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Effect of the Nutritional Supplement Synertox® on Lead - Induced Toxicity in Male Albino Rats

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Abstract: ***Background: Synertox® is a supplement of high nutritional value containing a cocktail of highly rich ingredients. Lead is one of the well - known hazardous heavy metals. The current study was conducted to investigate the probable role of synertox to ameliorate the lead - induced toxic effects. Materials and Methods: For this purpose, 60 male albino rats were randomly divided into 3 groups, of 20 animals each, designated groups 1, 2 and 3. Rats in group 1 served as untreated control, group 2 animals were intoxicated by lead and group 3 rats were exposed to lead and received synertox in their drinking water. Results: All hematological and biochemical parameters were altered in the lead intoxicated rats (group 2) compared to control group. Addition of synertoxin group 3 resulted in increasing the erythrocytic indices, including RBCs count, hemoglobin concentration and hematocrit %. Biochemical parameters of group 3 disclosed comparatively increased levels of total proteins and decreased levels of creatinine, urea, bilirubin and serum enzymes in relation to the levels recorded in group 2 (lead intoxicated animals). Conclusion: It was concluded that synertox as a highly concentrated nutritional supplement may provide an ameliorating effect on the lead induced toxicity as judged by the encountered hematological and biochemical profiles.***

Keywords: Lead toxicity, synertox, antioxidant, hematological changes, biochemical Profile

1. Introduction

Synertox®is a highly concentrated nutritional supplement containing rich essential ingredients including vitamins and organic acids. It is used as a soluble product for maintaining immune status, enhancing performance and sustaining the vital body physiological functions. The highly concentrated components and micronutrients contained in synertox includes; phosphoric acid, citric acid, lactic acid, aspartic acid, calcium lactate, papain, potassium and sodium tartrate, dried *Bacillus subtilis* fermentation extract, propylene glycol, calcium pantothenate, riboflavin, thiamine nitrate and pyridoxine hydrochloride.

Lead is one of the most hazardous environmental heavy metal that is globally distributed and widely used. [1] [2] [3]It possesses non - biodegradable nature, hence it is accumulated in the environment with increasing the possible hazards. Occupational exposure to lead and its compounds is the most common source of toxicity to human. Generally, lead poisoning takes place by ingestion of food and water contaminated with lead, followed by rapid absorption to reach blood stream and finally leadis localized in certain tissues. [4]Lead as a highly toxic metal affects all types of human and animal tissues. [5]

Long - term exposure to lead may provoke pronounced tissue changes as a consequence to the resultant chronic toxicity. [6] This is exemplified by the lead - induced histological, histochemical and metabolic hepatic and renal changes. [7] [8] [9] [10] [11] [12] Lead - induced toxic effects may also extend to the circulating blood cells. [13] [14]

The present study was carried out to investigate the probable ameliorating effect of synertox as a supplement of high nutritional value on lead - induced toxicity.

2. Materials and Methods

Experimental animals:
A total of 60 adult male albino rats, aging 4 months and weighing 165 - 220 g, were used in the present study. The rats were housed and maintained according tothe laboratory standards (12 - h dark - light cycle, ambient temperature 24 ± 1 ^{o}C, relative humidity of 30%to70%). All the official guides to care and use of laboratory animals stated by the Ethical Committee of Imam Mohammad Ibn Saud University, Saudi Arabia, were followed carefully.

Lead and Synertox:
Lead was used as lead acetate trihydrate (Pb $(CH_3COO)_2$ x3 H_2O) (Sigma - Aldrich, Germany). The commercially available synertox® (Agrarian Marketing Corporation, IN, USA) was procured and implicated in the experiment with its original concentration without dilution. Synertox ingredients as stated by the manufacturer are listed in Table (1).

Experimental design:
After one week acclimatization period, the rats were randomly allotted into three equal groups, of 20 animals each, designated groups 1, 2 and 3. Rats in group 1 served as untreated control, i. e., not exposed to lead and received only plain drinking water without the addition of any chemical. Group 2 animals were exposed daily to lead acetate trihydrate dissolved in water at the dose of 5 mg/Kg b. w. by an oral gavage in a volume of 1 mL/ kg b. w. Rats in group 3 were exposed to lead at the same dose and via the same

route, with the concomitant addition of synertoxto their drinking water at the level of 0.5 ml/L.

Experimentation period extended to 30 days, and throughout this period water and feed (dry ration) were available *ad libitum*. All experimental animals were observed for clinical signs, behavioral activity, feed consumption and water intake.

Hematological and biochemical assays:
At the end of experiment, Blood samples were collected from animals of all groups. Blood samples with anticoagulant (EDTA) were used to estimate the various hematological parameters, including RBCs and total WBCs counts, and other erythrocytic indices involving hemoglobin (Hb) concentration and packed cell volume (PCV) %.

Serum harvested from the coagulated blood samples was employed to assess the various biochemical parameters encompassing total proteins, albumin, globulin, creatinine, urea, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), total thiols and malondialdehyde (MDA).

Erythrocytic and total leucocytic counts were assessed using the convenient hemocytometer. Hemoglobin (Hb) concentration was assessed by Cyanmet - hemoglobin method as described by Benjamin (1978) and Coles (1986). Packed cell volume (PCV) % was estimated by a micro - hematocrit method.

Total thiols were estimated by using a total thiol colorimetric assay kit (Cell Biolabs Inc., USA). Measurement of Malondialdehyde was done by a MDA colorimetric assay kit (Elabscience, USA). All other biochemical parameters including serum enzymes were assessed using the relevant commercial colorimetric diagnostic kits (Interchim Diagnostics Biochemistry kits, France).

Statistical analysis:
All data were presented as means± S. D. The obtained data from all animals were analyzed using a statistical analysis SPSS software (SPSS Inc. Chicago IL, USA). P - values less than 0.05 ($P< 0.05$) were considered statistically significant.

3. Results

Starting from day 13 post - intoxication, all intoxicated rats in group 2 were less active, compared to control and group 3 animals. No mortalities occurred among the different groups.

Lead intoxicated rats in group 2 showed comparatively decreased hematological parameters, the erythrocytic indices including erythrocytic count, Hb concentration and PCV % were significantly lower than the control levels.

In comparison with control and group 3 animals, all biochemical parameters of lead intoxicated rats in group 2 were altered. Levels of total proteins, total thiols, albumin and globulin were decreased in group 2 animals. On the contrary, creatinine, urea, bilirubin serum enzymes (ALT, AST and ALP) and MDA levels were significantly increased in relation to control levels.

Lead intoxicated rats which received synertox (group 3) in their drinking water manifested comparatively increased levels of their hematological parameters if compared with group 2 animals which exposed to lead without receiving synertox. The relatively restored hematological parameters didn't reach the control levels, however, were standing higher than that of the intoxicated rats which didn't receive synertox.

With receiving synertox (group 3), total proteins, total thiols and MDA levels were comparatively increased in relation to group 2. The levels of other biochemical parameters including serum enzymes, creatinine, urea and bilirubin were comparatively decreased in intoxicated animals with receiving of synertox. The estimated relevant increments or decrements of biochemical parameters in case of intoxicated rats which received synertox, were comparatively shifted toward the control levels.

Tables (2 & 3) and **Figures (1&2)** show the various hematological and biochemical parameters assessed in rats intoxicated with lead and rats intoxicated with lead and received synertox, compared with the control untreated rats.

4. Discussion

The present study was designed to evaluate the capability of a highly concentrated nutritional supplement containing a mixture of valuable nutritive ingredients to alleviate the lead - induced toxic effects. In other words, the anti - toxic activity of synertox against one of the highly hazardous heavy metal toxicants was tested in the present investigation. This was mainly based upon estimation of the various hematological and biochemical parameters which can reflect the relevant alterations of tissues and organs.

Synertox contains a cocktail of highly potent ingredients including organic acids and essential micronutrients. It is enriched with enzymes, organic acids and extract of microorganism, and it is easily dissolved in water. Practically, high solubility of synertox in drinking water is an additional feature which enables experimental animals to receive enough amount of synertox in case of decreased feed intake.

Presently, all the estimated hematological and biochemical parameters in lead intoxicated rats which didn't receive synertox were altered at varying degrees. Generally, these parameters have been reported to be reliable factors for the assessment of health status. [15]Abnormal hematological and biochemical profiles including elevated serum enzymes levels are indicators to cell and tissue damage due to toxicity. [16]

The currently reported decrease in hematological indices is in accordance with the results of previous studies focused on heavy metals toxicity. [3] [17] [18] [19] It is worth mentioning that chronic lead toxicity may result in anemia through hindering iron incorporation into protoporphyrin and thus preventing heme synthesis. [13] [14]

The presently encountered decreased erythrocytic and total leucocytic countsmight interpreted by the ability of lead to affect metabolism and function of mature circulating blood cells, and also its possible direct effect on the process of hematopoiesis. It has been stated that hematopoiesis and circulating blood cells are highly sensitive to toxicity with the result of inhibited cell synthesis and/or abnormally produced cells. [20] [21] [22]

The current data showed significantly altered biochemical parameters in lead intoxicated rats which didn't receive synertox. This was presumably originated as a consequence to damage of the involved tissues and cells. Lead - induced tissue damage may be ascribed to the ability of lead to react with the interstitial protein structures as well as with the concerned tissue enzymes. This damaging effect triggers the release of reactive oxygen species (ROS) and initiation of local inflammatory response. [22] The lead - induced subcellular damage was explained by the ability of lead to interact with the nuclear and organellar structures. [11] [[23] [24] [25] Total thiols were significantly decreased in the presently lead intoxicated rats which didn't receive synertox. Thiols through acting as a scavenger for free radicals contribute to the antioxidative activities. Decreased total thiols level undoubtedly contributes to failure of the anti - oxidation mechanisms. MDA is a marker of lipid peroxidation, and increase in its level obviously points to an oxidative damage. Consequently, the state of oxidative stress is continuously exaggerated and cell necrosis could be resulted at the advanced stage of toxicity. [26]Moreover, the process is accentuated by the intracellular accumulation of lead to form complexes with the fine cell structures, and this ultimately enhances the autophagic mechanism. [27]

Biochemical parameters are sensitive enough to reflect conditions of hepatic and renal damage. [30] [31]Our results demonstrated decreased levels of total proteins, albumin and globulin in the lead intoxicated animals. Decreased total proteins and its fractions may point to the hepatic involvement in the lead toxicity. Decreased total proteins was also reported in cadmium intoxicated animals. [17] [28] Also, increased serum enzymes levels, as currently demonstrated in lead intoxicated rats, are most probably related to the lead - induced hepatic damage. Elevation of serum enzymes levels, including AST, ALT and ALP, usually resulted as a sequel of hepatocytes damage which gives the opportunity for the release of lysosomal enzymes through the leaky cell membranes. [11] [29] [30]

Kidney is documented as a target of the toxic effects exerted by heavy metals. [31] [32] [33] Increased creatinine and urea levels in the presently intoxicated animals are indicators to the lead induced renal damage. Elevated levels of urea and creatinine are usual biochemical change in cases of impaired renal functions. [17] [31]

The presently lead intoxicated rats which received synertox exhibited relative improvement of the hematological and biochemical parameters toward the control levels. This ameliorating effect of synertox on lead toxicity is most probably attributed to its highly rich constituents. The suggested anti - toxic effect of synertox might partly related to calcium pantothenate (Pantothenic acid, vit. $B_{5)}$ which is one of the synertox ingredients. The rich ingredient Pantothenic acid can induce adrenocortical activation which in turn contributes to alleviate the oxidative stress created by the heavy metal toxicity. [27] [28] Moreover, the Presently positive effect of synertox to improve the erythrocytic indices, including Hb concentration, is related to its content of pantothenic acid which is a part of Coenzyme A. Succinic acid, as a product of citric acid cycle, when linked with Coenzyme A, it is activated and cooperates with glycine in heme biosynthesis [27]Additionally, pyridoxine hydrochloride (vit. B_6) as an ingredient of synertox, promotes incorporation of iron in hemoglobin synthesis. In this way, synertox can contribute to alleviate the toxic effects of lead on heme synthesis and subsequently Hb concentration.

In the presently intoxicated rats which received synertox, there was comparative increase in the total blood proteins. This increase may be explained by the role of pantothenic acid (vit. B_5) and thiamine mononitrate (vit. B_1) included in the composition of synertox.

Vit. B_5 is essential for synthesis of albumin fraction of plasma proteins and vit. B_1 acts as a Coenzyme in citric acid cycle to produce the energy needed for protein synthesis. [29] [30]

Briefly, Synertoxwas found to sustainthe kidney functions in the currently lead intoxicated rats. This was evidenced by the improved levels of creatinine and urea which are sensitive parameters to evaluate kidney functions. [31] [32] This is could be also applied to the liver functions as indicated by the improved levels of ALT and AST. [25] [33]

Conclusively, the presently demonstrated improved hematological and biochemical profiles in lead intoxicated rats may point to the capability of synertox to ameliorate the toxic effects of lead. This is most probablyascribed to the rich formula of synertox. The ingredients of synertox presumably act synergistically in case of oxidative damage to sustain the organs integrity and functions, partly through a proposed anti - oxidant activity. Also, synertox ingredients can enhancethe vital metabolic processes such as protein and heme synthesis.

5. Conclusion

The present data might provide an evidence of the anti - toxic property of synertox as a nutritional supplement. This property can be practically employed to counteract the expected negative toxic effects of hazardous environmental toxicants including heavy metals. Basing upon the present results, it is recommended that synertox can be added to the drinking water in localities where the risk of environmental pollutants is suspected. Future research work should focus on the detailed molecular antioxidant mechanisms which could be triggered by the synertox ingredients.

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Legends for Figures

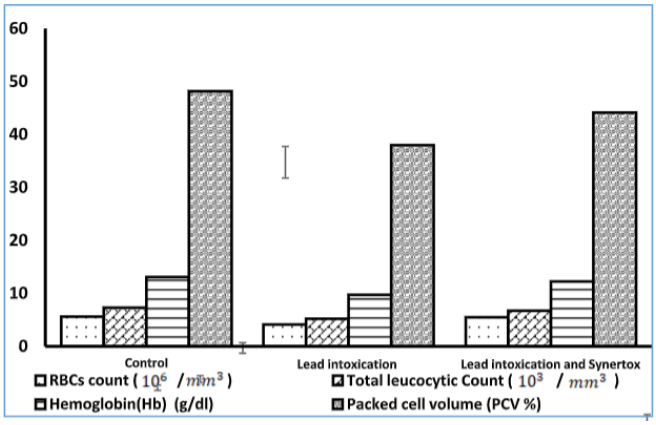


Figure (1): Hematological parameters of rats intoxicated by lead and rats intoxicated by lead and received Synertox® compared with untreated control animals.

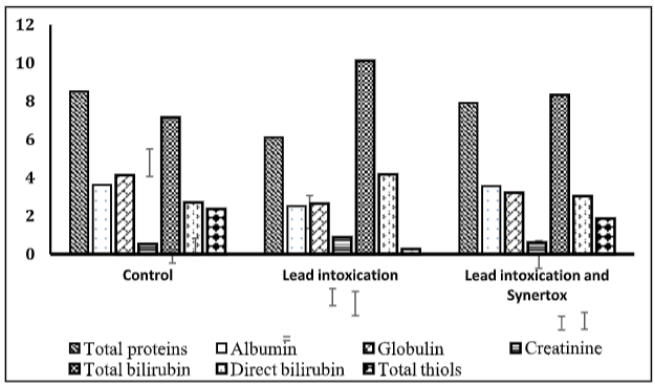
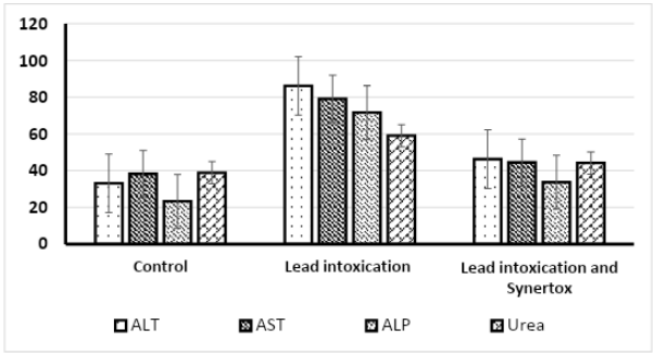


Figure (2): Biochemical parameters of rats intoxicated by lead and rats intoxicated by lead and received Synertox® compared with untreated control animals

a) Levels of total proteins (g/dL), albumin (g/dL), globulin (g/dL), creatinine (mg/dL), total bilirubin (mg/dL), direct bilirubin (mg/dL), total thiols (mmol/L) and malondialdehyde (MDA) (nmol/mL).



b) Levels of serum alanine transferase (ALT) (IU/L), aspartate transferase (AST) (IU/L), alkaline phosphatase (ALP) (IU/L) and urea (mg/dL).

Table 1: Synertox® ingredients (/1000 mL and g/L)) as stated by the manufacturer

Ingredient	(/ 1000 ml)	Ingredient	(g/ L)
Citric acid	80	Sodium citrate	40
Phosphoric acid	65	Potassium citrate	40
Malic acid	4	Papain	40
Tartaric acid	5	Sodium potassium tartrate	40
Disodium EDTA	15	Thiamine mononitrate	3
Propylene glycol	100	Calcium pantothenate	3
Aspartic acid	24	Riboflavin	3
Lactic acid	80	Pyridoxine hydrochloride	3
Dried *Bacillus subtillisfermentation extract*	250		
Calcium lactate	25		

Distilled water (180) up to 1 L

Manufacturer: Agrarian Marketing Corporation (Middlebury, IN, USA).

Table 2: Hematological parameters of rats intoxicated by lead and rats intoxicated by lead and received Synertox® compared with untreated control animals

Parameter	Control (untreated)	Lead intoxication	Lead intoxication and Synertox
RBCs count (10^6/mm^3)	5.61 ± 0.06	4.11*± 0.16	5.49**± 0.04
Total leucocytic Count (10^3/$mm^{3)}$	7.28 ± 0.05	5.21*± 0.38	6.71**± 0.08
Hemoglobin (Hb) (g/dl)	13.10 ± 0.25	9.73*± 0.48	12.23**± 0.57
Packed cell volume (PCV %)	48.17 ± 0.37	38.31*± 0.83	44.13**± 0.64

Values are means ± S. D., N=20. *Significantly different from control (P < 0.05), **Significantly different from lead intoxication.

Table 3: Biochemical parameters of rats intoxicated by lead and rats intoxicated by lead and received Synertox ® compared with untreated control animals

a) Levels of total proteins (g/dL), albumin (g/dL), globulin (g/dL), creatinine (mg/dL), urea (mg/dL), total bilirubin (mg/dL), direct bilirubin (mg/dL), total thiols (mmol/L) and malondialdehyde (MDA) (nmol/mL)

Parameter	Control (untreated)	Lead intoxication	Lead intoxication and Synertox
Total proteins	8.51 ± 0.13	6.11*± 0.17	7.91**± 0.15
Albumin	3.62± 0.04	2.51*± 0.13	3.55**± 0.17
Globulin	4.13± 0.06	2.64* ± 0.21	3.21± 0.22
Creatinine	0.53 ± 0.11	0.88*± 0.43	0.61**± 0.39
Urea	38.87 ± 0.67	59.03*± 0.52	44.15**±0.89
Total bilirubin	7.15 ± 0.42	10.12*± 0.33	8.33**± 0.56
Direct bilirubin	2.70 ± 0.29	4.17* ± 0.43	3.04 ± 0.37
Total thiols	2.36 ± 0.31	0.269*± 0.057	1.86**± 0.46
MDA	331.17 ± 3.61	412.31 ± 3.11	357.27 ± 2.97

Values are means ± S. D., N=20. *Significantly different from control (P < 0.05), **Significantly different from lead intoxication.

b) Levels of serum alanine transferase (ALT) (IU/L), aspartate transferase (AST) (IU/L) and alkaline phosphatase (ALP) (IU/L)

Parameter	Control (untreated)	Lead intoxication	Lead intoxication and Synertox
ALT	33.19 ± 0.44	86.14*± 0.47	46.27**± 0.55
AST	38.15 ± 0.85	79.18*± 0.77	44.39**± 0.61
ALP	23.31 ± 0.63	71.63*± 0.56	33.68**± 0.51

Values are means ± S. D., N=20. *Significantly different from control (P < 0.05), **Significantly different from lead intoxication.

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