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 possess 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, dystoxia (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 mycotoxycosis 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, (T2-Gr), which was assigned as the syrbiotic 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-Sol) was given the T-2 contaminated chow and (PoultryStar® Sol) in the tap water with normal drinking water for 90 days, and Group 4 (T2+PSS-Gr) which was given the T-2 contaminated chow and (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 end the of 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 generative and antioxidant potentials of (PoultryStar® Sol) in coping with T2 toxin mediated hepatotoxicity in rats.

Keywords: T2 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. sporotrichoids, 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 T2 mycotoxicosis and the associated pathogeneity. Among these wide spectrum agents, polyunsaturated fatty acids (PUFAs), 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 Biomim® anti-mycotoxin product called (Mycocif plus) can modify the epoxide ring of trichothecenes into a diene. Some bacteria like Devosiamutans (17-2-E-8) and Mycocif-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 oxidation [10], epimerization [11] glucosylation [12] and de-epoxidation [13]. Biotransformation reactions include hydrolysis, acetylation, deamination, glucosylation, ring cleavage, and decarbonylation.
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 pose an enzymatic conversion 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 toxins by some lactic acid bacteria (Lactobacillus sakei, 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 trichotheccenes.

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 symbiotic 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 oxidative/reductive / antioxidant 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 Beitar 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 symbiotic (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 under distinctive 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 premix (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 (PSS-Gr), which was assigned as the symbiotic (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 rodents’ chow ad libitum at a level of (470 ppb) along with symbiotic (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 (45th and 90th) dayof 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-Mérieux 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 H₂O₂ 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) was estimated in the RBC’s hemolysate by using the method set out by Eillman 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- 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) followed by post hoc Brunner 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 significant.

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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 Days</td>
<td>90 Days</td>
<td>45 Days</td>
</tr>
<tr>
<td>CN-Gr</td>
<td>119.66±4.53</td>
<td>120.14±4.44</td>
<td>125.15±2.96</td>
</tr>
<tr>
<td>PPS-Gr</td>
<td>117.61±3.65</td>
<td>118.52±2.90</td>
<td>124.22±3.50</td>
</tr>
<tr>
<td>T2-Gr</td>
<td>140.18±3.93</td>
<td>318.15±7.88</td>
<td>140.23±2.55</td>
</tr>
<tr>
<td>T2+PPS-Gr</td>
<td>138.41±3.52</td>
<td>316.25±6.80</td>
<td>133.23±2.91</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>At 45 day (n=7)</th>
<th></th>
<th>At 90 day (n=7)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA nmol/ml</td>
<td>TOC (μmol H₂O₂ Eq/L)</td>
<td>TAC (TroloxEqmol/L)</td>
<td>OSI (TOC/TAC) (arbitrary unit)</td>
<td>MDA nmol/ml</td>
<td>TOC (μmol H₂O₂ Eq/L)</td>
<td>TAC (TroloxEqmol/L)</td>
<td>OSI (TOC/TAC) (arbitrary unit)</td>
</tr>
<tr>
<td>CN-Gr</td>
<td>18.54±1.11 A</td>
<td>13.3±3.52 A</td>
<td>1.61±0.27 A</td>
<td>0.12±0.81 A</td>
<td>19.88±1.30 A</td>
<td>14.8±3.68 A</td>
<td>1.45±0.21 A</td>
<td>0.10±0.75 A</td>
</tr>
<tr>
<td>PPS-Gr</td>
<td>17.96±1.44 A</td>
<td>12.9±2.78 A</td>
<td>1.90±0.46 B</td>
<td>0.14±0.68 B</td>
<td>19.50±1.55 A</td>
<td>15.5±2.95 A</td>
<td>1.36±0.52 B</td>
<td>0.08±0.66 B</td>
</tr>
<tr>
<td>T2-Gr</td>
<td>26.35±2.35 B</td>
<td>19.5±3.64 B</td>
<td>0.99±0.64 AC</td>
<td>0.05±0.02 AC</td>
<td>38.39±2.17 B</td>
<td>23.4±3.61 B</td>
<td>0.86±0.22 C</td>
<td>0.04±0.95 C</td>
</tr>
<tr>
<td>T2+PPS-Gr</td>
<td>22.54±1.58 C</td>
<td>13.4±4.35 C</td>
<td>1.52±0.99 AD</td>
<td>0.11±0.78 AD</td>
<td>24.27±1.55 C</td>
<td>19.3±3.06 C</td>
<td>1.48±0.20 AD</td>
<td>0.08±0.84 AD</td>
</tr>
</tbody>
</table>

(CN-Gr) = negative control group, (PPS-Gr) = PoultryStar® Sol treated group, (T2-Gr) = T2 intoxicated group and (T2+PPS-Gr) = T2 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 than that of the negative control.

Table 3: Serum activities of Glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) and Catalase (CAT) for 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).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>At 45 day</th>
<th>At 90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-GSH (μmol/m)</td>
<td>GSH-Px (U/ml)</td>
</tr>
<tr>
<td>CN-Gr</td>
<td>22.07±1.11a</td>
<td>113.4±5.7a</td>
</tr>
<tr>
<td>PPS-Gr</td>
<td>22.20±1.12a</td>
<td>114.7±6.3a</td>
</tr>
<tr>
<td>T2-Gr</td>
<td>20.98±1.07a</td>
<td>110.9±4.5a</td>
</tr>
<tr>
<td>T2+PPS-Gr</td>
<td>21.25±1.73a</td>
<td>113.8±3.4a</td>
</tr>
</tbody>
</table>

(CN-Gr) = negative control group, (PPS-Gr) = PoultryStar®Sol treated group, (T2-Gr) = T-2 intoxicated group and (T2+PPS-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 rats 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 (TG), Total cholesterol, Low density lipoprotein (LDL) and High-Density Lipoprotein (HDL) on days 45 and 90 of the experimental periods. (The results are expressed as mean ± SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>At 45 day</th>
<th>At 90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglyceride (TG) mg/dl</td>
<td>Total cholesterol mg/dl</td>
</tr>
<tr>
<td>CN-Gr</td>
<td>82.8±9.14a</td>
<td>70.21±2.10a</td>
</tr>
<tr>
<td>PPS-Gr</td>
<td>81.3±14.0a</td>
<td>69.57±1.91a</td>
</tr>
<tr>
<td>T2-Gr</td>
<td>133.4±4.30a</td>
<td>150.36±3.41a</td>
</tr>
<tr>
<td>T2+PPS-Gr</td>
<td>88.5±1.33c</td>
<td>103.5±2.86c</td>
</tr>
</tbody>
</table>

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

3-5 Liver Histopathological findings

The histopathological examination of liver sections obtained from both control groups, (CN-Gr) and (PPS-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 rropy 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 absence 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 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 hepatocytes 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).
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 GSH-P-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 hepatocytes 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 pyknotis (condensation of chromatin material), karyorrhexis (fragmentation of nuclear chromatin) and eventually cytoplasmalysis 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 antioxidant 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

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).
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 in high-grade oxidative 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, centrolobular 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 symbiotic 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 prominenace 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 probiotic 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 antioxidoreductive 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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