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Synbiotic (PoultryStar® Sol) Protects Rat Liver from Oxidoreductive Stress Induced by T2-Mycotoxicity

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ABSTRACT
Mycotoxins are secondary metabolic byproducts of molds that posses harmful and detrimental impact on animal and human health. Fungal T-2 mycotoxin is the most efficacious trichothecene mycotoxin. It can induce hepatic and intestinal damages through eliciting severe oxidoreductive stress. Consequently, dysbiosis (alteration in the compositional balance of intestinal microbiota) occurred that can play an important role in the pathogenesis of several liver diseases. Recently, the using of pro and prebiotics was proven to exert antioxidative stress potentials in liver and gut ecosystem. To explore, the efficacy of using synbiotic (PoultryStar® Sol) during chronic T-2 mycotoxicosis course in rat, this study was designed. Rats were allocated into 4 main groups, (CN-Gr), which was treated as the negative control and was allowed for the free access to the normal rats chow and the tap water for 90 days, (PSS-Gr), which was assigned as the synbiotic positive control group and was allowed for the free access to normal rat chow and (PoultryStar® Sol) in the tap water (20mg/rat every 5 days) for 90 days. Group 3 or (T2-Gr) and was given the T2 contaminated chow *ad libitum*at a level of (470 ppb) along with normal drinking water for 90 days, and Group 4 (T2+PSS-Gr) which was given the T-2 contaminated chow *ad libitum*at the same level of Group 3 along with synbiotic (PoultryStar® Sol) in the tap water the same dose of (PSS-Gr) for 90 days. Each one of the four groups was subdivided into two subgroups (n=7) that one was exsanguinated on day 45 (Subgroup A) meantime the remaining rats (Subgroup B) were kept until the end of the entire study period (90 days). In T2 intoxicated group, liver histopathological findings on day 45 revealed lesions of reversible types (hydropic and fatty degenerations) only. Concurrently, on day 90 the changes comprised apoptosis and some necroinflammatory lesions. In T2+PSS-Gr, the ultimate efficacy of synbiotic success in preventing hepatic necroinflammatory changes and limiting the apoptotic activity which were in accordance with the amelioration of antioxidative status, liver function enzymes and other serum biochemical estimated tests. Summing up, the total results of this study has been clarify the regenerative and antioxidant potentials of (PoultryStar® Sol) in coping with T2 toxin mediated hepatotoxicity in rats.

Keywords: T-2 mycotoxin, oxidoreductive stress, hepatotoxicity, PoultryStar® Sol, Histopathology.

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INTRODUCTION

Mycotoxins are secondary toxigenic metabolites of fungal origin that claim a significant threat to the animals and human beings. Fungi belong to the genus *Fusarium* are well known mycotic agents that can produce the highly food contaminants occurring mycotoxins called Trichothecenes.

T_2-toxin which is affiliated to non macrocyclic type-A trichothecenes, is a potent cytotoxic secondary metabolite yielded by multiple species of the genus *Fusarium* (*F. poae*, *F. sporotichioides*, *F. equiseti, and F. acuminatum*). It represents a prime toxic trichothecene of human and animal concern [1]. Obviously, this non volatile toxin is highly resistant to ultraviolet light and heat [2]. T-2 mycotoxin has been categorized as a biological neurotoxic weapon due to its serious toxicity, unique stability and specific capability to pass through the blood brain barrier by disturbing its permeability [3]. Human alimentary toxic aleukia (ATA) disease has been related originally to the contamination of edible cereals with T_{-2} toxin. Furthermore, T-2 mycotoxin is a radiomimetic toxin , and, in turn can aggravates the aftermath of ionizing radiation exposure [3].In China and other countries, the HT_{-2} and T-2 mycotoxins contaminated grains is also a hazardous and critical problem [4]. Beside the oral route of T-2 entry, lung and skin are further confirmed organs to be additional pathways for its exposure. Up to date, tremendous data of vast research works indicates that oxidoreductive stress is the cardinal toxic mean of T_2 mycotoxicosis [5]. Since oxidoreductive stress contributes effectively to the pathogenesis of trichothecenes mycotoxicity, a lot of research work are essaying to recognize factors with antioxidative efficacy to interdict oxidative stress mediated T_2 mycotoxicosis and the associated pathogenecity. Among these wide spectrum agents, polyunsaturated fatty acids ($PUFA_s$), organic acids, oligosaccharides, vitamins, quercetin, amino acids, some herbal extracts, nucleotides, antimicrobial peptides and bacteria strive for potentiating the anti-oxidative status against the deleterious impact of T-2 and other trichothecenes toxins. Furthermore, the protective potentials of supplementation of *B. subtilis* in the aflatoxin-contaminated broilers and layers diets has an exactitude results [6,7] .Interestingly, Fuchs *et al*. (2002) found that *Eubacterium* BBSH 797 present in Biomin® anti-mycotoxin product called (Mycofix plus) can modify the epoxide ring of trichothecenes into a diene. Some bacteria like *Devosiamutans* (17-2-E-8) and Mycofix-Plus 3 and 5 can induce biotransformation of vomitoxin (*deoxynivalenol*) into less-toxic metabolites, and subsequently participating in diminishing the intestinal lesions produced by T-2 mycotoxin in duodenum [8].

It is not worthy to find that *Mycobacterium tuberculosis*, a virulent life threatening bacteria has develop in vivo planning to fight free radicals action by displaying several antioxidative enzymes and thus detoxify different reactive oxygen species [9]. Different microbial biodegradation pathways of T2 toxin and other trichothecenes has been clarified. These pathways include oxygenation [10], epimerization [11] glucosylation [12] and de-epoxidation [13]. Biotransformation reactions include hydrolysis, acetylation, deamination, glucosylation, ring cleavage, and decarboxylation.

Latterly, the utilization of probiotic bacteria, yeast and enzymes to alleviate, adsorb or biodegrade most mycotoxins is a recent and novel plan used for mycotoxins detoxification.Beneficial microorganisms have been confirmed to poses an enzymaticalconversion of different types of mycotoxins including T-2 to less toxic products. The detrimental specificity of T-2 intoxication is the presence of 12,13-epoxide ring in its structure. Seemingly, deepoxidation of this ring from T-2 structure represent the mode to overcome its toxicity. In rat, the deepoxy T-2 mycotoxin (DE T-2) was substantiated to be 400 times nethermost toxic than T-2 toxin. Furthermore, diverse *Rhodococcus* species are poignant in the disintegration of aromatic mycotoxins. Obviously, some kind of moulds, yeast, bacteria and enzymes have the capability to transform and subsequently detoxify T-2 toxin [5,14].

Interestingly, the treatment of vomitoxin and T-2 toxin by some lactic acid bacteria (*Lactobacillus sake*i, *Pediococcus pentosaceus* and *Pediococcus acidilactici*) led to significant lessening of their mycotoxicity more than 30%-50% respectively [15]. Concurrently, in the same year Gao *et al*. (2018) [16] isolated unconventional bacterium named *Eggerthella* sp. DII-9 from avian guts and confirmed its capability to biotransform of T-2 triol, T-2 tetraol and some other trichothecenes.

In different animal species including poultry and rats, the toxic lesions of T-2 mycotoxin can be categorized as genotoxic, immunotoxic and cytotoxic for digestive system (specially liver and intestine), nervous system and skin. In liver, as other intermitotic tissues, the active dividing hepatocytes are pronounced to be more sensitive to T2 toxin [17]. Furthermore, T-2 toxin can promote apoptosis in liver and intestinal crypts of Lieberkuhn in mice [18].

PoultryStar®Sol is a synbiotic product of Biomin® GmbH company that patented by it as a highly selective and synergistic probiotic strains (preparation of dried probiotic bacteria, *Enterococcus sp., Bifidobacterium sp., Pediococcus sp.,* and *Lactobacillus spp.*) plus prebiotic fructooligosaccharides (FOS). This multi species symbiotic promotes poultry gut health. Using *lactobacillus* with levofloxacin revealed an efficacious synergism in the field of diarrhea treatment [19]

The current study is designed to assess the possible role of PoultryStar® Sol in amelioration of T-2 mycotoxicosis in rats by evaluating the oxidoreductive / antioxidative stress biomarkers and the liver histopathological findings during long course of T-2 experimental intoxication.

MATERIALS AND METHODS

2-1 Ethics Statement

All animal experimental studies were carried out and complied with the regulations and rules by the Animal Ethics Committee of College of Pharmacy/University of Baghdad.

2-2 Materials

The T2 contaminated diet was obtained from Scientific Laboratories of Beetar Sun Co Ltd. (The Group) Company, Baghdad-Iraq. A T2 contaminated diet level of (470 ppb) was determined by (Veratox® brand of diagnostics from Neogen® which are quantitative microwell enzyme-linked immunosorbent assay (ELISA) tests).

The synbiotic (PoultryStar® Sol) produced by BIOMIN GmbH-Austria, is based on tangible materials incorporation of dried probiotic bacteria (*Enterococcus sp., Bifidobacterium sp., Pediococcus sp*., and *Lactobacillus spp*.) product contains at least 5 x 10^{12} CFU/kg. plus prebiotic (fructooligosaccharides).

2-3 Chemicals

All the used chemicals were of analytical grade quality and were manufactured by Sigma-Aldrich. Exclusively, all the working solutions were formulated using deionized water. SOD, CAT, and GSH-PX assay kits were obtained from Nanjing Key Gen Biotech. Co. Ltd. (Nanjing, China). Concurrently, alanine aminotransaminase (ALT, GPT), aspartate aminotransaminase (AST, GOT) and alkaline phosphatase (ALP) enzymes assay kits were purchased from (Bio-Merieux Laboratory Reagents and Products, France).

2-4 Animals

Fifty six adult male Wistar albino rats of nine weeks age and 155-175 g body weight were get from Animal House in College of Pharmacy, University of Baghdad. The rats were accommodated in clean plastic cages and allocated to acclimatize to the laboratory environment for one week underdistinctive balanced conditions of photoperiod (12-h dark:12-h light cycle), humidity of 40% and room temperature 23±0.5 ◦C. Throughout the acclimatization and the experimental periods, *animals had ad libitum access* to the rodent's chow and water. The rats were offered a standard rodents chow. The rats aliment diet contained protein (12%), fat (10%), sugars (5%), vitamin premixe (1%), salt mixtures (4%), fiber (4%) and starch (64).

2-5 Experimental design

T-2 mycotoxicosis was induced by continuous feeding rats with T-2 contaminated rodents chow at a level of (470 ppb). The animals were allocated into 4 main groups (n=14), viz; Group 1 or (CN-Gr), which was treated as the negative control and was allowed for the free access to the normal rats chow and the tap water for 90 days, Group 2 or the (PSS-Gr), which was assigned as the synbiotic (PoultryStar® Sol) positive control and was allowed for the free access to normal rat chow and (PoultryStar® Sol) in the tap water (20mg/rat every 5 days) for 90 days,. Group 3 or (T2-Gr) and was given the T-2 contaminated diet *ad libitum*at a level of (470 ppb) along with normal drinking water for 90 and Group 4 (T2+PSS-Gr) which was given the T2 contaminated rodent's chow *ad libitum* at a level of (470 ppb) along with synbiotic (PoultryStar® Sol) in the tap water (20mg/rat every 5 days) for 90 days. Each one of the four groups was subdivided into two subgroups (n=7) that one was exsanguinated on day 45 (Subgroup A) meanwhile the remaining rats (Subgroup B) were kept until the end of the entire study period (90 days).

Twice blood and tissue collections were done at the end of (45^{th} and 90^{th} day)of the experimental period. The rat's blood was collected by cardiac puncture under the D.C.E.

anesthesia at a ratio of (3:2:1,Diethyl Ether; Chloroform: and Ethanol, respectively). The blood samples were processed for serum collection and running of the biochemistry analyses. The blood samples were withdrawn in the fasting state and processed within 30 minutes of collection, centrifuged at 3000 rpm for 10 min to separate the serum and transferred in Eppendorf tubes for analysis

The animals were ethically euthanized via decapitation and their organs (livers) were collected and put in 10% neutral buffered formalin for the histopathologic evaluation

2-6 Biochemistry Analyses

2-6-1:Total Serum Proteins Estimation.

In all serum samples, total proteins were measured by using Bradford reaction method (Bradford, 1976).

2-6-2 Biochemical Analyses for Evaluation of the Serum Lipid Profile and Liver Function Tests

The serum samples were used to obtain cholesterol, low density lipoproteins, high density lipoproteins and triglycerides. Furthermore, alanine aminotransaminase (ALT, GPT), aspartate aminotransaminase (AST, GOT) and alkaline phosphatase (ALP) activities were detected using the commercial kits obtained from (Bio-Merieux Laboratory Reagents and Products, France).

2-7 Determination of Oxidative/Antioxidative Stress Biomarkers

The blood samples were used to determine the oxidative stress biomarkers like malondialdehyde (MDA) and total oxidative stress capacity. Serum malondialdehyde level was measured using thiobarbituric acid reactive substance (TBARS) method as described by Kikugawa *et al*. 1992 [20] with simple modifications. The serum concentration of MDA was expressed in nmol/ml. Each of the total oxidant (TOC) and total antioxidant (TAC) capacities were measured using the automated colorimetric measurement method found by (Erel, 2004: Erel, 2005) [21,22] and the commercial kits obtained by (Rel Assay Diagnostics®, Gaziantep, Turkey).

The oxidative stress index (OSI) was expressed as the ratio of the TOC value to TAC value. Certainly, OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L)

Furthermore, The serum activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) enzymes of all animals were analyzed by spectrophotometrical methods of the Nanjing Key Gen Biotech. Co. Ltd.

2-7.1 Determination of the reduced glutathione (r-GSH) level

The reduced form of the glutathione (r-GSH) concentration was estimated in the RBCs hemolysate by using the method set out by Ellman in (1959) [23]. This method is based on the reaction of the thiol groups in the sample with the Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid or (DTNB), splitting the disulfide bond to produce 2-nitro-5-thiobenzoate (TNB^-), which ionizes to a complex colored material called TNB^{2-}dianion which spectrophotometrically, can be measured at 412 nm[23].

2-8 Histopathological Studies

After completion of the blood samples collection, the euthanized animals were decapitated ethically and livers were fixed for 96 hours in 10% neutral buffered formalin saline. The tissues were stabilized in paraffin and sectioned at 5-μm thickness by utilization a rotary microtome. According to Luna (1968), sections were stained with hematoxylin-eosin (H&E) [24].

2-9 Statistical Analysis

The data were expressed as mean ±standard error of the mean (SEM), after analyzing with one-way ANOVA and the Least Significant Difference (LSD's) followed by post hoc Branferroni test. All tests were done using the SPSS version 20 statistical package software (SPSS Inc. Chicago, IL, USA). When *P*- value of less than *0.05* it was considered as statistically sign

RESULTS

3-1 Liver function tests

T-2 Mycotoxin administration in Gr3 (T2-Gr) produced a marked and a statistically significant elevation in the serum level of the hepatic enzymes especially on day 90 after the toxin administration (*P<0.05*). The PoultryStar® Sol coadministration along with the mycotoxin failed to ameliorate the deterioration in the Alkaline Phosphatase (ALP) level that stayed significantly different as compared to the control (*P<0.05*), but it succeeded in changing the serum level of the hepatic enzymes to a level significantly different as compared to both the positive and the negative controls.

Table 1: Hepatic enzymes (Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)levels in serum of rats of all groups at day 45 and 90 of the experimental study.(The data are expressed as mean ± SEM).

Groups	ALP (IU/L)		AST (IU/L)		ALT (IU/L)	
	45 Days N=7	90 Days N=7	45Days N=7	90 Days N=7	45 Days N=7	90 Days N=7
CN-Gr	119.66±4.53^A	120.14±4.44^A	125.15±2.96^A	127.33±3.33^A	28.44±1.90^A	30.35 ±1.84^A
PPS-Gr	117.61±3.65^A	118.52±2.90^A	124.22±3.50^A	126.55±2.95^A	27.88±1.05^A	29.30±1.22^A
T2-Gr	140.18±3.93^B	318.15±7.88^B	140.23±2.55^B	277.43±7.22^B	30.93±1.56^A	101.34±3.77^B
T2+PPS-Gr	138.41±3.52^B	316.25±6.80^B	133.23±2.91^C	155.71±3.65^C	27.38±2.18^A	65.23±1.97^C

(CN-Gr) =negative control group, (PSS-Gr) = PoultryStar® Sol treated group, (T2-Gr) = T-2 intoxicated group and (T2+PSS-Gr) = T-2 intoxicated group+PoultryStar® Sol treated group respectively.

(Each value that is not sharing a common superscript letter signifies a statistically significant difference with *P<0.05*).
The sample size is 7 rats for each group.

3-2 Levels and Activities of Oxidative Stress and Antioxidant Biomarkers

The results revealed a time dependent significant and prominent increase in the serum levels of the oxidative stress markers. This change was limited along with the coadministration of the PoultryStar® Sol as it could have prominently reduced the oxidative stress level. When the PoultryStar® Sol was given alone, a decline in the oxidative stress was noticed as compared to that of the negative control and this was deduced from the statistically significant change in the values of TAC and OSI (*P<0.05*). When the PoultryStar® Sol was co-administered, the oxidative stress declined to a level comparable to that of the control as seen in the results of some of the parameters like TAC. Although, the other parameters, like the MDA, were still significantly higher than that of the negative control, it is obvious that malondialdehyde level in the PoultryStar® Sol+T2 group is significantly lower than that in T-2 intoxicated group only.

Table 2: Biochemical measurements of Serum Malondialdehyde (MDA), Total Antioxidative Stress Capacity (TAC), Total Oxidant Capacity (TOC) and Oxidative Stress Index (OSI) for all groups at day 45 and 90 of the experimental study. (The results are expressed as mean ± SEM)

	At 45day (n=7)				At 90 day (n=7)			
	MDA nmol/ml.	TOC (µmol H_2O_2 Eq/L)	TAC (TroloxEqµmol/L)	OSI (TOC/TAC) (arbitrary unit)	MDA nmol/ml.	TOC (µmol H_2O_2 Eq/L)	TAC (TroloxEqµmol/L)	OSI (TOC\TAC) (arbitrary unit)
CN-Gr	18.54±1.11^{A}	13.3±3.52^{A}	1.61±0.27^{A}	0.12±0.81^{A}	19.88±1.30^{A}	14.8±3.68^{A}	1.45±0.21^{A}	0.10±0.75^{A}
PPS-Gr	17.96±1.44^{A}	12.9±2.78^{A}	1.90±0.46^{B}	0.14±0.68^{B}	19.50±1.55^{A}	15.5±2.95^{A}	1.36±0.52^{B}	0.08±0.66^{B}
T2-Gr	26.35±2.35^{B}	19.5±3.64^{B}	0.99±0.64^{C}	0.05±0.02^{C}	38.39±2.17^{B}	23.4±3.61^{B}	0.86±0.22^{C}	0.04±0.95^{C}
T2+PPS-Gr	22.54±1.58^{C}	13.4±4.35^{A}	1.52±0.99^{A}	0.11±0.78^{A}	24.27±1.55^{C}	19.3±3.06^{C}	1.48±0.20^{A}	0.08±0.84^{B}

(CN-Gr) =negative control group, (PSS-Gr) = PoultryStar® Sol treated group, (T2-Gr)= T-2 intoxicated group and (T2+PSS-Gr)= T-2 intoxicated group+PoultryStar® Sol treated group respectively.

(Each value that is not sharing a common superscript letter signifies a statistically significant difference with $P<0.05$). The sample size is 7 rats for each group.

3-3 Serum activities of the antioxidant enzymes

T-2 mycotoxin administration for 90 days produced a gradual decline in the level of the antioxidant enzymes. The change was milder for glutathione as it was insignificantly changed on day 45 but the change was significant in day 90. The other enzymes showed a significant change in both days 45 and 90 after the toxin administration. The PoultryStar® Sol succeeded in performing a partial restoration in the level of the antioxidant enzymes as their values were still significantly less that that of the negative control.

Table 3: Serum activities of Glutathione peroxidase (GSH-Px), Super oxide dismutase (SOD) and Catalase (CAT)) antioxidative stress enzymes together with the RBCs hemolysate reduced glutathione (r-GSH) concentrations at days 45 and 90 of the experimental period. (The results are expressed as mean ± SEM).

GROUP	At 45 day				At 90 day			
	r-GSH (µmol/m)	GSH-Px (U/ml)	SOD (U/ml)	CAT (U/ml)	r-GSH (µmol/m)	GSH-Px (U/ml)	SOD (U/ml)	CAT (U/ml)
CN-Gr	22.07±1.11^{A}	113.4±5.7^{A}	84.54±2.5^{A}	2.99±0.13^{A}	21.05±1.15^{A}	116.5±5.2^{A}	88.83± 2.66^{A}	2.95± 0.16^{A}
PPS-Gr	22.20±1.12^{A}	114.7±6.3^{A}	85.22±2.8^{A}	3.07±0.13^{A}	22.55±1.18^{A}	117.4±6.2^{A}	87.13± 2.13^{A}	3.09± 0.11^{A}
T2-Gr	20.98±1.07^{A}	110.9±4.5^{B}	66.85±1.5^{B}	2.23±0.18^{B}	12.24±1.08^{B}	74.9±5.3^{B}	47.33± 2.33^{B}	1.11± 0.17^{B}
T2+PPS-Gr	21.25±1.73^{A}	113.8±3.4^{A}	77.64±2.4^{C}	2.19±0.13^{B}	15.77±1.01^{C}	105.9±3.8^{C}	68.45± 2.20^{C}	2.11± 0.13^{C}

(CN-Gr) =negative control group, (PSS-Gr) = PoultryStar® Sol treated group, (T2-Gr)= T-2 intoxicated group and (T2+PSS-Gr)= T-2 intoxicated group+PoultryStar® Sol treated group respectively. r-GSH, GSH-Px, SOD and CAT refer to the blood hemolysate reduced glutathione level, serum level of the glutathione peroxidase, superoxide dismutase and catalase respectively.

(Each value that is not sharing a common superscript letter signifies a statistically significant difference with *P<0.05*). The sample size is 7 for each group.

3-4 Lipid Profile Findings

A prominent and a significant hyperlipidemic effect was observed for mycotoxin as seen in the results of the serum level of triglyceride, cholesterol, LDL and HDL *(P<0.05)*. The impact was more significant on day 90 as compared to that on day 45. The PoultryStar® Sol could have hindered the progression of this dilemma as the changes were less when they were co-administered along with the mycotoxin as compared to that of the mycotoxin alone treated group. Although, they failed to produce a complete restoration as the values of the lipid profile parameters were still significantly different as compared to that of the negative control.

Table 5: Measurements of Serum lipid profile (Triglyceride (T.G), Total cholesterol, Low density lipoprotein (LDL) and High-Density Lipoprotein (HDL) on days 45 and 90 of the experiment periods. (The results are expressed as mean ± SEM)

GROUP	At 45 day				At 90 day			
	Triglycerie (TG) mg/dl	Total cholesterol mg/dl	LDL-cholesterol mg/dl	HDL-cholesterol mg/dl	Triglyceride (TG) mg/dl	Total cholesterol mg/dl	LDL-cholesterol mg/dl	HDL-cholesterol mg/dl
CN-Gr	82.8±1.91^{A}	70.21±2.10^{A}	120.57±0.88^{A}	43.18±1.18^{A}	79.8±1.23^{A}	75.55±2.61^{A}	122.37±0.85^{A}	42.15±1.14^{A}
PPS-Gr	81.3±1.40^{A}	69.57±1.91^{A}	119.59±0.67^{A}	48.16±1.31^{B}	78.3±1.11^{A}	73.11±2.43^{A}	121.45±1.01^{A}	55.72±2.03^{B}
T2-Gr	133.4±4.30^{B}	150.36±3.41^{B}	154.59±1.77^{B}	29.21±1.15^{C}	199.4±3.24^{B}	211.10±5.70^{B}	195.18±1.57^{B}	25.55±1.14^{C}
T2+PPS-Gr	88.5±1.33^{C}	103.51±2.86^{C}	132.71±1.03^{C}	36.22±1.17^{D}	98.9±2.17^{C}	144.33±3.86^{C}	131.71±1.83^{C}	31.12±1.15^{D}

(CN-Gr) = negative control group, (PSS-Gr) = PoultryStar® Sol treated group, (T2-Gr) = T-2 intoxicated group and (T2+PSS-Gr) = T-2 intoxicated group+PoultryStar® Sol treated group respectively.

(The value that is not sharing a common superscript letter signifies a statistically significant difference with *P<0.05*). The sample size is 7 rats for each group

3-5 Liver Histopathological findings

The histopathological examination of liver sections obtained from both control groups, (CN-Gr) and (PSS-Gr) reveals normal hepatic tissue architecture with complete preservation of hepatocyte outline and nuclear staining (Figure-1).

In T-2 intoxicated group, (T2-Gr) after 45 days from the commencement of this experiment, liver histopathological findings comprised an advanced degenerative changes as (hydropic degeneration and microvesicular (mostly) and macrovesicular (occasionally) steatosis), remarkably (Figure-2). The severe cellular swelling is attributed to the

acinar ballooning degeneration and subsequently cytoplasm rarefaction due to accumulation of water vacuoles that appeared with irregular hazy boundaries. Additionally, the presence of variable sizes, clear, unstained, and rounded fat droplets participate also in this swelling. These lesions are predominant in both portal triad area and hepatic lobule structure. Some sections appeared to develop focal apoptotic body formation without any sign of inflammatory reaction. Obviously, this finding represents apoptosis rather than coagulative type necrosis. An acidophilic intracytoplasmic solitary hyaline Mallory Denk bodies were also noticed during this period (Figure-3)

On day 90, features of subacute hepatitis are noticed as the most predominant lesion, meanwhile, less apoptotic bodies formation activities were developed. However, some hepatic lobules developed apoptotic disarray pattern. Additionally, individual cell necrosis and/or spotty (cluster cells necrosis) is well developed in portal area (mainly) and in the centrilobular area (Zone 3) with an occasional variable degrees of centrilobular glycogen depletion (Figure-4). Obvious and pronounced disruption in the hepatic cords radiation architecture is seen in the triad (portal) area. Seemingly, in this area, the necrotic hepatocytes revealed multiple stages of nuclear changes (pyknosis, karyorrhexis and karyolysis) and cytoplasmolysis. Some other hepatocytes undergo non inflammatory apoptotic appearance (Figure-5). Interestingly, some liver sections (on day 45 and 90) revealed occasionally the presence of solitary an acidophilic intracytoplasmic inclusions called Mallory Denk bodies. These ropy hyaline non specific bodies represent an intermediate damaged microfilaments in the T-2 intoxicated hepatocytes.

In T-2 intoxicated rats that simultaneously treated with PoultryStar® Sol in (T-2+PSS-Gr), the histopathological changes are restricted to those of mild reversible degenerative types with almost absences of the necroinflammatory lesions. Most of the hepatocytes appeared to have intermitotic activity criteria, binucleation and active looking autophagic vacuoles inside their cytoplasm. A few hepatocytes in this group are still appear to have minimum degree of apoptotic activity (Figure-6).

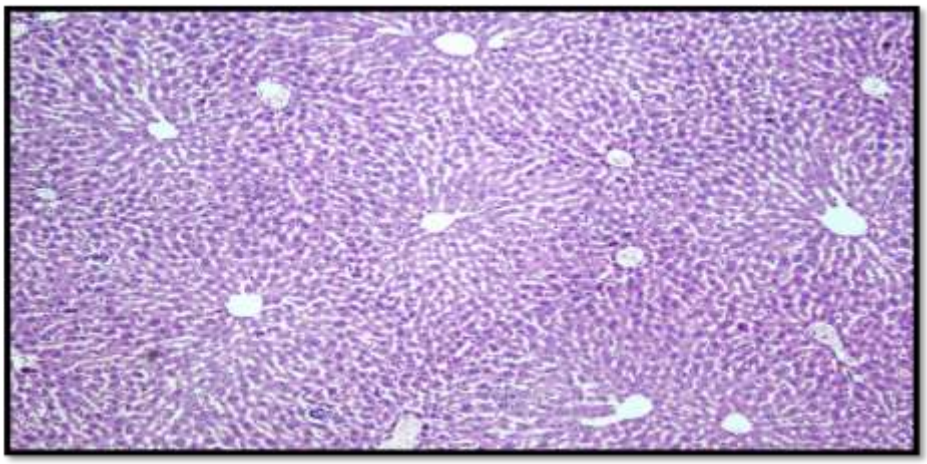

Figure 1: Photomicrograph of liver of rat from Control Group (CN-Gr). Normal multilobular architecture with obvious hepatic cord radiations from central vein toward lobular periphery (10X)

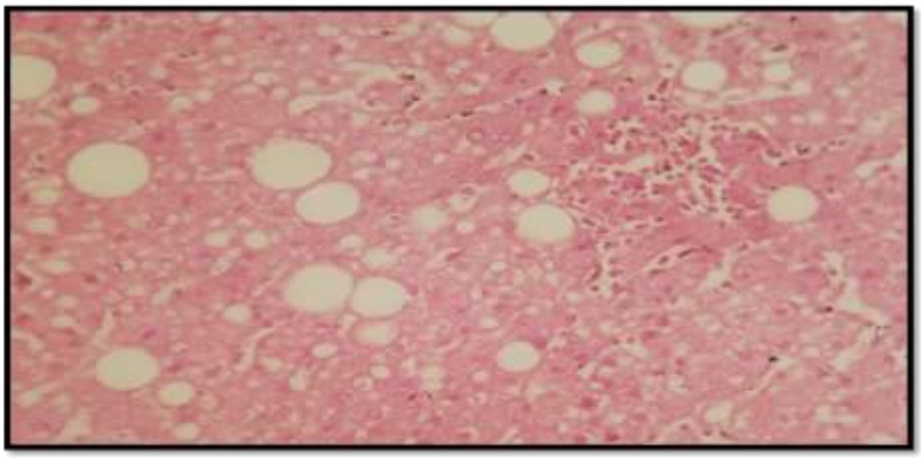

Figure 2: Photomicrograph of liver of rat from T-2 intoxicated group on day 45. Presence of microvesicular steatosis (numerous small size fat droplets) and macrovesicular metamorphosis (engorgement of the cytoplasm by a large fat vacuole) that push nucleus aside give the cell ring like appearance. Other hepatocytes appear with an intracytoplasmic water vacuoles with hazy boundaries which represent hydropic degeneration. Few apoptotic hepatocytes and some congested sinusoidal spaces are seen also (20X).

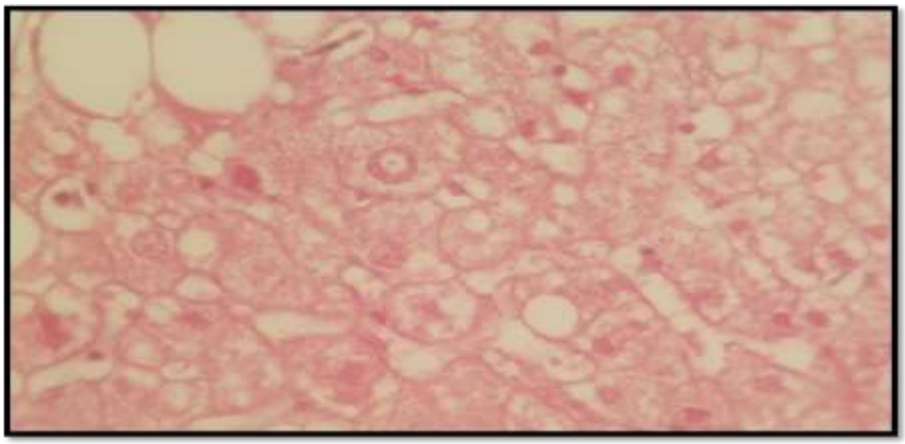

Figure 3: Photomicrograph of liver of rat from T-2 intoxicated group on day 45. Acute cellular swelling due to either micro or macro vesicular steatosis (fatty change) or hydropic degeneration. Some hepatocytes undergo shrinkage and fragmentation into apoptotic bodies without any inflammatory reaction surrounding them. An intracytoplasmic acidophilic solitary hyaline Mallory Denk bodies are also noticed (40X)

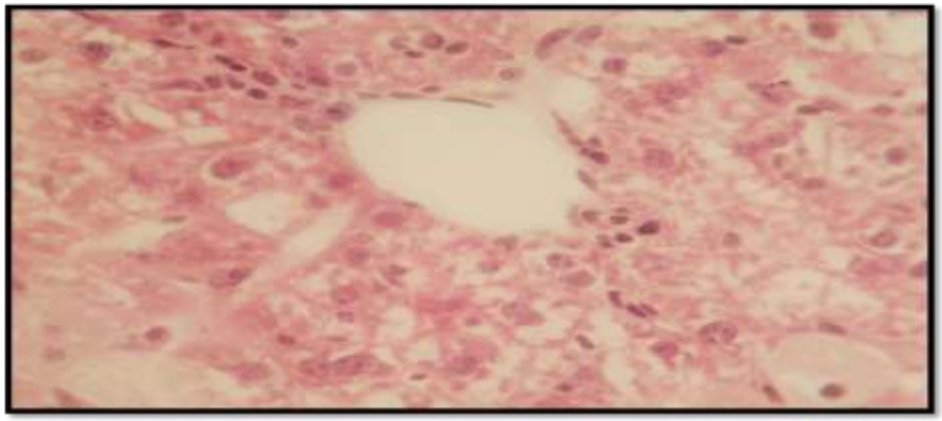

Figure 4: Photomicrograph of liver of rat from T2 intoxicated group on day 90. Perivenular necroinflammatory lesions (homicidal cells) in zone-3 consist of coagulative necrosis with sub acute lymphocytic infiltration and mild degree prominence of Kupffer cells (liver specific macrophages). Besides, some hepatocyes appeared as shrink suicidal apoptotic cells (40X).

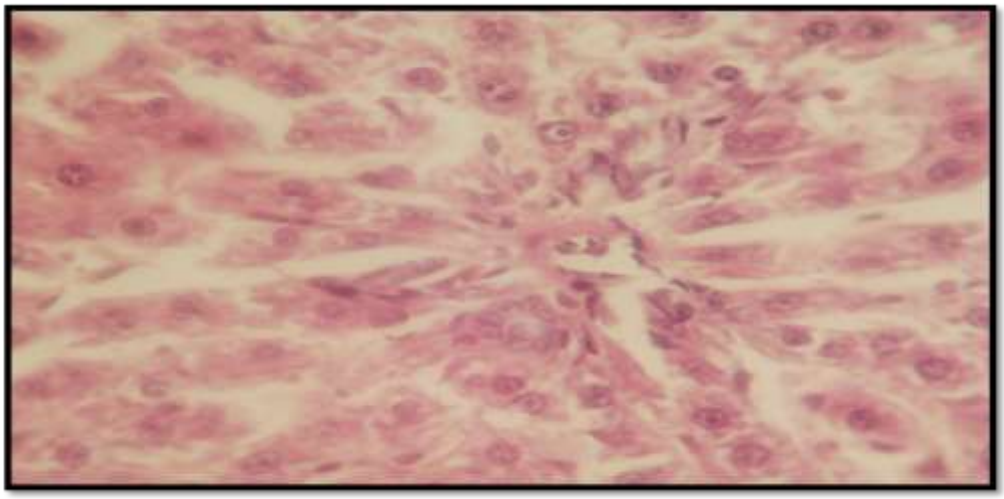

Figure 5: Photomicrograph of liver (portal triad area) of rat from T-2 intoxicated group on day 90. Pronounced disruption in the hepatic cord radiation architecture. Most hepatocytes revealed multiple stages of nuclear necrotic changes (pyknosis, karyorrhexis and karyolysis) and cytoplasmolysis. Some other hepatocytes undergo non inflammatory apoptotic appearance (40X).

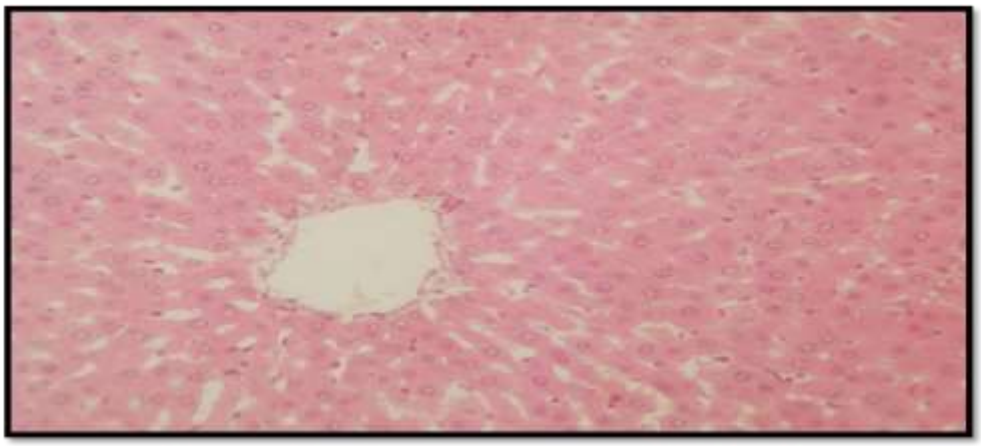

Figure 6: Photomicrograph of liver of rat from (T-2+PSS-Gr) on day 90. Preservation of normal hepatic cord orientation together with well differentiated active looking intermitotic hepatocytes appearance. Concurrently, very few apoptotic cells are noticed (20X).

DISCUSSION

The T-2 trichothecene toxin is the most cytotoxic agent that provoking numerous toxic alterations in different tissues, cells and in various cellular reactions which are responsible for cell cycle and oxidoreductive stress mediated apoptosis.

In the current study, after 45 days of commencement of this experiment., only the concentration of MDA was increased significantly with a frank decrease in the activity of catalase enzyme only. Meanwhile, the up-regulation of MDA and total oxidative capacity at the end of the study period together with the critical decreases in concentration of r-GSH, activities of GSHP-x and catalase reflected that rats had been impelled by progressive of oxidoreductive stress induced by T2 toxin by a dose and time dependant manner. These biochemical findings proposed that the pernicious impact of T-2 intoxication was at least created by oxidoreductive stress.

Besides, it was obvious that the histopathological changes of T2 toxin against rat liver were dose and time-dependent findings that verify the previous research work results [4, 27], remarked that T-2 toxin triggers toxic reactions in the hepatocytes involving sophisticated interactions between apoptosis and autophagy in a dose dependant manner. Seemingly, a low cumulative doses of T-2 toxin after 45 days can evokes activation of autophagy to be an initial event in the liver and then minimizes or inhibits apoptosis. This process (autophagy) is revealing the active regenerative capabilities of intermitotic hepatocytes to develop a repair mechanism against T2 toxin damage. Furthermore It was also obviously found that T-2 toxin can contradict with the plasma membrane phosphorylation and initiate lipid peroxidation in liver [28]. Accordingly, the Na K/ATPase depending pump will be affected by T-2 toxin and ceased. This cessation in turn will cause the entrance of water inside the hepatocyes and hydropic degeneration will be establish. This lesion in liver was the most remarkable one seen in the histopathologic sections examined after the first period.

Meanwhile, after long duration (90 day) of T2 toxin exposure, the cumulative overwhelming doses of T-2 toxin motivated the pro-apoptotic events to be the dominant process with deleterious and irreversible hepatic toxic effects in the form of either apoptosis without any inflammatory reaction or coagulative necrosis that coincided with infiltration of sub acute to chronic inflammatory cells, as confirmed histopathologically in the current study. Additionally, and depending on the findings of previous studies [29,30] the current work was found that T-2 toxin induced programmed cell death and/or coagulative necrosis. by inhibition of protein synthesis and promoting nuclear pyknosis (condensation of chromatin material), karyorrhexis (fragmentation of nuclear chromatin) karyolysis (dissolution of chromatin) and eventually cytoplasmolysis of the necrotic hepatocytes due to prolonged T-2 intoxication. However, other factors may participate in exacerbation and complementation of chronic T2 toxicity by elevating the expression level of certain caspases enzymes which involved in the mitochondrial dysfunction pathway [30]. Though it is of controversial roles, tumor protein (p53), is a nuclear transcription agent that controls the expression of several genes involved in apoptosis and cellular oxidoreductive stress [29]. Accordingly, it was previously found that (p53) up-regulated during T-2 intoxication which further affirm this possibility [30]. Furthermore, T-2 toxin could inhibit protein synthesis via its harmony linkage with trans-peptidase (ribosomal subunits) and thus halt RNA and DNA biosynthesis [1,33]. This linkage can interpret the pronounced down regulation of intracellular r-GSH which was previously noticed by (Bouaziz *et al*., 2006; Chaudhari, 2009) [34,35] together with significant reduction in the activities of its related antioxidative GSH-Px enzyme, a findings which completely match the biochemical status of the current study. Obviously, T-2 hepatotoxicity oppresses glutathione S-transferase the drug metabolizing enzymes and thus opposed its de-epoxidation which is an important route for T2 detoxification in animals. Unlike, chickens which have no deepoxidation capability, rats displaying high deepoxidation activity [36], rendering this rodent species more resistant to T-2 toxicity and need prolonged duration course as the case of this study.

Glutathione peroxidase and catalase are an intermediaries in the regenerative system of the oxidoreductive damage induced by the aggression of excess ROS produced by

prolonged T-2 exposure. These enzymes revealed at the end of this study, a severe decline in their activities. This decline can be attributed to the continuous depletion of r-GSH the master intracellular antioxidant and precursor of GSH-Px. On the other hand, catalase as enzyme that is mainly situated in the sub cellular peroxisomes and cytosol, beside its ability to catalyze the conversion of hydrogen peroxide to water, it posses peroxidase activity. Catalase is very effectual and potent in high-grade oxidoreductive stress [37]. This enzyme is especially important in the case of limited r-GPX availability as the case of current study and plays a significant role in the development of tolerance to oxidative stress as adaptive reaction of the intoxicated hepatocytes. Obviously, after post adaptive stage of T2 intoxication, catalase undergoes pronounced suppression. Aspartate aminotransferases (AST) is concentrated in the liver, where 20% of its total activity restricts in the cytosolic part and 80% in the mitochondria [38]. This enzyme is also diffusely available in the kidneys, heart, brain striated muscle, and erythrocytes. Concurrently alanine aminotransferases (ALT) is highly concentrated in liver (localized solely in the cellular cytoplasm) with very low concentrations in striated muscles and kidney [39]. Consequently, the elevation in alanine aminotransferases serum levels is more precise indicator for hepatic damage. As stupendous finding in the current study, ALT level was not significantly increased after 45 day where the hepatic damage was characterized by reversible degenerative lesions only. On the contrary, an intensive elevation in the level of ALT was associated with irreversible necrotic or apoptotic hepatocytes. In the liver, centrilobular zone of the hepatic acinus has a higher concentration of AST. Seemingly, T-2 toxin damage (coagulative necrosis) to this area (zone 3) after 90 day from the commencement of the experiment resulted in greater alterations to AST levels as confirmed by the histopathological and biochemical findings. Furthermore, the pattern of T-2 intoxication in this study which is quite similar to ischemic hepatitis, appeared when the AST alterations are more greater than ALT ones.

The efficacy of PoultryStar® Sol on liver enzymes during this study revealed an ameliorative effects. Hypothetically, the probiotic content of this product may use certain enzymatic mechanisms to block and modify T-2 toxin receptors. Additionally, the using of this symbiotic product appeared to have significant antioxidoreductive activities by its unique total antioxidative capacity.

Adjustment of gut microbial ecosystem by using synbiotic product to alleviate the T-2 mycotoxicosis and its deleterious complications might be a novel attempt to cope with this type of toxicity. Probiotics have potential as prevention strategies or therapies for multiple digestive system disorders, including liver diseases of different causative agents.

The prominence of apoptosis rather than necrosis at the end of the current study may attributed to the action of varying probiotic strains included in PoultryStar® Sol that may enhance the secretion of anti inflammatory like (IL-10) and modulate proinflammatory like (IL-12) cytokines by liver immune cells (Kupffer cells and monocyte-derived macrophages) that ensure proper liver homeostasis [40]. Seemingly, the protective effect of (PoultryStar® Sol) against T-2 toxin stress was through protecting r-GSH content and metabolism. Additionally, these beneficial probiotic bacteria ensuring a well balanced gut homeostasis through the notion of competitive exclusion of pathogenic microorganism which in turn will minimize the liver pathogenic bacteria and T-2 toxin burden. Moreover, it seems to be that T-2 toxicity was alleviated through selective activating autophagy by elevating the antioxidant capacity as confirmed by the results of current study with subsequent reversion of the degenerated hepatocytes [40]. Fructooligosaccharides (FOS) as prebiotic substance included within (PoultryStar® Sol) may participate, like other anti hepatic damage plants [42,43] in restoring serum lipid profile constituents and subsequently in liver protection.

CONCLUSION

The exact mechanisms that determine the relationship between probiotic bacteria and the T2 toxin remain vague. Several peculiar criteria of using probiotic bacteria and prebiotic agents can be addressed here and collectively can ameliorate T-2 toxicity. Firstly, PoultryStar® Sol had been restore the reduced glutathione concentration, ameliorate some liver function enzymes and potentiate the total antioxidative capacity together with diminishing the lipid peroxidation status. Secondly it may can support the gut barrier function by increasing the activities of goblet cells to produce more mucus which in turn prevents T-2 adherence and/ or translocation to the blood stream, besides the indirect competitive exclusion effect of PoultryStar® Sol against diverse pathogenic microorganisms in the gut ecosystem. In conclusion, summing up, current findings demonstrated that PoultryStar® Sol efficaciously blocked the oxidoreductive stress effects and the cell necrosis commence by significant and potential inhibition of apoptosis elicited by T-2 mycotoxin in the rat hepatocytes.

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CONFLICT OF INTEREST

All the authors confirm that there is no any conflict of interest regarding publication of this manuscript.

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