



EFFECT OF ADMINISTRATION OF SODIUM SELENITE ON BIOCHEMICAL AND HISTOMORPHOLOGY OF THE LIVER IN WISTAR RATS (*RATTUS NORVEGICUS*)

- A.S. Muhammad** Human Anatomy Department,
Faculty of Basic Medical Sciences,
Federal University Dutse, Jigawa, Nigeria
- D.U. Jamil** Human Physiology Department,
Faculty of Basic Medical Sciences,
Federal University, Dutse, Jigawa, Nigeria
- M.I. Umar** Human Anatomy Department,
Faculty of Basic Medical Sciences,
Bayero University Kano, Nigeria
- Ibrahim Ahmad Atiku** Human Anatomy Department,
Faculty of Basic Medical Sciences,
Federal University Dutse, Jigawa, Nigeria
- Ibrahim Bukar Mai Siyama** Human Anatomy Department,
Faculty of Basic Medical Sciences,
Federal University Dutse, Jigawa, Nigeria
- H.O Umar** Human Anatomy Department,
Faculty of Basic Medical Sciences,
Federal University Dutse, Jigawa, Nigeria
- T.W. Jacks** Human Anatomy Department,
College of Medicine,
University of Maiduguri, Nigeria
-
-



Abstract

Liver being one the major site of metabolism, continuously get exposed to xenobiotics which can alter many important metabolic functions. Distortion of metabolic functions can lead to various form of hepatic injury making the liver vulnerable and implicated in so many health problems. This research was aimed at studying the effect of different doses of sodium selenite on some liver function markers using Albino Wistar rats. Twenty Albino Wistar Rats were randomized into four Groups (Group A, B, C and D) of 5 rats each, group A receiving 1ml/kg body weight of normal saline, B, C and D receiving 5mg/kg, 10mg/kg and 15mg/kg body weight of sodium selenite (Na_2Se) treatment respectively given orally per day. At the end of the sixth week, the animals were sacrificed; Liver enzymes and some serum electrolytes, serum cholesterol, serum albumin and serum protein were quantified. The result indicates significant ($P < 0.05$) increased in plasma levels of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), while the Sodium Na^+ , Potassium K^+ , Chlorine Cl^- concentrations were significantly ($P < 0.05$) decreased across the entire treatment group receiving sodium selenite. Microscopic examination of liver showed slight abnormality in the cytoarchitecture of the liver when the treatment groups (especially group D) was compared with the control. Therefore, the results of this study suggest that hepatic damage induced by sodium selenite could be determined by the amount of the sodium selenite consumed.

Keywords: Sodium selenite, Serum Electrolytes, liver function, Liver enzymes, liver histology

INTRODUCTION

The liver regulates many important metabolic functions, so the hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). Chronic liver injury leads to a progressive wound healing response that eventually results in liver fibrosis characterized by both quantity and quality alteration of hepatic extracellular matrix, ECM (Liu *et al.*, 2006). Liver is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. Toxins absorb from the intestinal tract gain access first to the liver resulting in a variety of liver ailment. Thus, liver diseases remain one of the serious health problems. Liver damage ranges from acute hepatitis to hepatocellular carcinoma, through apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression and regeneration (Loguercio and Federico, 2003). Loguercio and Federico (2003) stated that all processes that involve hepatocyte, Kupffer, stellate and endothelial cells which induce liver disease are related to the crucial role of reactive oxygen and nitrogen species. Oxidative stress contributes a decisive generating factor in the pathogenesis of acute and chronic liver diseases (Tanikawa and Torimura, 2006; Cesaratto *et al.*, 2004; Tuma, 2002). The main sources of free radicals are represented by hepatocyte mitochondria and cytochrome P450 enzymes, by endotoxin-activated macrophages (Kupffer cells) and by neutrophils. After liver cell injury, recruitment of leukocytes will take place (Marra, 1999). Leukocytes together with kupffer cells will produce compounds that modulate stellate cell behavior. Nitric oxide (NO) and inflammatory



cytokines, such as tumor necrosis factor β (with stimulatory ability on the stellate cell for collagen synthesis) will be produced by monocytes and macrophages. In addition, Kupffer cells can stimulate matrix synthesis by stellate cells through the actions of transforming growth factor β (TGF- β) and reactive oxygen species (ROS) (Stalnikowitz & Weissbrod, 2003; Granger & Kubus, 1994).

The most famous individual markers for assessment of liver fibrosis are: liver function tests (Aspartate Aminotransferase: AST and Alanine Aminotransferase: ALT) that reflect hepatocyte damage, bilirubin and alkaline phosphatase that show biliary obstruction and albumin and prothrombin time (PT) that reveal biosynthetic function of liver. These simplified enzymes may reflect the extent of hepatocellular necrosis. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) play an important role in amino acid metabolism by catalyzing reversible transfer of the α -amino group. Transaminases are expressed in varying concentrations in organs and physiologically detectable in the serum. While high activities of ALT are present in the periportal region of the liver, AST is found in high concentrations in numerous other organs. In general, serum activities of transaminases are increased in various liver diseases, for example hepatitis, cirrhosis, infections, carcinoma, or alcohol abuse. Serum activities of aminotransferases, however, are only of limited prognostic value and do not reflect the extent of liver cell necrosis appropriately (Reichling and Kaplan, 1988). Alkaline phosphatase is involved in the hydrolysis of organic phosphate esters and, though not exclusively expressed in the liver, used as a marker of cholestasis (Kaplan, 1972). Serum activity of alkaline phosphatase is elevated in acute or chronic hepatitis, cirrhosis and intrahepatic or extrahepatic malignomas (Reichling and Kaplan, 1988). Alkaline phosphatase is also an enzyme useful for follow-up of liver diseases associated with cholestasis, that is, occlusive jaundice.

Selenium (Se) as an essential dietary trace element, plays an important role in a number of biological processes in both humans and other species (Fraga, 2005), its deficiency can induce some pathological conditions, such as cancer, coronary heart disease, and liver necrosis (Tang *et al.*, 2012; Ognjanovic *et al.*, 2008; Soudani *et al.*, 2011). Studies have shown that selenium and zinc efficacy on immune system, increase response to influenza and HBV vaccine (Janbakhsh *et al.*, 2013). Sodium selenite has also been observed to decrease levels of lipid peroxidation (LPO) and NOPs (nitric oxide products) and increase activities of superoxide dismutase, GR (glutathione reductase), and GPX (glutathione peroxidase) in heart of a diabetes-induced rats (Ayaz and Turan, 2006). At low dose of about 1mg/kg Na₂Se, have an anti diabetic complimentary treatment (Hassan and Ali, 2015), neuro protection in cerebral ischemia and improves antioxidant capacity in patients with coronary artery disease (Ahmad *et al.*, 2011; Schnabel *et al.*, 2008).

Selenium forms the prosthetic group of some critical selenocysteine containing enzymes, such as glutathione peroxidase, iodothyronine 5'-deiodinase, and thioredoxin reductase



(Stadtman, 1996). Sodium selenite is protective agent against a number of toxicants. At low dose of about 0.25mg/kg, sodium selenite highly protected rats from mercuric chloride toxicity due to it high activity of antioxidant enzymes stimulation (Youcef *et al.*, 2013) which in turns reduced liver damage. Selenium (Se) at supranutritional levels can enhance the activity of glutathione S-transferase (GST), whose gene is a target of nuclear factor erythroid-2 related factor 2 (Nrf2) which transiently increases hepatic TrxR1 activity (Zhang, *et al.*, 2008). Other studies reported that supranutritional Selenium had no such effect on hepatic TrxR1 activity. However, its excessive intake could cause potential toxic effect (Combs and Gray, 1998). In view of this discrepancy, the present research investigates the hepatotoxic effect of excessive and prolong intake of selenium using animal model (Albino Wistar rats).

MATERIALS AND METHODS

Animals

Albino Wistar rats (180-220g) were procured from Department of pharmacology and physiology University of Maiduguri, Nigeria, and used throughout the study. They were housed in microlon boxes and acclimatized for two weeks in a controlled environment (temperature 25±2 °C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*.

Experimental protocol

Twenty wistar rats weighing about 180-220g each were randomly divided into four groups of 5 animals each. The first group (A) which is the control received nothing but 1ml/kg body weight of normal saline, Group (B) received 5mg/kg, Group (C) received 10mg/kg and Group (D) received 15mg/kg body weight of Na₂Se (from Sigma-Aldrich (St. Louis, MO, USA) respectively given orally and daily for six weeks using a modified method of Youcef *et al.*, (2013). At end of the sixth week, the animals were sacrificed by cervical dislocation. Liver function biochemical enzymes, serum electrolytes variables, serum cholesterol, serum albumin, serum protein and liver histopathology were assessed.

Serum Collection

Blood samples of the animals in all of the groups were collected through orbital venous plexus (Timm, 1979) method approved by IACUC, (2011), placed in lithium heparinized (LH) sample tubes and centrifuged at 4000 revolutions per minute for 15 minutes after which the serum was decanted in plain sample bottles for analysis.

Liver Enzymes Analyses

Serum analysis for the presence of liver cell enzymes was thereafter done. Biochemical parameters i.e., aspartate amino transferase (AST) alanine amino transferase (ALT), and alkaline phosphatase (ALP) were measured according to the reported methods of Atef M. Al-Attar, 2012).



Protein Quantification

Protein was measured by the method of Lowry, 1951 and Bradford (1976), using bovine serum albumin as the standard.

Serum Electrolytes Assay

Sodium and Potassium were determined by flame photometry method at 590nm and 770nm wavelength respectively, bicarbonate was determined by method of titration, while Serum chloride was estimated by the method of Schales and Schales (1941).

Estimation of Serum Cholesterol and Albumin

Serum Cholesterol and Albumin levels were estimated using spectrophotometer by the methods of Zlatkis *et al.*, (1953) and Rodkey, (1965) respectively.

Histopathological Studies

The livers of all the rats were fixed in 10% formalin and processed by the usual method for paraffin embedding at histopathology Department, University of Maiduguri, Nigeria. Section of 4-5 μm thickness by microtome was taken, stained with hematoxylin and eosin stain for histopathological examination through light microscope (Banchroft *et al.*, 1996).

Statistical Analysis

Student t-test and one-way analysis of variance (ANOVA) were used to analyze the data. The results were expressed as mean \pm standard deviation (SD). The difference of the means was considered significant at $*=P<0.05$ and $P^{**}=P<0.01$.

RESULTS

Behavioral Observation

Administration of Na_2Se induces some behavioral response such as weakness and withdrawn responses when compare with the control. The animal slept longer than the control and the degree of weakness was reduce before the end of the research indicating that the animals had become more tolerant.

3.2 Biochemical Analysis

Table 1: Effect of Administration of different concentration of Na_2Se (mg) on Liver Enzymes in Rats

Liver Enzymes (IUL)	Group A(control)	Group B (5mg/kg)	Group C (10mg/kg)	Group (15mg/kg)
AST	70.00 \pm 7.12	71.00 \pm 5.90*	74.17 \pm 0.12*	65.50 \pm 8.87*
ALT	38.75 \pm 6.60	37.83 \pm 7.22	46.50 \pm 8.60*	42.33 \pm 8.21*
ALP	109.00 \pm 34.03	122.83 \pm 34.28*	143.17 \pm 36.93*	136.70 \pm 30.34*

Mean \pm SD*= $P<0.05$, P**= $P<0.01$

Na_2Se increases serum level of aspartate amino transferase (AST) in group B and C while in group D a decrease was observed but not significant when compared with control. Alanine



amino transferase (ALT), and alkaline phosphatase (ALP) level was also increasing across the group significantly.

Table 2: Effect of Administration of different concentration of Na₂Se (mg) on Serum Electrolyte in Rats

Serum Electrolytes (m/mol/L)	Group A(control)	Group B (5mg/kg)	Group C (10mg/kg)	Group D (15mg/kg)
Na ⁺	140±0.1	13.17±1.63	134.33±2.34*	65.5±8.87*
K ⁺	6.4±0.27	5.8±0.91	5.83±0.68	5.6±0.46**
Cl ⁻	105±1.16	101.33±2.07*	101±2.76*	100.53±3.45*
HCO ₃ ⁻	21±0.82	19.5±0.8**	17.33±2.16**	19.00±2.00

Mean±SD*=P<0.05, P**=P<0.01

This Table shows that Na₂Se caused a reduction in the serum values of the electrolytes (Na⁺, K⁺, Cl⁻, HCO₃⁻) at different dosage levels. However, the reduction was only statistically significant (P< 0.05-0.01) at C and D for Na⁺, at D for K⁺, B, C, and D for Cl⁻, while B and C for HCO₃⁻.

Table 3: Effect of Administration of different concentration of Na₂Se(mg) on Biochemical Variables in Rats

Biochemical variable	Group A(control)	Group B (5mg/kg)	Group C (10mg/kg)	Group D (15mg/kg)
Total Protein (g/l)	78.00±2.83	74.17±6.15	75.17±5.38	65.50±8.87*
Albumin (g/l)	40.25±3.78	34.67±3.88*	36.00±1.10	36.33±2.66
Cholesterol (mmol/L)	2.05±0.10	2.05±0.54	2.07±0.31	2.47±0.42

Mean±SD*=P<0.05, P**=P<0.01

There was slight decrease across the group for serum total protein and albumin. The statistical significance was only observed at group D for Total Protein, at group B for Albumin. There was no observable difference observed for cholesterol across the group except the decrease observed at D which was not significant.

Histological Studies

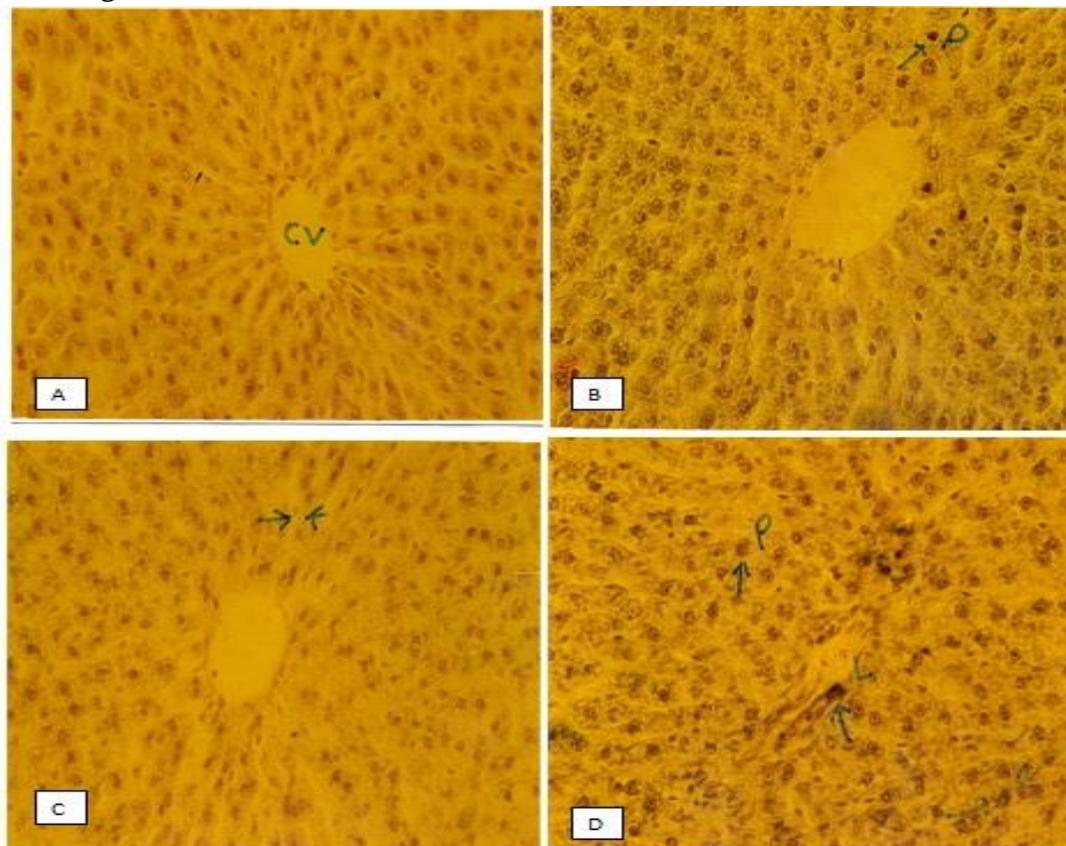


Figure 1: H&E staining of Liver Tissue (Mgx100)

Group A (Control): control group without treatment showing normal histology, the central vein (CV) is well defined with sinusoid radiating normally with the hepatocytes forming anastomosing plates that runs radially from the central vein CV. The cytoplasm of the hepatocytes shows granular appearance with spherical nuclei that are well defined and centrally located.

Group B (5mg/kg body weight of Na₂Se): This group shows some feature related to the control, only that there were very few pyknotic nuclei (P) observed in some hepatocytes.

Group C (10mg/kg body weight of Na₂Se): Mild degeneration was observed in the hepatocytes. The hepatocytes show pyknotic nuclei (P) and mild congestion of the sinusoids(S) toward the periphery.

Group D (15mg/kg body weight of Na₂Se): This group shows necrosis of the hepatocytes (N) and congested sinusoid (S), the pyknotic nuclei were more pronounced.

DISCUSSION

The present investigation revealed that high dose of Sodium selenite causes significant increase in AST, ALT and ALP activities (table 1), and significant decrease in the serum level of Na, K, Cl and HCO₃ (table 2), albumin and total protein (table 3) also reduced significantly. The principal toxic effects of prolong use of high dose selenium might involve



interaction with a number of cellular processes, including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative stress as might be suggested from similar studies involving mercury (Stacey *et al.*, 1982).

Significant decrease in serum protein and albumin levels was recorded. The decreased in the protein concentration of Na₂Se treated rats might be due to changes in protein synthesis and/or metabolism as result of high dose sodium selenite. This oppose the findings of Franziska *et al.*, (2015) that revealed increases colonic selenoprotein expression in all high dose selenium-supplementation even though their area of concentration is the colon but our expectation was to be similar as colon and liver represent a very important part of the gastrointestinal tract. However, evidence from Agustin *et al.*, (2014), indicated that infected liver lowered serum levels of proteins due to disrupted architecture and cells of the liver (figure 1) by diminishing the hepatic ability to synthesize proteins (Agustin *et al.*, 2014).

Na₂Se also caused a reduction in the serum values of the electrolytes (Na⁺, K⁺, Cl⁻, HCO₃⁻) (table 2) at different dosage levels. This observation was in agreement with work of Baldus *et al.*, (1964); Anjum *et al.*, (1983), that reported that Impairment in electrolyte metabolism (i.e Hypokalaemia, Hyponatremia, may be associated with the severity of liver disease, although, Anjum *et al.*, (1983), further stated that Hypokalaemia and low urinary sodium appear (as seen in table 2) to be bad prognostic findings in cirrhosis (Anjum *et al.*, 1983).

This toxicity level elicited by sodium selenite (Na₂Se) might be due to low activity of antioxidant enzymes as a result of complexes formed by the high doses of the compound Na₂Se in the liver which might cause a decreasing content of antioxidant like GSH, and the liver is hypothesized to be highly susceptible to oxidative stress. This is in agreement with the work of Combs and Gray, (1998) that reported that selenite could cause potential toxic effect and also Xinghua *et al.*, (2017) report that sodium selenite may slow down the growth of rats and lead to organic damage to some extent.

All these possible mechanisms of sodium selenite toxicity may lead to the formation of reactive oxygen species (ROS), may also lead to membrane biochemical and functional alterations and thus induced liver cell damage as evidenced by significant elevation in serum level of AST, ALT and ALP activities.

The result of the current study is in agreement with the work of Zhang *et al.*, (2008) that explain that high-dose sodium selenite indeed can enhance hepatic thioredoxin reductase 1 TrxR1 activity which in turn enhance the activity of glutathione S-transferase (GST). These finding correlates with liver Injury observed in carbon tetrachloride (CCl₄) toxicity (Jamil *et al.*, 2015).

This increase liver enzymes observed may also be due to cellular necrosis of hepatocytes as evidenced by the results of histopathology of the liver at C and D treatment groups, which



causes increase in the permeability of cell resulting to release of transaminases and ALP in blood stream as explained by Franziska *et al.*, (2015), that a selenite supplementation during acute colitis has no health benefits but may even aggravate the course of Inflammatory bowel disease (IBD)(Colitis). This confirms that sodium selenite intoxication could alters liver cytoarchitecture as revealed by the result of our histological findings.

It was observed that sodium selenite when given at high dose increases the transaminases, increase ALP activities, decreases total protein and albumin levels in serum. However, it expected that high dose Na_2Se will elevated the level of GSH-Px in serum thereby providing hepatoprotection through enhancing the antioxidant ability which in turn reduces the liver enzyme activities. However, but this result contradicts findings of the previous literatures.

CONCLUSION

Prolong administration of sodium selenite could cause behavioral changes, weight gain and hepatocyte injury in rats. The results of this study suggest that hepatic damage induced by sodium selenite could be determined by the amount of the sodium selenite consumed.

ACKNOWLEDGEMENT

The Authors wishes to acknowledge the contribution of the academic and the non-academic staff of Department of Anatomy, University of Maiduguri, Nigeria throughout the course of the study.



REFERENCES

- Agustín Albillos, Margaret Lario, Melchor Álvarez-Mon, (2014) Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance. *Journal of Hepatology* vol. 61 j 1385–1396.
- Ahmad A, Khan MM, Ishrat T, Khan MB, Khuwaja G, Raza SS, et al. (2011). Synergistic effect of selenium and melatonin on neuroprotection in cerebral ischemia in rats. *Biol Trace Elem Res.*139(1):81–96.
- Anjum Shahid, Hurna Quresbi, Fatima Nizami, Sarwar J. Zuberi, (1983) Electrolytes in Liver Disease-A Preliminary Study. *JPMA* 33: 289.
- Atef M. Al-Attar, (2012). Attenuating Effect of Ginkgo biloba Leaves Extract on Liver Fibrosis Induced by Thioacetamide in Mice, *Journal of Biomedicine and Biotechnology* Volume 2012: 1- 9.
- Ayaz M, and Turan B. (2006). Selenium prevents diabetes-induced alterations in [Zn²⁺]i and metallothionein level of rat heart via restoration of cell redox cycle. *Am J Physiol Heart Circ Physiol.* 290(3):H1071–80.
- Baldus, W.P., Feichter, R.N., Summerskill, W.HJ., Hunt, J.C. and Wakim, K.G. (1964) The kidney in cirrhosis. II. Disorders of renal function. *Ann. Intern. Med.*, 60:366.
- Banchroft, J., Stevens, A. and Turner, D., (1996). *Theory and Practice of Histological Techniques*, fourth ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
- Bradford, M.A. (1976) Rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 72, 248-54.
- Cesaratto, L., Vascotto, C., Calligaris, S., Tell, G. (2004). The importance of redox state in liver damage. *Ann. Hepatol.*, 3: 86-92.
- Combs, G.F. and Gray, W.P. (1998) Chemopreventive agents: selenium. *Pharmacol Ther.* 79, 179-192.
- Fraga CG. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med.* 26(4-5):235–44.
- Franziska Hiller, Lisa Oldorff, Karolin Besselt and Anna Patricia Kipp (2015). Differential Acute Effects of Selenomethionine and Sodium Selenite on the Severity of Colitis *Nutrients*, 7, 2687-2706; doi:10.3390/nu7042687.
- Ghaffari T, Nouri M, Irannejad E, Rashidi MR. (2011). Effect of vitamin e and selenium supplement on paraoxonase-1 activity, oxidized low density lipoprotein and antioxidant defense in diabetic rats. *Bioimpacts.* 1(2):121–8.
- Granger DN, Kubes P. (1994). The microcirculation and inflammation modulation of leukocyte- endothelial cell adhesion. *J Leukocyte Biol.*, 55, 662–675.
- Hassan Ahmadv and, Ali Khosrowbeygi (2015). Effect of Sodium Selenite on Lipid Peroxidation and Glutathione in Alloxan Induced Diabetic Rats. *Zahedan J Res Med Sci.* 17(3): e24471.
- Jamil Dauda Usman, Fasanmade A.A and Nwenfulu Kester. Effects of silymarin on reproductive variables in male wistar rats with carbon tetrachloride (ccl4) - induced



- liver fibrosis. *The American Journal of Innovative Research and Applied Sciences*. 2015; 1(6):214-221.
- Janbakhsh A, Mansouri F, Vaziri S, Sayad B, Afsharian M, Rahimi M, et al. (2013). Effect of selenium on immune response against hepatitis B vaccine with accelerated method in insulin-dependent diabetes mellitus patients. *Caspian J Intern Med*. 4(1):603-6.
- Kaplan MM. (1972). Alkaline phosphatase. *Gastroenterology*, 62:452- 468.
- Liu S. Q., J. P. Yu, H. L. Chen, H. S. Luo, S. M. Chen, and H. G. Yu, (2006). "Therapeutic effects and molecular mechanisms of *Ginkgo biloba* Extract on liver fibrosis in rats," *The American Journal of Chinese Medicine*, vol. 34, no. 1, pp. 99-114.
- Loguercio, C. and Federico, A., (2003). Oxidative stress in viral and alcoholic hepatitis. *Free Radical Biol. Med.*, 34, 1-10.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the folin phenol reagent. *The J Biol Chem.*; 193: 265-275.
- Marra F. (1999). Hepatic stellate cells and the regulation of liver inflammation. *J Hepatol*, 31, 1120-1130.
- Ognjanovic B.I, Markovic S.D, Pavlovic SZ, Zikic RV, Stajn AS, Saicic ZS. (2008). Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. *Physiol Res*. 57(3):403-11.
- Reichling JJ and Kaplan MM. (1988). Clinical use of serum enzymes in liver disease. *Dig Dis Sci.*, 33:1601-1614.
- Rodkey F. Lee, (1965). Direct spectrophotometric determination of albumin in human, *Clinical Chemistry*, Vol 2 No 4.
- Schales, O. and Schales, S.S. (1941) cited by Varley, H. Practical clinical biochemistry. 4th ed. Heinemann, London, 1967.
- Schnabel R, Lubos E, Messow CM, Sinning CR, Zeller T, Wild PS, et al. (2008). Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease. The Selenium Therapy in Coronary Artery Disease Patients (SETCAP) Study. *Am Heart J*. 156(6):1201 e1-11.
- Soudani N, Troudi A, Bouaziz H, Ben Amara I, Boudawara T, Zeghal N. (2011). Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. *Ecotoxicol Environ Saf*. 74(3):513-20.
- Stacey, N.H., Kappus, H. (1982) Cellular toxicity and lipid peroxidation in response to mercury. *Toxicol and App Pharmacol*. 63(1), 29-35.
- Stadtman, T.C. (1996). Selenocysteine. *Annu Rev Biochem*. 65, 83-100.
- Stalnikowitz DK and Weissbrod AB. (2003). Liver fibrosis and inflammation. A review. *Annals of Hepatology*, 2, 4, 159-163.
- Tang J, Tan W, Zhu Y, Wang L, Zhai L. (2012). Effect of selenium on the protection of myocardial cells from injuries induced by overloaded reactive oxygen species, and on the expression of actin in myocardial cells. *Wei Sheng Yan Jiu*. 41(1):2-5.
- Tanikawa, K. and Torimura, T. (2006). Studies on oxidative stress in liver diseases: Important future trends in liver research. *Med. Mol. Morphol.*, 39:22-27.



- Timm, K.I. (1979). *Orbital Venus Anatomy of the rat*. Laboratory Animal Science. 29:636-638.
- Tuma, D.J. (2002) Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic. Biol. Med.*, 32, 303-308.
- Wolf, P., (1999). Biochemical diagnosis of liver diseases. *Ind. J. Clin. Biochem.*, 14:59- 90.
- Xinghua Wang, Yukun Yang, Hening Zhang, and Ju Liu. (2017). Safety Assessment and Comparison of Sodium Selenite and Bioselenium Obtained from Yeast in MiceBioMed Research International Volume 2017, Article ID 3980972, <https://doi.org/10.1155/2017/3980972>
- Youcef Necib, Ahlem Bahi, Sakina Zerizer, Cherif Abdennour, and Mohamed Salah Boulakoud (2013). Hepatoprotective Role of Sodium Selenite Against Oxidative Damage Induced by Mercuric Chloride in Rat Albinos Wistar *Journal of Stress Physiology & Biochemistry*, Vol. 9 No. 4, pp. 230-240 ISSN 1997-083.
- Zhang J, (2008). Further insight into the impact of sodium selenite on selenoenzymes: high-dose selenite enhances hepatic thioredoxin reductase 1 activity as a consequence of liver injury. *Toxicology Letter*.
- Zlatkis A, Zak B, Boyle AJ. (1953) A method for the determination of serum cholesterol. *J Lab Clin Med.*; 41: 486-490.