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EFFECT OF LOW AND HIGH ESTERIFIED PECTIN ON ARSENIC TOXIFICATION IN RATS

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ABSTRACT

The main purpose of the present study is to evaluate the efficiency of each low and high esterified pectin on arsenic absorption, retention and removal in male albino rats (Sprague Dawley strain). Fifty male albino rats were divided into ten groups (5 rats for each), the first group was fed on based diet (b d) as a (-) control group (NC), groups (2-4) received b d with 4.0, 8.0 and 16 mg/Kg sodium arsenite (SA) /Kg diet daily as which represents 1/100, 1/50 and 1/25 from sodium arsenite, LD_{50} in the diet as control (+) 1,2 and 3 respectively, groups (5-7) , received bd with 8% high esterified pectin (HEP, DE 73.5) with the previously SA doses and groups (7-10), received bd with 8% low esterified pectin (LEP, DE 31%), and the same SA doses. Rats were fed with LEP with 4.0 mg SA/Kg diet resulted in the best improvement of the nutritional valus in addition to the histopathological changes. At the end of the experiment (30 day) the mean value of serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), uric acid, urea, creatinine and lipid peroxide (TBARS) decreased in the groups were fed HEP or LEP in the presence of the previously SA doses when compared with the same positive control. The best results of the previously parameters obtained in group was fed with 8% LEP with 4.0 mg SA/Kg diet except ALT which obtained with 8.0 mg SA/Kg diet when compared with negative control group. These results were (23.92 VS. 24.08 U/L), (102.3 vs. 82.44 U/L), (120.9 VS. 110.5 IU/L), (4.19 VS. 3.79 mg/dL), (13.46 vs. 14.05 mg/dL), (0.56 vs. 0.43 mg/dL) and (6.17 vs. 6.28 n mol/ml) respectively. On the other side, total protein and albumin were increased in all groups fed with HEP or LEP in the presence of all previously concentration of SA. Maximum improvement of total protein and albumin were obtained in the group fed with LEP with 4.0 mg SA/Kg diet when compared with negative control which amounted (7.19 vs. 7.21 g/dL) and (4.1vs. 4.6 g/dL), respectively.

On the other hand, arsenic retention in kidney, brain and liver reduced in all groups were fed with HEP or LEP as compared with positive control. The highest removal of arsenic from the previously organs specially in liver were obtained in the group was fed with

LEP with 4.0 mg SA/Kg diet when compared with negative control which amounted (0.532 vs. 0.000155 ppm), (0.317 vs. 0.000075 ppm) and (0.074 vs. 0.000009 ppm) respectively. On contrast, the concentration of arsenic exerted in feces increased gradually by increasing the doses presented in the diet. Maximum arsenic exerted in feces was in the group was fed 8% LEP with 16 mg SA/Kg diet which amounted 8.549 ppm. The histopathological examination is in good agreement with biochemical measurements. It could be recommended from these results that LEP as a powerful bind material of arsenic should be incorporated into human food consumption daily to reduce the hazard toxicity of arsenic pollution of food and water.

INTRODUCTION

Arsenic is a naturally occurring metal that is present in food, soil and water; it is released in the environment from both natural and man-made sources (Tchounwou *et al.,* 1999). It is considered as a human carcinogen (Wang and Huang, 1994), cellular mechanism of arsenic toxicity involves generation of reactive oxygen species (Chen *et al.,* 1998). Moreover, reactive oxygen species is involved in genotoxicity caused by arsenic (Jha *et al.,* 1992), tissue degenerative changes (Prasad and Rossi, 1995). Exposure to arsenic compounds is associated with several human diseases, including Blackfoot disease, hypertention and cancer (Lyn *et al.,* 1998)

Liver and kidneys are the primary target organs for toxic effects of arsenic as evidenced by clinical manifestations and biochemical alterations (Santra *et al.,* 2000). Alanine aminotranferase (ALT) and aspartate aminotransferase (AST) are the two main serum parameters for liver function and released from the liver cells into the bloodstream, often in the liver disease, resulting in abnormally high serum levels, that may not return to normal for days or weeks. Rats inorganic arsenic exposure decrease the concentration of brain glutathione (Kannan and Flora, 2004), but increase the oxidant production (Shila *et al.,* 2005a).

Pectin is the one of the most common sources of dietary fiber in human nutrition. It is a constituent of plant cell walls in vegetable and fruits or generally presented in an isolated form in jams, jellies, or dairy products.

Pectins are the ionic plant polysaccharides functioning as hydrating agents and cementing substances for the cellulose network. The backbone of pectin is composed of 1→4 linked α-D-galactosyluronic acid residues, some of which are methyl-esterified (Schols and Voragen, 1996). Dietary pectin can bend heavy metals and toxic metabolites (Rose and Quarteman, 1987), pectin with less than 50% of carboxyl groups esterified are called low-esterified pectin.

The number of esterified carboxyl groups determines the degree of esterification, which is one of the important chemical parameters of pectins (Schols and Voragen, 1996).

Many functional properties of pectin such as rheological behavior, galetion, or their binding capacity are dependent on the degree of esterification as well as on other structural parameters such as molecular weight and distribution of free and methoxylated carboxyl groups within the galacturonan chains (Dongowski, 1995). The present study was aimed to determine the effects of low and high esterified pectins on tissue oxidative stress, biochemical variables indicative of hepatic and renal function and concentrations of arsenic in rats treated with doses of arsenic.

MATERIALS AND METHODS

1.Materials:

1.1. Sodium arsenite ($NaAsO_2$, molecular weight 129.9, sigma chemicals, USA)in dose of 0.4, 0.8 and 1.6 mg/kg body weight corresponding to 1/100, 1/50 and 1/25 of LD_{50}, respectively (Brown and Kitchin , 1996) were put in 100g diet which equal to 4.0, 8.0 and 16.0 mg/kg diet, respectively.

1.2. Nitric acid 69% analar BDH.

1.3. Perchloric acid 70% Aldrich.

1.4. Genu® citrus pectin type 105 rapid set has high degree of estrification (DE) 73.5% and partially deesterified Genu® citrus pectin type LM 104 As (31%DE) were obtained from Copenhagen pectin, Lille Skenved, Denmark.

1.5. Sodium hydroxide pellets 99% Riedle-Dehaën.

1.6. Calcium carbonate 99% Fisher Scientific U.K.

1.7. Hydrochloric acid Panreac 37%.

1.8. Kits used to determine serum AST, ALT, ALP, Uric acid, urea, creatinine, total protein, albumin and lipid peroxide was obtained from Biodiagnostic Company.

1.9. Study animals:

Fifty male Sprague-Dawley albino rats weighting 150±5gm each were obtained from animal house of Food Technology Research Institute, Agriculture Research Center, Giza. All rats were allowed to acclimatize to laboratory environment and fed on basal diet for two weeks (adaptation period) prior to the study. The basal diet consisted of protein (casein) 10%, Cellulose 5%, salt mixture 4%, vitamin mixture 1%, corn oil 10% and corn starch 70% according to Lana Peter and Pearson (1971).

On the other hand, all treatments fed with HEP and LEP at concentration of 8% according to Kim *et al.* (1996).

Also, the rats were kept on plenty of diet and sufficient of deionized water till the end of the experiment.

After the adaptation period, rats were fed as the following experimental design:

2. METHOD:

2.1. Experimental design

For experiments, rats were randomly selected into ten groups containing five rats in each.

Group 1: negative control (fed with basal diet [Bd]).

Group 2: positive control 1 (basal diet + 1/100 of sodium arsenite (SA) LD_{50} 4.0mg/kg diet).

Group 3: positive control 2 (basal diet + 1/50 of SA LD_{50} + 8.0mg SA/kg diet).

Group 4: positive control 3 (basal diet + 1/25 of SA LD_{50} 16.0mg SA/kg diet).

Group 5: treatment 1 (basal diet + 1/100 SA LD_{50} + 8.0% high esterified pectin [HEP]).

Group 6: treatment 2 (basal diet + 1/50 SA LD_{50} + 8.0% HEP).

Group 7: treatment 3 (basal diet + 1/25 SA LD_{50}) + 8.0% HEP.

Group 8: treatment 4 (basal diet + 1/100 SA LD_{50} + 8.0% low esterified pectin [LEP]).

Group 9: treatment 5 (basal diet + 1/50 SA LD_{50} + 8.0% LEP).

Group 10: treatment 6 (basal diet + 1/25 SA LD_{50} + 8.0% LEP).

After the period of treatment 30 days, all animals groups were sacrificed under light chloroform anesthesia by cervical dislocation which is one of recommended physical method of euthanasia by the institutional animal, Ethics committee of India (Mukherjee *et al.,* 2006).

3.1. Collections of serum and tissues:

3.1.1. Blood sampling and serum preparation:

Blood samples were collected by orbital venous Plexuses a heparinized fine capillary glass tube into a dry clean glass tubes to prepare serums.

Blood was left for 15 min. at room temperature, then the tubes was centrifuged for 10 min. at 1500 rpm and clean supernatant serum was kept at -20ºC till analysis.

3.1.2. Organs preparation

Liver, kidney and brain tissues were removed, rinsed in cold saline, blotted, weighed and used for histological analysis and metal concentration measurements.

Half portion of tissues from each rat was kept in formaline 10% solution for histological analysis according to Yoon *et al.* (2001), another portion was kept at -20ºC for wet digestion and analysis of arsenic content (Nandi *et al.*, 2006)

4.1. Arsenic analysis

Organs (Kidney, brain and liver) arsenic levels were estimated according to the method of Parker *et al.* (1967). To accurately weight of tissue in 125 ml Erlenmeyer flask, add glass beads and 25ml deionized water, add 10 ml 1:2 (equal volumes) mixture of concentrated HNO_3 and $HClO_4$. Boil the sample until the solution is clear. Transfer the solution quantitatively to 100 ml volumetric flask. Dilute to volume with deionized water and mix well.

Aliquote of this were used to estimate arsenic by using inductivity coupled plasma (ICP, Optima 2000DV-perkin Elmer) at 193.7nm wavelength. The volumes were expressed in µg/g of wet tissue.

5.1. Serum biochemical parameters

Activity of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was estimated following the method of Reitman and Frankel (1957), and they were calculated extrapolating the optical density (O.D.) in the relative standard curve that was prepared using different concentration of respective enzymes and the values were expressed as unit/ml of serum.

Also, the serum samples were inspected for protein quantitatively using biuret method according to the technique described by (Gornal *et al.,* 1949). Albumin was determined colorimetrically according to the method of Doumas *et al.* (1977). Creatinine was measured spectrophotometrically using alkaline picrate (Larsen, 1972). Lipid peroxides was estimated following the method of Ohkawa, *et al.* (1979). Finally, urea, alkaline phosphatase (ALP) and uric acid were determined according to the methods of Fawcett and Scott (1960), Belfielf and Goldberg, (1971) and Barham and Trinder, (1972), respectively.

6.1. Measurement of galactouronic acid content and degree of esterification

6.1.1. Measurement of galactouronic acid

The pectin content of high and low esterified pectins were determined as galactouronic acid by high performance liquid chromatography as described by Hicks *et al.* (1985) with modification

6.1.1.1. Galactouronic acid extraction.

200 mg of low and high esterified pectin, dispersed in 1 ml of iced-cold 80% sulfuric acid, was allowed to set at 25 ºC for 18 hours. The sample was then diluted to 13 ml, sealed in a vail, and placed in a boiling water bath for 5 hours. The resulting dark solution was neutralized with solid calcium carbonate, filtered (0.2 µm) and injected into the chromatography.

6.1.1.2 Determination of galactouronic acid:

Galactouronic acid was identified by a Hewlett Packard HP 1050 High

performance liquid chromatography (HPLC) equipped with refractive index 1047 HP, Column compartment was set at 85 ºC, degaser and autosmpler. The chromatograph was fitted with Bio Rad HPX-87-C model (30cm× 7.8m mid.) Isocratic elution system was used by deionized water at the flow rate 0.8ml/min.

6.1.2. Measurement of degree of esterification:

Methoxyl content was determined by the measurement of methanol liberated on saponification of the pectin according to the method described by Speirs *et al.* (1980) as follow:

2 g of tested high and low pectins were homogenized with 150 ml water; the samples were saponified by the addition of 20 ml of 1M NaOH and allowed to stand for 30 min. at room temperature. The alkali was then neutralized by addition of an equivalent amount of 0.5M HCl. The resulting acid mixture was transferred to 250ml volumetric flask and made up to the volume with distilled water. The contents of the flask were centrifuged at 12000 rpm for 20 min. the supernatant was decanted off and retained until analysis methanol released by using gas chromatography (Knuth, *et al.,* 1984) and (litchman and Upton, 1972).

Gas chromatography condition

Analysis was performed on Hewlett-Packard Model 5890 gas chromatography equipped with flame ionization detector. The instrument was also equipped with HP-1 column (cross-linked methyl silicone) 30m × 0.53mm × 0.88µm film thickness.

Detector and injection port temperature was 250 ºC for each, nitrogen (15ml/min) was used as a carrier gas and hydrogen (15 ml/min and air 240ml/min was used for the flame operation. On-column injections of 1.0µl were used for all samples and standards. Columns were pre-conditioned with a nitrogen flow (15 ml/min) for 1 hr. at 30 ºC, then programmed at 4 ºC/min to 150 and held for 15 min.

The reactive area under the peaks obtained from the chromatogram may be used to calculate the methoxyl content of the sample using the following relationship.

$$\% \text{ (W/W) methoxy content} = \frac{1.211A_1}{A_2 \times W}$$

Where:

A_1 = area under the peak of sample.
A_2 = area under the peak of standard.
W = weight of sample (g).

From this:

Degree of esterification (DE) =

$$\frac{\% \text{ methoxyl content} \times 612}{\% \text{ galacouronic acid}}$$

7.1. Statistical analysis:

The standard analysis of variance procedure in completely randomized design was applied for the present data according to Gomez and Gomez (1984).

Least significant differences (LSD) and Duncan's tests were done to compare a pair of group means. The level of statistical significant was set at $p<0.05$.

RESULTS AND DISCUSSION

1. Pectin substrate.

The galactouronic acid concentration in high and low esterified pectin were 75.72 and 78.53%, respectively. The assay showed degree of esterification to be approximately 73.5 and 31% respectively in high and low esterified pectin respectively.

Effects of feeding different doses from sodium arsenite alone or in combination with high and low esterified pectin on body weight (g) and body weight gain % of rats.

The effect of different doses of SA in the presence of HEP and LEP on body weight and body weight gain % were tabulated in table (1). The initial body weight at the zero time showed insignificantly deference between all groups. On the other hand, at the end of the experiment, the body weight of all (+) control rats except G2 (fed with basal diet + 4.0mg sodium arsinite) were significantly lower than the (-) control rats. The obtained results were parallel with those obtained by Chavez *at al.* (2006) who reported that from the ninth day of exposure (10 mg sodium arsenite/kg bw/day) and beyond, significant reduction in water and food intake was observed in arsenite treated group as compared with the control rats and body weight of these animals were decreased vs. control in tenth day of exposure.

Table (1): effect of feeding different doses from sodium arsenite with or without high or low esterified pectin on body weight (g) and body weight gain percentage of rats.

Parameter / Group	Body weight (g)		
	Initial Zero time	Final After 30 days	Gain%
NC	148.6^{A} ±0.466	164.30^{A} ±1.049	15.69^{A} ±1.183
G2	148.20^{A} ±0.465	163.90^{A} ±1.28	15.73^{A} ±0.927
G3	148.60^{A} ±1.599	158.90^{BCD} ±1.577	10.37^{E} ±0.941
G4	148.20^{A} ±2.447	155.80^{D} ±2.958	7.61^{F} ±0.559
G5	148.70^{A} ±3.225	161.60^{AB} ±3.492	12.94^{BCD} ±1.556
G6	148.40^{A} ±1.549	159.80^{ABCD} ±1.405	11.38^{DE} ±0.743
G7	148.40^{A} ±4.006	156.80^{CD} ±3.224	8.43^{F} ±0. 1.107
G8	148.60^{A} ±2.136	162.81^{AB} ±2.291	14.26^{AB} ±0.467
G9	150.50^{A} ±7.342	162.30^{AB} ±3.190	13.79^{BC} ±1.388
G10	148.00^{A} ±1.551	160.40^{ABC} ±1.652	12.31^{CD} ±0.145
LCD	**5.351**	**4.055**	**1.688**

Group2-4 = Positive control
Group 5-7 = HEP + 4.0, 8.0 and 16mg SA/kg diet
Group 8-10 = LEP + 4.0, 8.0 and 16mg SA/kg diet
Value in each column which have the same letters are significantly different ($P<0.05$).
All results are expressed as ± standard deviation.

Table (1) also shows insignificantly different in body weight between groups received 4, 8 mg SA/Kg diet in the presence of 8% HEP and the (-) control (161.6 and 159.8g) vs. (164.3) respectively at the end of the experiment. The rats were fed with dose 16 mg SA /kg diet recorded significant decrease in body weight when compared with the (-) control. The corresponding value was 156.8 gm. on the other hand, all treatments with 4, 8 and 16 mg SA /kg diet in the presence of 8% LEM pectin recorded non significant differences compared with (-) control (162.81, 162.3 and 160.4 vs. 164.3g), respectively, at the end of the experiment. The properties of pectin such as rheological behavior, gelation, or their binding capacity are dependent on the degree of esterification as well as parameters such as molecular weight and distribution of free and methoxylated carboxyl group within the galactouronan chain (Dongowoski, *et al.,* 1997).

From the same table it was observed that there was a significant decrease ($P<0.05$) for all groups when compared with (-) control except (+) control group was fed with 4.0 mg/ kg diet (15.73 ± 1.28g) compared with (-) group (15.69 ± 1.183 g).

From the data presented in table (1) it could be clearly observed that the BWG% of all treatments with 4, 8 and 16 mg / kg diet in the presence of LEP were higher than the rats were fed with HEP. The corresponding values were (14.26, 13.79 and 12.1%, vs. 12.94, 11.38 and 8.43%), respectively. The highest mean value of BWG % was found in the (+) control group which treated with 4.0 mg SA/day alone, which recorded value of (15.73 g) vs. 14.26% in the group was fed with LEP in the same dose of SA. The present results were in the line with those of Castenmiller and West (1998) and Zanutto *et al.,* (2002) they reported that one of the effects of dietary fiber on lipid metabolism is centralized on its interaction with bile acids, resulting in an increased excretion of them, thus reducing the absorption of fat and fat soluble substances such as cholesterol. On the other hand, Chávez, *et al.* (2006) found that animals had decreased body weight when the rats were exposure with 10mg sodium arsenite / kg bw /day and beyond.

Effect of feeding different doses from sodium arsenite alone and in the presence of high or low esterified pectin on liver function activities in rats:

In this study, the AST and ALT were determined as indicator hepatocellular injury but the ALP was estimated as indicator of cholestatic injury Zavala *et al.,* (1998). From the data tabulated in table (2) it was observed that the (+) control groups had shown a significant increase in the mean value of ALT, AST and ALP with increasing the dose of sodium arsenite administrated, it was clearly observed that the worst treatment was 16 mg/kg diet sodium arsenite which the results were higher than the (-) control by about (198.3%, 77.1% and 102.17%) for ALT, AST and ALP respectively. On the other hand, feeding rats with high and low esterified pectin caused significant decrease ($P<0.05$) in mean values of AST, ALT and ALP, as compared with control (+) group at the end of the experiment. The best treatment that was (8% low esterified pectin + 4 mg sodium arsenite /kg diet) for AST and ALP which the results lower from the first control (+) group by about (11.12%) and 17.14%), respectively, while ALT activity in the group was fed on (8% low esterified pectin + 8 mg sodium arsenite) was lower than the second control (+) group by about 50.62%.

Table (2): Effect of feeding different doses from sodium arsenite alone and in the presence of high or low esterified pectin on liver function activity in rats

Parameter / Group	ALT (U/L)		AST (U/L)		ALP (IU/L)	
	Zero	End	Zero	End	Zero	End
NC	24.28^{H} ±0.38	24.08^{H} ±0.475	82.82^{H} ±1.35	82.44^{H} ±1.431	110.8^{J} ±1.56	110.5^{J} ±0.575
G2	24.05^{H} ±1.495	36.24^{F} ±1.223	82.45^{H} ±1.087	115.1^{E} ±1.56	110.5^{J} ±0.744	145.9^{F} ±1.404
G3	24.67^{H} ±1.020	48.44^{D} ±0.963	82.68^{H} ±1.173	126.90^{D} ±1.895	111.60^{J} ±0.196	170.70^{D} ±2.514
G4	24.18^{H} ±1.239	71.83^{A} ±0.912	82.88^{H} ±0.941	146.00^{B} ±1.219	110.20^{J} ±1.509	223.4^{A} ±2.154
G5	24.00^{H} ±0.781	29.38^{G} ±0.768	82.99^{H} ±0.436	108.10^{F} ±1.571	111.31^{J} ±1.722	141.21^{G} ±2.146
G6	24.30^{H} ±0.677	41.12^{E} ±1.015	82.46^{H} ±0.884	138.4^{C} ±1.431	110.11^{J} ±2.018	162.11^{E} ±1.952
G7	24.31^{H} ±0.69	64.82^{B} ±0.931	82.96^{H} ±1.962	168.30^{A} ±1.851	110.50^{J} ±1.227	211.10^{B} ±2.973
G8	24.46^{H} ±1.755	24.72^{H} ±0.807	82.63^{H} ±1.105	102.30^{G} ±1.357	111.50^{J} ±1.217	120.90^{I} ±1.458
G9	24.36^{H} ±1.096	23.92^{H} ±1.965	82.50^{H} ±1.622	110.30^{F} ±1.565	110.32^{J} ±1.011	135.71^{H} ±1.390
G10	23.88^{H} ±1.905	50.87^{C} ±1.712	83.03^{H} ±1.121	116.90^{E} ±1.833	111.10^{J} ±1.594	186.90^{C} ±1.486
LCD	1.946		2.344		2.754	

Group2-4 = Positive control
Group 5-7 = HEP + 4.0, 8.0 and 16mg SA/kg diet
Group 8-10 = LEP + 4.0, 8.0 and 16mg SA/kg diet
Value in each column which have the same letters are significantly different (P<0.05).
All results are expressed as ± standard deviation.

Table (2) show that there were a significant decrease P< 0.05 in AST, ALT and ALP for all treatments with 4.0, 8.0 and 16 mg/kg diet in the presence of low esterified pectin instead of high esterified pectin e.g. the ALT corresponding values were (24.72, 23.92 and 50.87 U/L) vs. (29.38, 41.12 and 64.82 U/L), respectively.

The present results were in the line with those of Li, *et al.* (2007) which reported that arsenite had obvious effect on serum levels of ALT and AST activities which were significantly raised so that the liver function become abnormal in the course of exposure time in the arsenic-treated group and become significantly higher than those in the control groups. On the other hand, high esterified pectins require large amounts of sugar and low pH for gel formation. While low- ester pectins form gel with or without sugar in the presence of divalent

cations (Kim, *et al.*, 1978). So that the quantity of metal bound to pectin is determined by the number of free carboxyl groups (Serguschentog *et al.*, 2007), this could be enhanced concentration on arsenite in feces of rats treated with low esterified pectin than high esterified pectin.

Effect of feeding different doses from sodium arsenite with or without high or low esterified pectin on uric acid, urea and creatinine in rat's serum.

Uric acid, urea nitrogen and creatinine are used as an estimation for glomerular filtration and renal function [(Ronald and Koretz, 1992) and (Finco, 1997)]

Data in table (3) showed that at the end of the experiment serum uric acid increased significantly P<0.05 in all positive control groups when compared with the negative group (6.21, 7.56 and 9.01) vs. (3.79) mg/dL respectively. In contrast, all treated groups fed with 8% LEP in the presence of 4.0, 8.0 and 16 mg/kg diet showed non significant differences in uric acid serum as compared with control (-) group. While, substituted LEP with HEP in the presence of the same previously dose of SA recorded significant increase as compared with negative control group. Moreover, the groups were fed with LEP with all the previously SA doses showed a significant decrease when compared with the same group fed with SA alone in control (+) group but, feeding rats with HEP instead of LEP with the same concentration of SA were revealed non significant differences, as compared with the same positive control group while these groups showed a significant increase when compared with the same groups fed with LEP with the same doses of SA. The corresponding values were (5.87, 7.04 and 8.59 mg/dl) vs. (4.19, 4.41 and 5.72 mg / dL) respectively. The lowest mean value of uric acid was found in the group which was treated with 4 mg / kg diet SA with 8% LEP which gave 4.19 mg/dL uric acid.

From the same table at the end of the experiment the control positive groups 1, 2 and 3 showed urea nitrogen serum about 20.63, 34.25 and 43.79 mg / dL), respectively which represent (146.83, 243.77 and 311.67%), respectively when compared with those of the control (-) group. Besides, all treated groups had significant increase in serum urea nitrogen except the group that was fed with 4.0mg sodium arsenite / kg diet with 8% low easterified pectin which gave non significant decrease (P<0.05) (13.46 ± 0.766 mg/dl) compared with the negative control group (14.05±0.454 mg/dl)

Table (3) also illustrated that the serum creatinine level for all treated groups was significantly increased as compared to control (-) group by gradually increasing the dose of sodium arsenite except the group that received 8% low easterified pectin with 4.0mg sodium arsenite / kg diet which recorded 1.31 times, compared with those of the control (-) group.

Serum uric acid, urea and creatinine levels were significantly increased in rats fed with sodium arsenite alone indicates initiation of renal infliction due to the persistent exposure to it, these results are in agreement with those reported by Nandi *et al.* (2006). On the other hand, renal function increased significantly by feeding rats with LEP instead of HEP which provides ion exchange activity of the free carboxyl groups, the more the number of free carboxyl groups in the molecule, the more intensive ion exchange capacities the pectin has. Therefore, pectin with the low degree of esterification helps to reduce the blood arsenic level and diminish its adverse effects. The present results were in the line with those of (El-Zoghby and Sitohy, 2001).

Table (3): Effect of feeding different doses from sodium arsenite alone and in the presence of high or low esterified pectin on uric acid, urea and creatinine in rat's serum.

Parameter / Group	Uric acid (mg/dL)		Urea (mg/dL)		Creatinine (mg/dL)	
	Zero	End	Zero	End	Zero	End
NC	3.33^{F} ±0.345	3.79^{DEF} ±0.232	14.06^{H} ±0.416	14.05^{H} ±0.454	0.427^{F} ±0.047	0.43^{F} ±0.032
G2	3.78^{DEF} ±0.220	6.21^{BC} ±0.340	14.16^{H} ±0.519	20.63^{F} ±0.832	0.45^{F} ±0.061	0.960^{D} ±0.085
G3	3.65^{EF} ±0.305	7.56^{AB} ±0.289	14.61^{H} ±0.553	34.25^{C} ±1.244	0.430^{F} ±0.026	1.35^{B} ±0.155
G4	3.88^{DEF} ±0.125	9.01^{A} ±0.108	13.93^{H} ±0.465	43.79^{A} ±1.502	0.44^{F} ±0.079	1.56^{A} ±0.155
G5	3.96^{DEF} ±0.243	5.87^{BCD} ±0.596	14.58^{H} ±0.545	16.35^{G} ±0.535	0.447^{F} ±0.057	0.743^{E} ±0.127
G6	3.86^{DEF} ±0.186	7.04^{B} ±0.229	14.03^{H} ±0.238	28.54^{D} ±0.509	0.427^{F} ±0.051	0.927^{D} ±0.060
G7	3.91^{DEF} ±0.269	8.59^{A} ±0.475	13.81^{H} ±0.341	36.73^{B} ±0.470	0.41^{F} ±0.044	1.433^{AB} ±0.110
G8	3.59^{EF} ±0.296	4.19^{CDEF} ±0.421	13.85^{H} ±0.498	13.46^{H} ±0.766	0.43^{F} ±0.059	0.560^{F} ±0.056
G9	3.49^{F} ±0.241	4.41^{CDEF} ±0.095	14.03^{H} ±0.721	21.74^{F} ±0.584	0.43^{F} ±0.040	0.760^{E} ±0.04
G10	3.72^{EF} ±0.250	5.72^{BCDE} ±0.358	13.38^{H} ±0.248	26.31^{E} ±0.900	0.41^{F} ±0.040	1.177^{C} ±0.166
LCD	1.819		1.140		0.1381	

Group2-4 = Positive control
Group 5-7 = HEP + 4.0, 8.0 and 16mg SA/kg diet
Group 8-10 = LEP + 4.0, 8.0 and 16mg SA/kg diet
value in each column which have the same letters are significantly different (P<0.05).
All results are expressed as ± standard deviation.

Effect of feeding different doses from sodium arsenite alone and with high or low esterified pectin on total protein albumin and lipid peroxide in rat's serum:

Total protein and albumin in serum were used as indicator of biosynthetic liver capacity.

Data in table (4) expressed that at the end of the experiment, serum total protein showed a gradually significant decrease P<0.05 for 1, 2 and 3 control (+) groups that read (5.36, 4.79 and 3.36 g/dl) as compared with the (-) control group that recorded 7.21 mg / dL. All treated groups with high esterified pectin had a significant increase (P<0.05) in mean values of serum total protein in the presence of 4.0, 8.0 and 16 mg sodium arsenite / kg diet compared with the positive control groups (1, 2 and 3). The corresponding values were (5.79, 5.42 and 4.1 g/dl) vs. (5.36, 4.79 and 3.36 g/dl), respectively. On the other hand, rats were fed with the previously doses of sodium arsenite in the presence of low esterified pectin had a significant increase in mean of total protein compared with positive

control groups. Also, from the other side, there was a significant decrease in the mean value of albumin for all treated groups with the previously sodium arsenite concentration and fed with high esterified pectin except the group was fed with 4.0 mg sodium arsenite which showed insignificant differences when compared with control (-) group. In contrast, substituting high esterified pectin with low esterified pectin with the same previously three concentrations of sodium arsenite exhibited non significantly increasing as compared with (-) control, the corresponding values were (4.1, 4.31 and 4.18 vs. 4.6 g/dl), respectively.

Gradually decreased of total protein and albumin serum indicated that the biosynthesis liver efficiency of them decreased by increasing the dose of SA fed to rats, These may be due to that arsenic-induced lipid peroxidation in liver could possibly result from an enhanced microsomal oxidative capacity induced by arsenic. Thus, elevated level of cytochrom would lead to high rates of free radical, which, in turn, would favor increased rate or lipid peroxidation (Ramos *et al.,* 1995).

Also, alteration of oxidative stress markers due to over use failure of antioxidant defense system secondary to reactive oxygen species production (Ramanathan *et al.,* 2002). After that, cell death is thought to take place by apoptosis (programmed cell death) and nicrosis (an uncontrolled cell death). On the other hand, the efficiency of LEP to increase both the total protein and albumin than HEP may be due to that LEP in rats previously fed arsenic helping to increase the amount of arsenic excreted with feces higher than HEP because the high esterified pectin is characterized by a major part of carboxyl group in galacturonan pattern to be occupied with methyl radicals preventing interaction with metal (Serguschenko, *et al.* 2007).

The lipid peroxide (malondialdehyde) or thiobarbituric acid (TBARs) concentration is an end product indicative of the degree of lipid peroxidation (Nandi *et al.,* 2005).

Table (4) also reveals that sodium arsenite treatments with the doses 4.0, 8.0 and 16 mg / kg diet within 30 days increased the serum lipid peroxide levels significantly, when compared with control negative group (95.22%, 186.31% and 383.6% increase), respectively. Feeding rats with 8% high esterified pectin in the presence of the doses of sodium arsenite significantly decreased lipid peroxide as compared with the control negative group that read (11.34, 15.49 and 25.92 n mol /ml vs. 6.28 n mol /ml), respectively. While rats fed with low esterified pectin in the presence of the same concentration of sodium arsenite were extremely lower than the (+) ones by 49.67, 54.88 and 61.64%, respectively. The lowest mean value of lipid peroxide was found in the group which was treated with 8% low esterified pectin + 4.0 mg sodium arsenite/ kg diet.

The increment of lipid peroxide serum levels in rats by increasing doses of sodium arsenite in control (+) group could be due to that the mechanism of acute toxicity if arsenic is related to its chemical form, oxidative stress, specific functional groups within enzymes, receptors or co-enzymes such as thiol or vicinal sulfhydryl have a major role in the activity of these molecules (Hultberg *et al.,* 2001).

Also, arsenic induces free radical generation (liu *et al.,* 2001). Moreover, lipid peroxidation is the basic cellular deteriorating process induced by arsenic oxidative stress (Gupta, *et al.,* 2005 and Halliwell, 1994). On the other hand, lipid peroxide decrement when treated rats with LEP instead of HEP with the degree

of esterification about 31% exerted highest arsenic binding activity with three concentrations used in the study. these could be due to that the quantity of metal bound in pectin is determined by number of free carboxyl group.

In contrast, high esterified pectin is characterized by a major part of carboxyl group galactouronan pattern to be occupied with methyl radicals preventing interaction with metal. Therefore, the lowest binding activity registered in animals treated with HEP (Serguschenko, *et al.*, 2007)

Table (4): Effect of feeding different doses from sodium arsenite alone and in the presence of high or low esterified pectin on total protein, albumin and lipid peroxide in rats.

Parameter / Group	Total protein g/dL		Albumin g/dL		Lipid peroxide n mol/ml	
	Zero	End	Zero	End	Zero	End
NC	7.11^{A} ±0.095	7.21^{A} ±0.381	4.47^{AB} ±0.217	4.6^{A} ±0.214	5.99^{G} ±0.321	6.28^{G} ±0.199
G2	7.07^{A} ±0.354	5.36^{D} ±0.240	4.22^{AB} ±0.262	3.86^{BC} ±0.137	5.55^{G} ±0.566	12.26^{E} ±1.100
G3	7.02^{A} ±0.221	4.79^{E} ±0.415	4.14^{AB} ±0.225	2.64^{D} ±0.372	6.51^{G} ±0.472	17.93^{C} ±0.703
G4	7.34^{A} ±0.166	3.36^{G} ±0.304	4.18^{AB} ±0.257	1.39^{E} ±0.246	6.28^{G} ±0.777	30.37^{A} ±0.901
G5	7.13^{A} ±0.535	5.79^{C} ±0.232	4.21^{AB} ±0.340	4.06^{AB} ±0.164	6.34^{G} ±0.981	11.34^{E} ±0.338
G6	7.00A ±0.189	5.42^{D} ±0.411	4.07^{AB} ±0.289	3.46^{C} ±0.223	6.29^{G} ±0.267	15.49^{D} ±0.497
G7	7.30^{A} ±0.425	4.10^{F} ±0.100	4.00^{ABC} ±0.443	1.73^{E} ±0.135	6.52^{G} ±0.181	25.92^{B} ±0.266
G8	7.24^{A} ±0.379	7.19^{A} ±0.276	4.06^{AB} ±0.424	4.10^{AB} ±0.15	6.68^{G} ±0.345	6.17^{G} ±0.174
G9	6.94^{A} ±0.264	6.37^{BC} ±0.342	3.94^{BC} ±0.556	4.31^{AB} ±0.568	6.56^{G} ±0.546	8.09^{F} ±0.181
G10	6.87^{AB} ±0.248	6.05^{C} ±0.300	4.14^{AB} ±0.31	4.18^{AB} ±0.333	6.31^{G} ±0.748	11.65^{E} ±0.517
LSD	0.519		0.524		0.9494	

Group2-4 = Positive control
Group 5-7 = HEP + 4.0, 8.0 and 16mg SA/kg diet
Group 8-10 = LEP + 4.0, 8.0 and 16mg SA/kg diet
value in each column which have the same letters are significantly different ($P<0.05$).
All results are expressed as ± standard deviation.

Concentration of arsenic retention in some organs and feces of rats fed daily with different concentrations of sodium arsenic with or without HEP or LEP.

At the end of experiment the data presented in table (5) illustrated that concentration of arsenic retention in some organs of all treatments are in the following sequence: Kidney > brain > liver e.g. in group (7) arsenic content of kidney, brain and liver were 3.081, 2.052 and 0.742 ppm respectively which could be attributable to the different bio-kinetic pattern of its distribution (Kannan *et al.,* 2001). Moreover, the concentration of arsenic in the same organ of rats fed with 8% HEP or LEP with any concentration of sodium arsenite recorded significant decrease as compared with the same control (+) group e.g. the concentration of arsenic in kidney in the group were fed with LEP or HEP with 4.0 mg SA/Kg diet as compared with control (+) control. The corresponding values were (0.532 and 0.852 vs. 1.911 ppm) respectively. These results are in agreement with those reported by Serguschenko, *et al.* (2007) who said that foodstuffs and supplements containing pectin were proposed for usage as agent binding ions of heavy metals, thus reducing its retention in the body and diminishing its toxic effect. Also, the efficiency of LEP on removal arsenic from tested organ were higher than HEP in the presence of the same doses sodium arsenite e.g. the concentration of arsenite in brain of group fed with HEP or LEP with 8 mg SA were 1.724 and 0.562 ppm respectively. These may be due to that the presence of free carboxyl group in the pectin molecule provides the ion-exchange activity. Thus, more the number of free carboxyl groups in the molecule, the more intensive ion-exchange capacities the pectin has. Therefore, pectin with the low degree of esterification exerts considerable high sorption activity (Manunza et al., 1998).

Data tabulated in table (5) showed that the arsenic content in feces significant increasing in all treatments fed with HEP or LEP as compared with control (+) group in the same dose of sodium arsenite e.g. concentration of arsenic in rats feces fed with HEP or LEP in the presence of 16.0 mg sodium arsenite significant increase when compared with control (+) group which recorded 6.717, 8.549 vs. 3.761 ppm, respectively. Moreover, concentration of arsenic in all group fed with LEP were higher than others were fed with HEP e.g. the group was fed with LEP with sodium arsenite about 8 mg/Kg diet could be eliminate higher quantity of arsenic in feces than the groups were fed with HEP which recorded (5.925 VS. 5.351 ppm) respectively. These results are in agreement with Serguschenko, *et al.,* (2007) who reported that low esterified pectin is more effective than high esterified pectine for removed metal from organ. Thus, the concentration of metal enhanced in feces of rats treated with LEP than other was treated with HEP.

Histopathological examination:

To confirm the results obtained in this study, we made a histopathological analysis:

1. Histopathological changes in Kidney (K) samples of rats were fed for 30 days on HEP or LEP in the presence of different concentrations of sodium arsenite.

Histopathological changes in Kidney (K) samples of rats fed for 30 days on different doses of sodium arsenite with or without HEP or LEP are presented in fig. (1), where, microscopically examination of kidney (K) samples of rats fed with basal diet (-) control revealed the normal histology of renal parenchyma (Group 1K). On the other side, Kidney of rat fed with different doses of sodium arsenite alone (4.0, 8.0 and 16 mg/kg diet (Groups 2K, 3K and 4K [+] control)

showed slight atrophy of glomerular tufts (arrows) in group 2K [control (+1) group], marked dilatation and congestion of renal blood vessels (arrows) is noticed in group 3K [control (+2) group], and vacuolation of epithelial lining renal tubules (small arrows) and endothelial lining glomerular tufts (large arrow) are showed in group 4K [control (+3) group], respectively. Examined sections of kidney of rats fed with HEP with 4.0 mg SA/kg diet showed apparent normal renal parenchyma (Group 5K). Moreover, increasing the dose of sodium arsenite to 8.0 and 16.0 mg / kg diet revealed dilatation and congestion of renal blood vessels and pylenosis of some nuclei, respectively (Groups 6K and 7K). Finally, apparent normal histology of renal parenchyma in kidney of rats fed with LEP in the presence of 4.0, 8.0 and 16.0mg SA / kg diet are presented in groups 8K, 9K and 10K.

2.Histopathological changes in brain (b) samples of rats fed for 30 days on HEP or LEP in the presence of different concentrations of sodium arsenite.

Histopathological changes in brain (b) samples of rats were fed for 30 days on in the presence of different concentrations of sodium arsenite with or without HEP or LEP are presented in fig. (2), where, brain samples of the control (-) (normal group) showed no histological changes as observed in group 1b, brain of rats from control (+) groups which fed with 4.0, 8.0 and 16 mg SA / Kg diet alone revealed cellular edema (arrows) in group 2b (control + 1), nicrosis of purkenzi cells of the cerebellum (arrows) in group 3b (control + 2) and marked

Table (5): concentration of arsenic retention in some organs and feces of rats were fed daily with different concentrations of sodium arsenite with or without HEP or LEP

Parameter / Group	Arsenic concentration ppm			
	Kidney	Brain	Liver	feces
NC	0.155^{J}*	0.075^{J}*	0.009^{J}*	0.003^{J}*
G2	$1.911^{E}\pm0.3126$	$1.591^{E}\pm0.0038$	$1.273^{C}\pm0.0055$	$2.156^{I}\pm0.01322$
G3	$5.171^{B}\pm0.0036$	$3.932^{B}\pm0.0032$	$1.674^{B}\pm0.0030$	$2.417^{G}\pm0.0082$
G4	$6.411^{A}\pm0.0046$	$4.754^{A}\pm0.0035$	$1.808^{A}\pm0.0040$	$3.761^{E}\pm0.02973$
G5	$0.852^{H}\pm0.0036$	$0.637^{G}\pm0.0058$	$0.368^{F}\pm0.0195$	$2.374^{F}\pm0.0104$
G6	$2.963^{D}\pm0.0035$	$1.724^{D}\pm0.0032$	$0.598^{E}\pm0.0060$	$5.351^{C}\pm0.0109$
G7	$3.081^{C}\pm0.0026$	$2.052^{C}\pm0.0046$	$0.742^{D}\pm0.0061$	$6.717^{A}\pm0.0214$
G8	$0.532^{I}\pm0.0040$	$0.317^{I}\pm0.0043$	$0.074^{I}\pm0.0061$	$3.125^{H}\pm0.0218$
G9	$0.913^{G}\pm0.0104$	$0.562^{H}\pm0.0053$	$0.155^{H}\pm0.0106$	$5.925^{D}\pm0.0219$
G10	$1.782^{F}\pm0.0026$	$0.877^{F}\pm0.0076$	$0.208^{G}\pm0.0032$	$8.549^{B}\pm0.0338$
LCD	**0.01715**	**0.01715**	**0.01715**	**0.005**

*Concentration as ppb
Group2-4 = Positive control
Group 5-7 = HEP + 4.0, 8.0 and 16mg SA/kg diet
Group 8-10 = LEP + 4.0, 8.0 and 16mg SA/kg diet
value in each column which have the same letters are significantly different ($P<0.05$).
All results are expressed as ± standard deviation.

dilatation and congestion of blood vessels (small arrow) associated with hemorrhage in virchow Rubin space (large arrow) in group 4b (control +3), respectively. Meanwhile, brain of rats fed with 8% HEP with the previously doses of sodium arsenite showed cellular edema in the presence of 4.0 mg SA / kg diet (arrow) in group 5b. While the groups were fed with 8.0 and 16.0mg SA/kg diet revealed neuronophagia (arrow) as shown in group 6b and 7b. Besides, brain of rats fed withLEP with 4.0, 8.0 and 16.0 mg SA/ kg diet exhibited slight edema of some cells (arrows) as noticed in group 8b, pylenosis of some neurons (arrows) as shown in group 9b and finally, neuronophyagia of some neurons (arrows) were presented in group 10b, respectively.

3- Histopathological changes in liver (L) samples of rats were fed for 30 days on HEP or LEP in the presence of different concentrations of sodium arsenite.

Histopathological changes in liver (L) samples of rats fed for 30 days on different doses of sodium arsenite with or without HEP or LEP in the presence of different concentrations of sodium arsenite are presented in fig. (3), where, the examined section of liver samples of the control (-) (normal group) showed no histopathological changes was observed in group 1L, however, liver of rats from control (+) groups which fed with 4.0, 8.0 and 16 mg SA / Kg diet alone showed hydropic degeneration of hepatocytes (small arrows) associated with focal leukocytic cells aggregation (large arrow) in group 2L (control + 1), clearance of the cytoplasm of hepatocytes (arrow) in group 3L (control + 2) and focal area of hepatic necrosis associated with heucocytic cell infiltration (arrow) in group 4L (control +3), respectively. Examined sections of liver samples of rats treated with 8% HEP in the presence of 4.0 and 8.0 mg SA/Kg diet reduce the histopathological changes to hydrobic degeneration of some hepatocytes (arrow) as shown in groups 5L and 6L, hydrobic degeneration of hepatocytes (small arrow) associated with focal mononuclear cell aggregation (large arrow) as noticed in group 7L. Also, the feeding rats with 8% LEP instead of HEP with 4.0mg SA/ kg diet recorded significant improvement in histopathological changes with slight hydropic degeneration of some hepatocytes (arrows) as noticed in group 8L when compared with the same (+) control group. Meanwhile, hydropic degeneration of some hepatocytes (arrow) presented in group 9L and congestion of centrol vein (arrow) in group 10L was noticed when the dose of sodium arsenite was increased to 8.0 and 16.0 mg SA/ kg diet, respectively.

Generally, speaking, the results of histopathological examination are in good agreement with the biochemical and chemical measurement of rat's serum constituents.

We recommended that low esterified pectin was higher than high esterified pectin in accelerated removal of arsenic with feces through decrease of its intestinal absorption, prevention of tits accumulation and amelioration of its toxicity especially when used with low concentration of arsenic. We advise people to consume vegetable and fruit rich in high and low esterified pectin, fresh or in their products such as Blenheim apricots, Montmorency cherries, Royal anne, Cherries, July Elberta peaches, Santa rosa plums, sugar beet and citrus which contain degree of esterification 69.4, 31.3, 62.1, 73.6,72.2, 47.2 and 76%, respectively (Gee *et al.,* 1958) and (Kalvons and Bennett, 1995).

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تأثير البكتين المنخفض والمرتفع الأستر على سمية الزرنيخ في الفئران

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الغرض الرئيسى من هذه الدراسه هو تقييم كفائة كل من البكتين المرتفع الأستر والمنخفض الأستر على امتصاص الزرنيخ وبقائه فى الأعضاء وامكانية ازالته منها فى ذكور الفئران الألبينو. حيث تم تقسيم خمسون من ذكور الفئران الألبينو إلى عشرة مجموعات تحتوى كل مجموعة على ٥ فئران . المجموعة الاولى تم تغذيتها على العليقة القياسية (كنترول سالب) المجموعة من (٢-٤) تم تغذيتها على عليقة قياسية مع تركيزات من زرنيخيت الصوديوم ٤- ٨- ١٦ ملليجرام/كجم عليقة يوميا كمجموعات مقارنة موجبة وهذه تمثل ١/١٠٠- ١/٥٠- ١/٢٥ من الجرعة التى تقتل نصف حيوانات التجارب وهذه المجموعة تمثل (كنترول موجب) ١- ٢- ٣. اما المجموعة من ٥-٧ فهى تمت تغذيتها على عليقة قياسية بالأضافة الى ٨% بكتين عالى الأستر (درجة الأستره ٧٣%) مع نفس التركيزات السابقة من زرنيخيت الصوديوم اما المجموعات من (٧-١٠) تم تغذيتها على عليقة قياسية مع ٨% بكتين منخفض الأستر (درجة الأسترة ٣١%) مع نفس التركيزات السابقة من الزرنيخ. الفئران التى تغذت على البكتين منخفض الأستر مع ٤ ملليجرام زرنيخيت الصوديوم هى التى اعطت تحسن فى القيم التغذويه وايضا فى التغيرات الهستولوجية. فى بداية التجربة (٣٠ يوم) انخفض متوسط قيم الأسبرتيت أمينو ترانسفيريز والألانين امينو ترانسفيريز والألكين فوسفاتيز وحمض اليوريك واليوريا والكرياتينين وبيروكسيدات الدهون فى المجموعات التى غذيت على البكتين المرتفع أو المنخفض الأستر فى وجود كل التركيزات السابقة من زرنيخيت الصوديوم عند مقارنتها بنفس الكنترول الموجب المقابل لها. وكانت أحسن النتائج فى الأختبارات السابقة ما عدا الألكلين فوسفتيز تم التحصل عليها عند تغذية الفئران ب ٨% بكتين منخفض الأستر مع ٤ ملليجرام زرنيخيت الصوديوم اما أحسن نتيجة بالنسبة للألكلين فوسفاتيز فكانت بأستخدام عليقة محتوية على ٨% بكتين منخفض الأستر فى ٨ ملليجرام زرنيخيت الصوديوم عند مقارنتهم الكنترول السالب وكانت نتائجهم مقابل الكنترول السالب كالتالى (٢٣,٩٢ مقابل ٢٤,٠٨ وحدة/ لتر) و (١٠٢,٣ مقابل ٨٢,٨٢ وحدة/ لتر) و (١٢٠,٩ مقابل ١١٠,٥ وحدة دولية/ لتر) و (٤,١٩ مقابل ٣,٧٩ ميكرومول/ لتر) و (١٣,٤٦ مقابل ١٤,٠٥ ملليجرام/ ديسيلتر) و (٠,٥٦ مقابل ٠,٤٣ ملليجرام/ ديسيلتر) و (٦,١٧ مقابل ٦,٢٨ نانومول/ مل) على التوالى . وعلى الجانب الأخر البروتين الكلى والألبيومين زادت فى كل المجاميع المغذاه على بكتين مرتفع الأستر والبكتين المنخفض الأستر فى وجود كل التركيزات السابقة من زرنيخيت الصوديوم . الحد الأعلى للتحسن فى البروتين الكلى والألبيومين تم التحصل عليه فى المجموعة التى تم تغذيتها على بكتين منخفض الأستر مع ٤ ملليجرام زرنيخيت الصوديوم بالمقارنة بالكنترول السالب والتى أعطت (٧,١٩ مقابل ٧,٢ جم/ ديسيلتر) و ٤,١ مقابل ٤,٦ جم/ ديسيلتر) على التوالى.

ومن الناحية الاخرى بقاء الزرنيخ فى الكلى والمخ والكبد قل فى جميع المجاميع التى تم تغذيتها على البكتين المرتفع أو المنخفض الأستر عند مقارنتة بالكنترول الموجب وكانت اعلى ازالة للزرنيخ من الأعضاء السابقة خاصة الكبد وذلك عند تغذية الفئران على بكتين منخفض الأستر مع ٤ ملليجرام زرنيخيت الصوديوم عند مقارنتها بالكنترول السالب حيث اعطت فى الأعضاء السابقة مقابل الكنترول (٠,٥٣٢ مقابل ٠,٠٠٠١٥٥ جزء فى المليون) و (٠,٣١٧ مقابل ٠,٠٠٠٠٧٥ جزء فى المليون) و (٠,٠٧٤ مقابل ٠,٠٠٠٠٠٩ جزء فى المليون) على التوالى . على العكس تركيز الزرنيخ المفرز فى براز الفئران زاد تدريجيا بزيادة تركيزات الزرنيخيت الموجودة فى العليقة. الحد الأقصى للزرنيخ المفرز فى براز الفئران كان فى المجموعة المغذاه على ٨% بكتين منخفض الأستر مع ١٦ ملليجرام زرنيخيت الصوديوم / كجم عليقة والذى كان ٨,٥٤٩ جزء فى المليون .

الاختبارات الهستولوجية كانت متوافقة مع القياسات البيوكيميائية ويمكننا ان نوصى وفقا لتلك النتائج ان البكتين المنخفض الميثوكسيل كمادة فعالة ورابطه للزرنيخ يجب ادخالها من ضمن الغذاء الذى يستهلكه الأنسان يوميا وذلك لتقليل مخاطر سمية الزرنيخ الملوث للغذاء والماء .