triangle matcher as the placement methodology to be used; London dG as Scoring methodology. The latter was adjusted to its default values. The MDB file of the ligand to be docked was loaded and Dock calculations were run automatically.

Statistical analysis

All data were expressed as mean value ± Standard error (SE). The data were statistically analyzed with one-way ANOVA followed by multiple comparisons using Tukey test. Probability level of less than 5% ($p< 0.05$) was considered significant.

Results and Discussion

Analytical HPLC study

Both fermented BTE and GTE were analyzed for their main active constituent (Fig. 1S) including caffeine and for 4 phenolic compounds (epigallocatechin, catechin, epicatechin and gallocatechin gallate), using HPLC (Fig. 2S-A & B). The results of HPLC for both extracts (Table 1S) revealed that the fermented black tea has higher content of caffeine (1.5 times) and higher amounts of phenolic compounds injected except gallocatechin gallate which has 5.1 µg/mL in green tea *vs* 2.77 µg/mL in BTE. In addition, the BTE showed higher total phenolic content (TPC) of 39.79 mgGAE/g extract (2.0 times) than GTE with content of 17.6 mgGAE/g extract (Table 2S). The result of TPC is in agreement of the results of free radical scavenging active using DPPH assay, BTE showed a lower IC_{50} of 26.82 µg/mL (Table 2S) *vs* 32.25 µg/mL for GTE.

Effect of Kombucha Tea Extracts on Liver Enzymes Activities in CCl_4-Induced Liver Injury in Rat Model

The CCl_4-intoxication resulted in liver injury in rats' model, which was observed by significant increase in liver enzymes activities such as AST level (66.29±2.69U/L *vs* 41.00 ± 1.51 in normal control, Fig. 1A) and ALT level (25.33±1.59 U/L *vs* 14.83 ± 1.11 in normal control, Fig. 1B). After the administration of BTE and GTE (100 mg/kg b.wt) for 7 consecutive days followed by CCl_4 intoxication, both extracts showed significant decrease in AST and ALT enzymes activities. Fermented BTE showed more potent effect in both AST and ALT levels than that of GTE relative to untreated animals with 42.5±1.765 U/L, 14.67±0.95 U/L, respectively and comparable to the normal control (Table 1). In conclusion b.wt, both extracts reduced liver enzymes activities significantly ($p< 0.05$) compared to CCl_4 group. When comparing the data obtained of both extracts versus CCl_4 intoxicated group, amelioration in serum activities were observed and the BTE was significantly ($p<0.05$) potent than GTE in hepatoprotective activity. In a related study, kombucha tea has been shown to attenuate the effect of acetaminophen on the levels of liver-related enzymes.[22,23] The same author showed also that kombucha tea attenuated hepatic lipid accumulation and damage in *db*/*db* mice with acute liver injury.[24] The observed potent effect of fermented BTE relative to GTE may be attributed to its higher total phenolic content (39.79 vs 17.6 µg/ml for GTE), which was supported by HPLC analysis of the individual phenolic compounds.[22, 23]

Effect of Kombucha Tea Extracts on GSH Level in CCl_4-Induced Liver Injury in Rat Model

The results revealed that the CCl_4-untreated group showed a significant reduction in the level of GSH with value of 10.17±0.36mg/dl (Figure 1C, $p<0.05$), compared to normal control group (14.51± 0.21mg/dl) which confirmed liver injury. On the other hand, both fermented BTE and GTE extracts revealed an improvement of glutathione levels. The level of GSH of BTE group (15.03±0.21mg/dl) was higher than that of GTE (13.48±0.49 mg/dl) and nearly comparable to the value of normal group (Table 1). In conclusion, the biochemical values of BTE group showed tendency towards normalization of ALT, AST and GSH values more than GTE (Figure 1A-C). The observed potent effect of fermented BTE relative to GTE may be attributed to its higher phenolic content of BTE (39.79 µg/ml) and its free radical scavenging activity (IC_{50} 26.82 µg/mL) which was comparable to ascorbic acid (IC_{50} 22.4 µg/mL).

Histopathological Changes Due to Effect of Kombucha Tea Extracts in CCl_4-Induced Liver Injury in Rat Model

The light micrographs of the liver sections (x400) of normal control group revealed a normal hepatic state of the structural components of the liver tissue, while the CCl_4-untreated group showed damage in the liver cells, inflammation of peritoneum, sinusoidal, and central vein dilatation (Figures 2B, 2C). However, these histopathological changes were significantly improved by the effect of both fermented extracts administration (Figures 2A-2G). The administration of fermented BTE showed a remarkable improvement and restoration of the hepatocytes structure (Figures 2F, 2G) compared to that of the fermented GTE (Figure 2D, 2E). The result of histopathological examination supported the effect of both extracts on biochemical parameters (ALT, AST and GSH) and the results of chemical analysis.

Table 1: Hepatoprotective effect of GTE and BTE in CCl_4-induced liver injury on serum level of AST, ALT, and GSH.

Biochemical parameters	AST (U/L)	ALT (U/L)	GSH (U/mg)
Control	41.00 ± 1.51	14.83 ± 1.11	14.51 ± 0.21
CCl_4 (1.25 mL/kg b.wt)	66.29 ± 2.69[a]	25.33 ± 1.59[a]	10.17 ± 0.36[a]
GTE (100 mg/kg b.wt) + CCl_4	55.33 ± 1.98[a,b,d]	21.5 ± 1.61[a,b,d]	13.48 ± 0.49[a,b]
BTE (100 mg/kg b.wt) + CCl_4	42.5 ± 1.765[b,c]	14.67 ± 0.95[b,c]	15.03 ± 0.21[b,c]

Data are *expressed* as mean ±SEM. (a) significant difference from the control group (at *P<0.05),* (b) significant difference from the CCl_4 group (at *P< 0.05).* (c) significant difference from the GTE group, (d) significant difference from the BTE group.

Table 2: Binding scores (ΔG Kcal/mol) and amino acids interaction of the docked polyphenols and caffeine on the active site of *hMPO* (PBD ID: 3ZS0).

Compound	Amino acid interaction	(ΔG Kcal/mol)
(-)-Gallocatechin gallate	HOH 2219 (C) H donor	-5.5842
	Arg333 (C) H acceptor	
	Leu167 (C) pi-H	
(-)-Epigallocatechin	Met175 (C) H donor	-4.9187
	Val171 (C) pi-H	
	Val171 (C) pi-H	
	Val171 (C) pi-H	
(+)-Catechin	Arg239 (C) H acceptor	-4.6227
	Arg333 (C) pi-cation	
(+)-Gallocatechin	Arg333 (C) H acceptor	-4.3385
	Hydrophobic interaction	
(-)-Epicatechin	Met175 (C) H donor	-4.0454
	Arg239 (C) H acceptor	
Caffeine	Arg424 (C) H acceptor	-3.6890

Molecular docking study

The fermented BTE and GTE were tested for antioxidant activity. Results illustrated high activity of extracts due to presence of polyphenolics. Generally, many polyphenolic compounds correspond to a wide variety of chemo-preventative agents,[25] and are responsible for undergoing redox chemistry including redox reaction as reported.[26] Epigallocatechin-3-gallate acts as a redox-dependent poison,[27] consequently, a molecular docking study has been carried out to verify the observed antioxidant activity of these polyphenolic compounds using Myeloperoxidase (MPO), since this enzyme is a member of subfamily of peroxidases. It is worthy mentioned, that the levels of NO and MPO were found to be significantly increased in liver tissue subjected to hepatotoxic materials. Explicitly, lipid peroxidation, resulting in liver injury has been reported to be induced experimentally by exposure to Diazinon.[28]

The MPO, which is the most abundant protein in neutrophils and catalyzes the conversion of hydrogen peroxide and chloride ions into hypochlorous acid, plays a role in downregulating the inflammatory response.[29] In non-infectious diseases, elevated level of MPO was observed with strong oxidative activity.

Molecular docking of polyphenols and caffeine were performed on the active site of (*h*MPO) enzyme co-crystallized with N-acetylglucoseamine (PDB: 3ZS0) using MOE docking suite. The docking model was developed to investigate the binding potential of the investigated polyphenols and caffeine. The selection of target (PDB: 3ZS0) was based on structural relevance of the co-crystallized ligand (N-acetylglucoseamine) with the studied compounds.

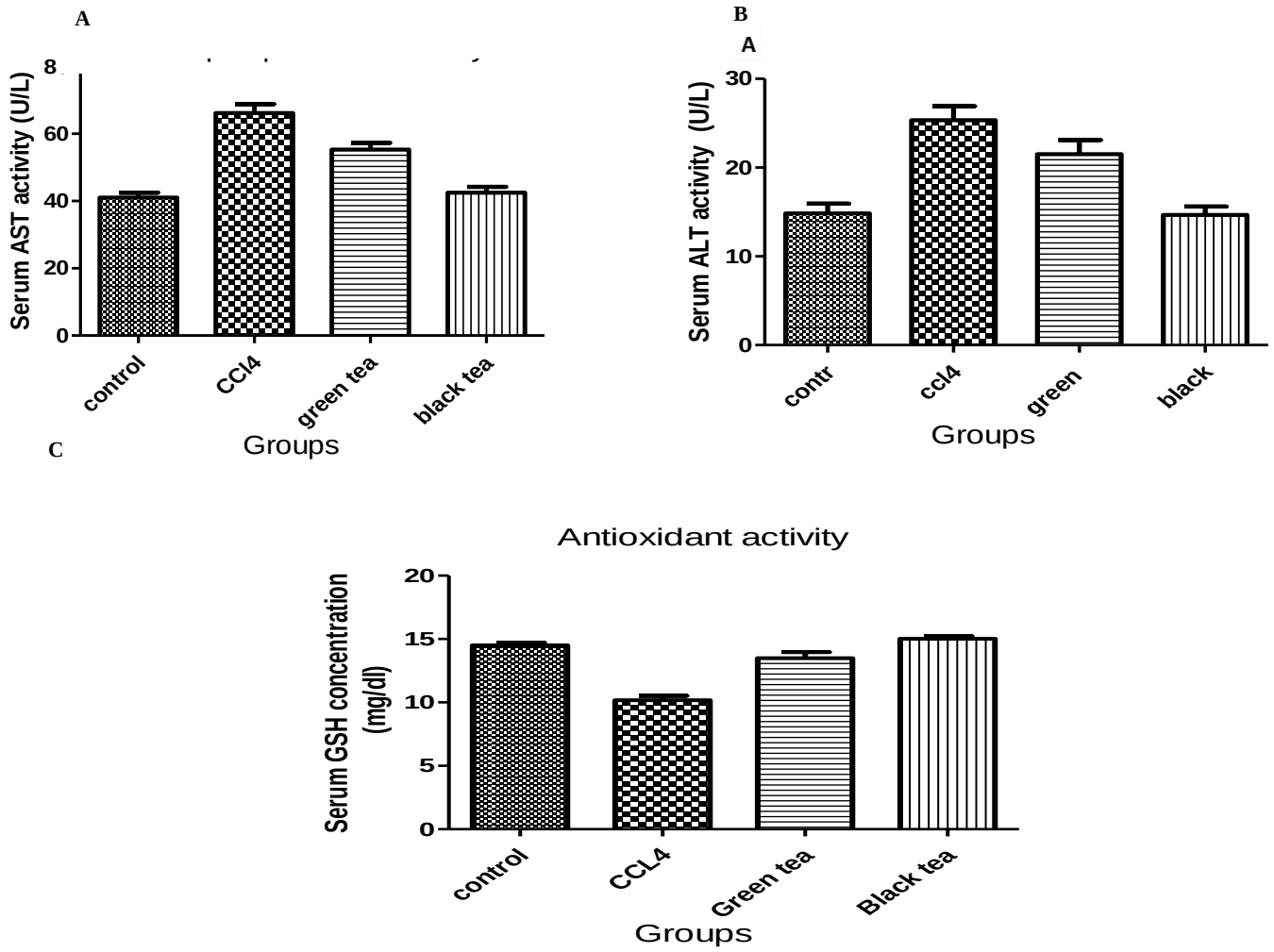

Figure 1: Effects of fermented GTE and BTE on CCl_4- induced damage on serum levels of AST (**A**), ALT (**B**), and GSH (**C**) in rats.

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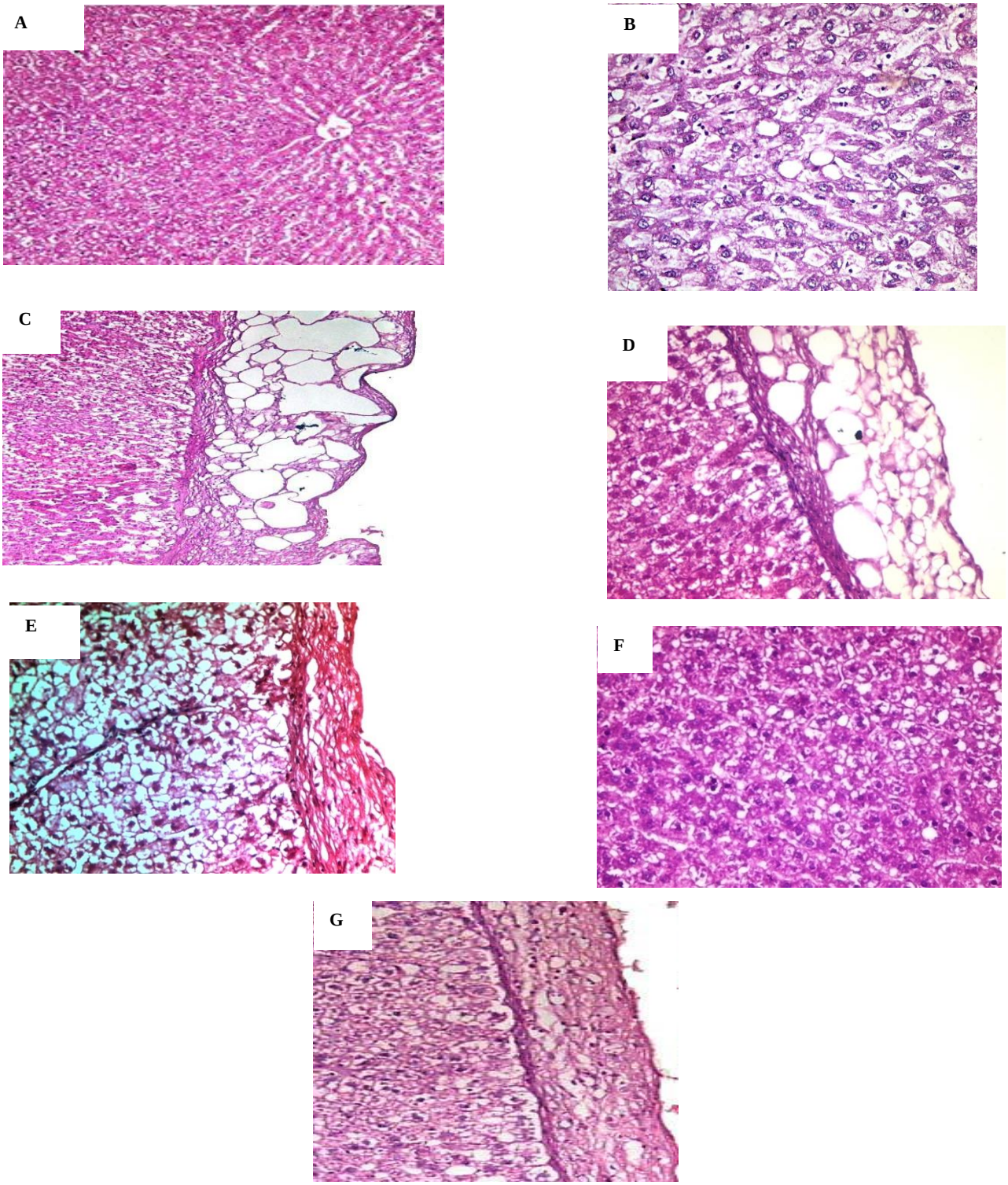

Figure 2: Histopathological sections of liver (x400) with CCl_4 induced liver damage stained with hematoxylin & eosin.
A: Normal control: The liver is normal and the cells are healthy; **B** and **C:** Positive control: cell atrophy is present with sinusoidal dilatation and mild steatosis, the appearance of peritonitis; **D** and **E:** Green tea extract (GTE): Improvement of peritonitis, Improvement of atrophy with hydrobic degeneration; **F** and **G:** Black tea extract (BTE): Improvement in liver cells, marked improvement in peritonitis.

The150-kDa *MPO* protein is a cationic homodimer consisting of two 15-kDa light chains and two variable-weight glycosylated heavy chains linked to a prosthetic heme group.[30,31] The light chains are glycosylated and contained the modified iron protoporphyrin IX in active site. In addition, the light and heavy chains form two identical 73-kDa monomers linked by a cystine bridge at Cys153. The protein forms a deep crevice which holds the heme group at the bottom, as well as a hydrophobic pocket at the entrance to the distal heme cavity which carries out its catalytic activity,[30] as indicated from its 3D structure (Figure 3). The 2D ligand interaction of the modified iron protoporphyrin IX in the active site of *h MPO* is illustrated in Figure 4.

The protocol involved the assessment of docking parameters as London dG energy score and refinement of the results based on Force field calculations. The molecular docking study of the polyphenolic compounds and caffeine was performed on the prepared *h* MPO (3ZS0). The binding scores (ΔG Kcal/mol) and the amino acid interactions of the docked polyphenols: (-)-gallocatechin gallate, (-)-epigallocatechin, (+)-gallocatechin, (+)-catechin and (-)-epicatechin as well as caffeine in the active site of *h MPO (3ZS0)* are recorded in (Table 2). It is well documented that hydrogen bond (donor and acceptor) interaction is the main binding forces of the docked compounds with 3ZS0, since the effect of hydrogen bonding interaction forces was more influential either by their allowed number or position within the ligand. The results showed that the binding scores of the polyphenolic compounds is higher [(ΔG - 5.584 to – 4.0454 Kcal/mol) than caffeine (ΔG = - 0.3689 Kcal/mol)]. This is attributed to the presence of hydroxyl groups which improve the binding of the polyphenols with the amino acids in active site of 3ZS0. (-)-Gallocatechin gallate showed the highest binding scores (ΔG = -5.584 kcal/mol) since the two oxygen atoms of hydroxyl groups of gallate moiety at C-3 and C-4 bind through hydrogen bonding with H_2O molecule and the amino group of Arg C333 in the active site of *3ZS0*, respectively (interaction diagram, Figure 5).

On the other hand, the binding diagram of (-)-epigallocatechin (Figure 6) revealed bindings with Met C175 and Val C171 in the active site (ΔG = -4.9187Kcal/mol). The interaction diagram showed hydrogen bond interaction between the hydroxyl group at C-3 of pyrogallol and Met C175. Moreover, there are pi-H interactions between the phenyl nucleus of pyrogallol and Val C171. In spite of (-)-epigallocatechin bounds with amino acids, it was not included in binding of the modified iron protoporphyrin IX in the active site of 3ZS0, a relatively good binding score was observed. In addition, its (+)-gallocatechin conformer (ΔG = -4.3385Kcal/mol) revealed that hydrogen bond interaction between the phenolic hydroxyl group at C-5 of chromene nucleus and Arg C333 in the active site where 3ZS0 is similar to the modified iron protoporphyrin IX of 3ZS0, in addition to the hydrophobic interaction (Figure 7).

The 2D interaction diagram of (+)- catechin (ΔG = -4.6227 kcal/mol) indicates hydrogen bonding interaction between hydroxyl group at C-3 of chromene ring and Arg C239 in addition to pi-cation interaction between phenyl nucleus of catechol and Arg C333 in the active site of 3ZS0 (Figure 8).

Molecular docking simulation of (-)- epicatechin with 3ZS0 revealed the participation of the 3-OH group of chromene nucleus and 4-OH group of catechol ring in hydrogen bonding with Arg C239 in the active site and Met C175, respectively (Figure 9). Comparing the 2D interaction diagram of (+)- catechin (Figure 8) with its epimer (-)-epicatechin (Figure 9) we observed that the latter failed to bind with Arg C333 in the active site which was associated with a decrease of its binding score (ΔG = - 4.0454 Kcal/mol). Generally, it was observed that all the polyphenols bound with Arg C333 in the active sites of MOP (3ZS0), but the epimers interacted with Met C175 instead.

Structural variation of caffeine molecule and absence of hydroxyl groups lead to decreasing of its binding score (ΔG = - 3.6890 Kcal/mol). Since caffeine is a small molecule, it occupied the active site of 3ZS0 and showed hydrogen binding of oxygen atom of carbonyl group at C-4 of purine nucleus with Arg C424 (Figure 10).

In conclusion, the molecular docking results revealed correlation between the number of hydroxyl group that participates in binding in the active site of 3ZS0 and their binding scores (ΔG Kcal/mol). Moreover, binding of polyphenols with Arg C333 in the active site improves the binding and this reflected on its binding score. 3D representation of the binding mode of (-)- gallocatechin gallate (green, higher ΔG = - 5.5842 Kcal/mol) with modified iron protoporphyrin IX (yellow) in the active site of 3ZS0 is illustrated in Figure (11). It is worthy to mention that the binding of the studied antioxidant polyphenols was in the active sites of 3ZS0, which contain the prosthetic heme group responsible for the observed antioxidant activity as shown in Figure 12. In addition, it showed the overlay of (-)- gallocatechin gallate (green) in the active site of MPO (3ZS0) with modified iron protoporphrin (red).

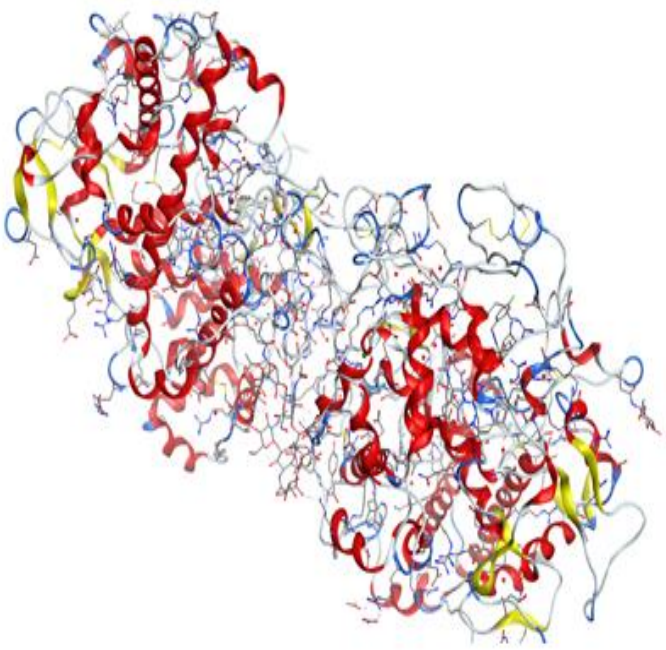

Figure 3: 3D structure of Myeloperoxidase (PDB ID: 3ZS0) containing the modified iron protoporphyrin IX active site.

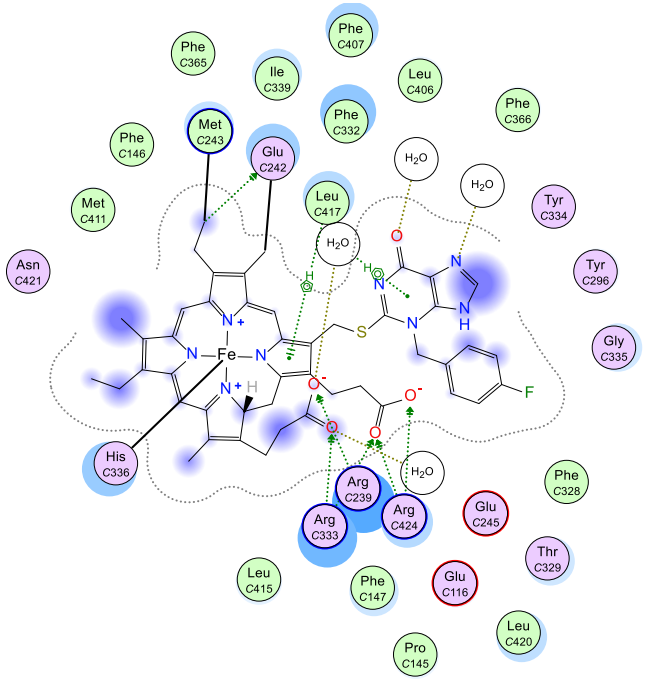

Figure 4: 2D ligand interaction of the modified iron protoporphyrin IX in the active site of *h MPO*.

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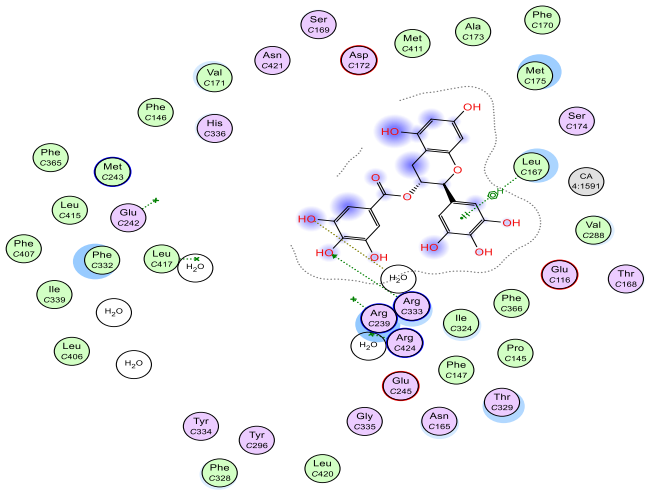

Figure 5: 2D ligand interaction of (-)-gallocatechin gallate with 3ZS0.

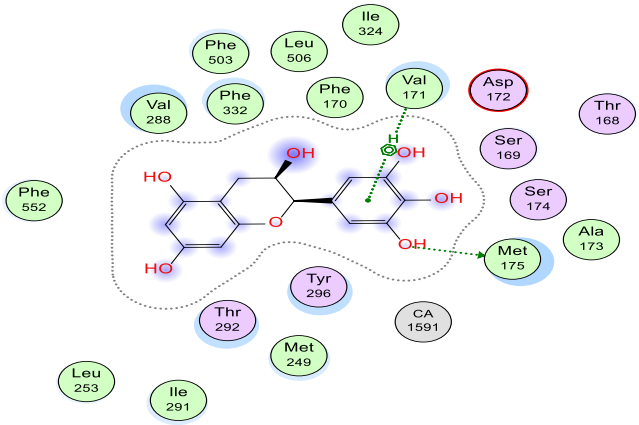

Figure 6: 2D ligand interaction of (-)-epigallocatechin with 3ZS0.

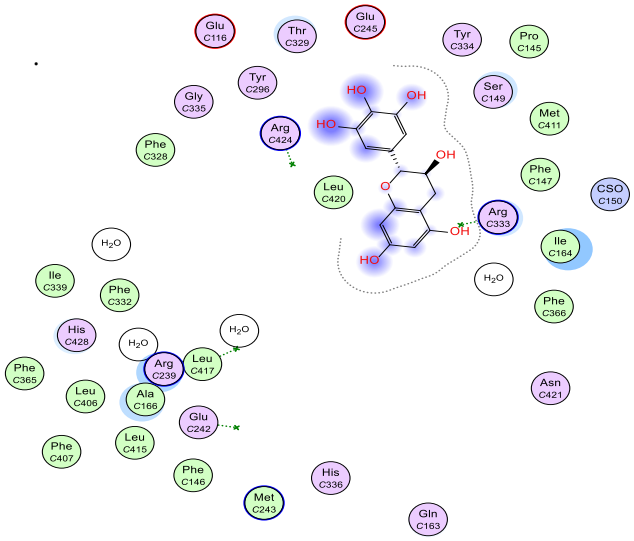

Figure 7: 2D ligand interaction of (+)-gallocatechin with 3ZS0.

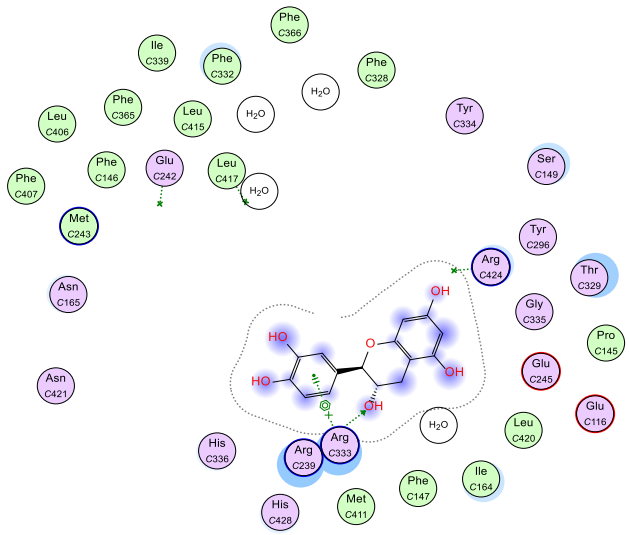

Figure 8: 2D ligand interaction of (+)-catechin with 3ZS0.

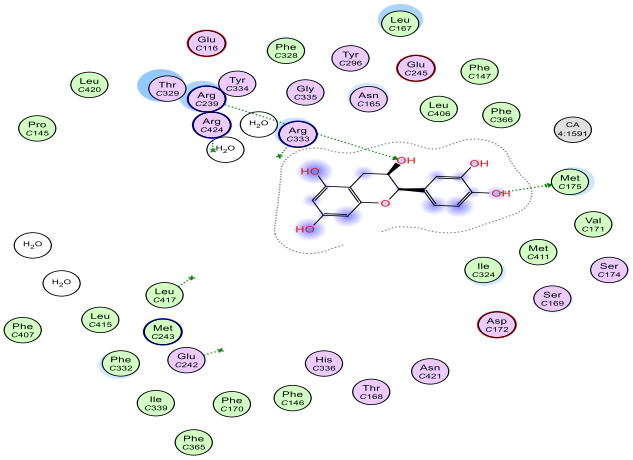

Figure 9: 2D ligand interaction of (-)-epicatechin with 3ZS0.

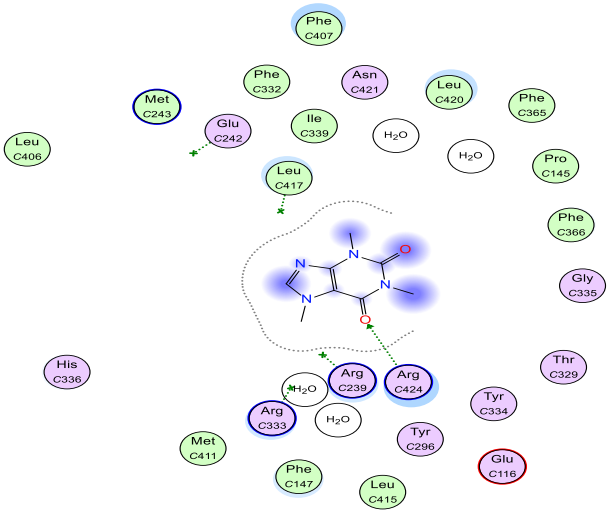

Figure 10: 2D ligand interaction of caffeine with 3ZS0.

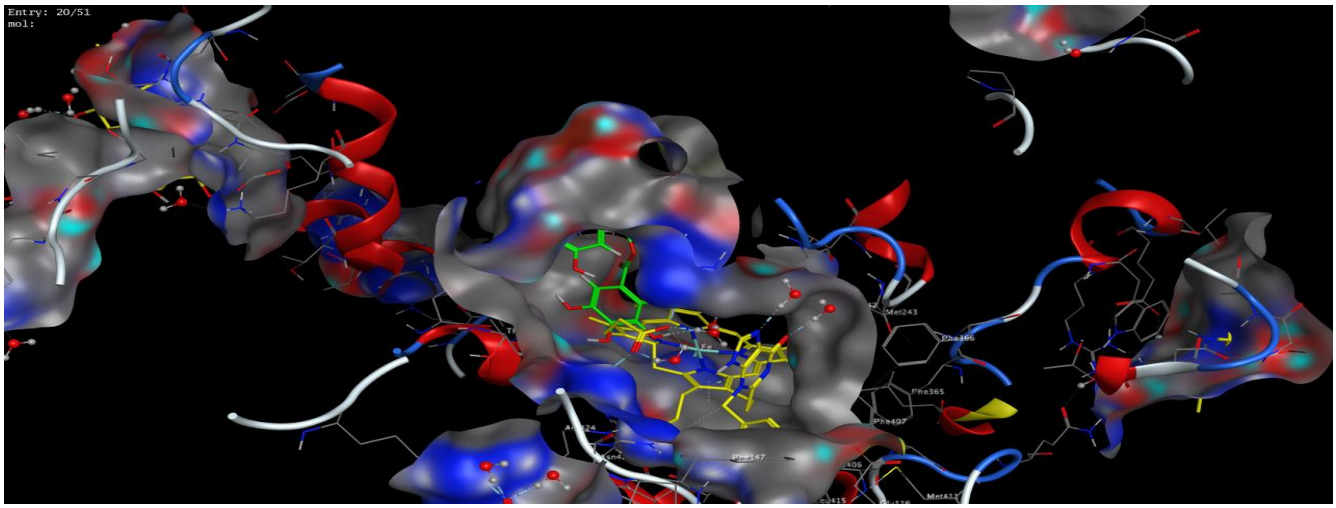

Figure 11: 3D representation of the (-)-gallocatechin gallate (green) and modified iron protoporphyrin IX (yellow) in the active site of 3ZS0.

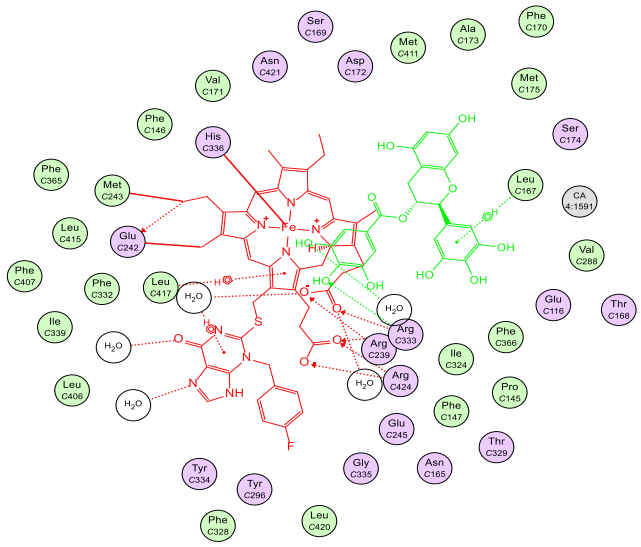

Figure 12: Overlay diagram of (-)-gallocatechin gallate (green) in the active site of *MPO* (3ZS0) with heme (red).

Conclusion

Three weeks fermentation using black tea and sucrose as sugar showed the best condition to have a potent antioxidant and hepatoprotective activities. This prompted the authors to investigate Kombucha as a targeted product to maintain a healthy liver and to prevent liver damage and introduce Kombucha to Egyptian market as functional drink. Results of the current study suggested that the antioxidant activity and hepatoprotective effect were identified to be mediated through its phenolic constituents. Molecular docking study of the polyphenolic compounds e.g. epicatechin, epigallocatechin, catechin, gallocatechin and gallocatechin gallate with Myeloperoxidase (3ZS0) enzyme confirmed the unique binding mode in its active site with the amino acids (Arg C239-Met C175), (Met C175-Val C171) and (Arg C333), respectively.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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