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Evaluation of Biochemical Changes in Rabbits Exposed to Sodium Cyanide

B. I. Elleh[1*], E. S. Bartimeaus[1], F. U. Igwe[2], D. G. Tamuno-Emine[1] and E. S. Agoro[3]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Biochemistry, Rivers State University, Port Harcourt, Nigeria.
[3]Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author ESB designed the study, performed the statistical analysis, Author BIE wrote the protocol and wrote the first draft of the manuscript. Authors BIE and ESA managed the analyses of the study. Authors FUI and DGT managed some literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of this study was to evaluate the biochemical changes in Rabbits due to Sodium Cyanide exposure.
Study Design: An experimental study.
Place and Duration of Study: This study was carried out at Animal House, Applied and Environmental Biology Department, Rivers State University, Port Harcourt, Rivers State, Nigeria, between April 2020 and November 2020.
Methodology: A total of forty eight (48) rabbits as indicated by Mead's formula constituted the sample size. The study was divided into three groups including the control group. With the exception of the control rabbits, others were treated daily with 0.05 mg/kg sodium cyanide for 30 days, 60 days and 90 days respectively. Cardiac blood samples were extracted from the rabbits using standard procedure. Biochemical parameters investigated include thyroxine (T_4), triiodothyronine (T_3), thyroid stimulating hormone (TSH), total proteins (TP), albumin (ALB), total bilirubin, conjugated bilirubin,

**Corresponding author: E-mail: benonisaiah74@gmail.com;*

aspartete aminotransferase (AST) and alanine aminotransferase (ALT) activities. Data were expressed as mean ± SD. Statistical differences between groups were computed using Graph pad prism 7.0 version developed by Graph pad software, San Siago, Califonia, USA. Results were analyzed using analysis of variance (ANOVA) and significance between groups was taken at *p<.05.*
Results: Biochemical results showed significant *(p<.05)* increase in levels of thyroid stimulating hormone, AST, ALT, total and conjugated bilirubin in day 30, 60 and 90 respectively as compared to control. Significant ($p < 0.05$) decrease was also observed in thyroxine, triiodothyronine, total protein and albumin concentrations following treatment with the cyanide.
Conclusion: Exposure to 0.05 mg/kg sodium cyanide may have harmful effect on biochemical and parameters due to damages done to organs such as liver and the thyroid gland.

Keywords: Sodium cyanide; biochemical changes; organ dysfunction; glands dysregulation.

1. INTRODUCTION

Cyanides, like sodium and potassium cyanide, are crystalline hygroscopic salts widely used in ore extracting processes, synthesis of organic and inorganic chemicals and production of chelating agents. Cyanides are well absorbed through the gastrointestinal tract or skin and rapidly absorbed through the respiratory tract. Once absorbed, cyanide is rapidly and ubiquitously distributed round the body, although the highest levels are specifically found in the liver, kidney, lungs, blood, and brain. The toxic effects of cyanide in humans and animals are generally alike and are assumed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration and histotoxic anoxia. Principal features of the toxicity profile for cyanide are, high acute toxicity by all routes of administration, with a very steep and rate-dependent dose-effect curve, and chronic toxicity, probably mediated through the main metabolite and detoxification product, thiocyanate [1].

The primary targets of cyanide toxicity in humans are the cardiovascular, respiratory and central nervous systems. The endocrine system is also a potential target for long-term toxicity, as function of continued exposure to thiocyanate, which prevents the uptake of iodine in the thyroid and acts as a goitrogenic agent [2]. In rabbits exposed to sodium cyanide in the diet at doses of 15mg CN^-/kg1day for 4 weeks or 20mg CN^- /kg1day for 40 weeks, hepatic toxicity (fatty degeneration and necrosis of the liver, increased serum levels of succinate dehydrogenase, alanine aminotransferase and alkaline phosphatase) and renal toxicity (tubular necrosis) were observed [3]. Besides acute lethal poisoning, cyanide chronic intoxication may also produce some pathologic effects on different tissues that precede alterations in biochemical parameters [4]. Consequently certain type of cells are damaged and leaked enzymes into the blood, where they can be measured as indicator of cell damage; Aspartate aminotransferase (AST), sometimes called serum glutanate oxaloacetate transaminase (SGOT) is one of such enzymes [4]. This enzyme is found in many tissues including the heart, muscle, kidney, brain and lung. The amount of AST in the blood is directly related to the extent of tissue damage. Alanine aminotransferase (ALT), formerly called serum glutamate pyruvate transaminase (SGPT), is produced mainly in the liver and small amounts are found in the heart, muscle and kidney. ALT catalyses the transfer of amino groups between L-alanine and glutamate [4]. Cyanide poisoning may produce some pathologic effects on different tissues that may manifest as alterations in biochemical parameters. The most widespread problems arising from cyanide are from chronic/sub chronic exposures [5]. Chronic cyanide toxicity is involved in the pathogenesis of some health problems [5]. In addition, chronic cyanide intoxication induces biochemical, histological and oxidative stress changes in some tissues in an experimental animal model [5]. Therefore, the aim of this study was to evaluate the biochemical changes in Rabbits due to Sodium Cyanide exposure.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of forty-eight (48), aged between 6 to 8 months, white rabbits (*Oryctolagus cuniculus*) that weighed between 1.2 - 1.5 kg were used for the study. The rabbits were purchased from a breeder in Port Harcourt, Rivers State, Nigeria. The rabbits were weighed and divided into three groups with matched control. Four rabbits were assigned to each group and the study lasted for 90 days as follows: group one (0 – 30) days,

Group two (0 – 60) days, Group three (0 – 90) days. Each rabbit in a group (treated group) was given 10ml of 0.05mg/kg sodium cyanide orally daily for 90 days. The matched control and treated rabbits were given water *ad-libitum* and feed daily. 0.05mg/kg is 10% of the value of LD50. The blood samples and liver were taken for analysis and histopathological investigations at day 30, 60 and 90 respectively.

2.1.1 Housing and nutrition

The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum* in the animal house. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.2 Procurement of Sodium Cyanide (NaCN)

Sodium cyanide, 98% purity, produced by Changsha Hekang Chemical Co. Ltd was purchased at Decosmiller Ventures, Ogbete, Enugu.

2.3 Sample Collection and Storage

At days zero, thirty, sixty and ninety, respectively, four rabbits from each group were sacrificed under chloroform anaesthesia. Blood samples were collected from the rabbits at intervals, 30days, 60days and 90days. The liver was harvested at 90 days from the rabbits. The blood was collected to test for serum total protein and albumin, AST, ALT and ALP activities, total bilirubin and conjugated bilirubin concentrations, TSH, T_3, T_4, and liver tissue for histological analysis. All the biochemical parameters were carried out at Nigerian National Petroleum Corporation (NNPC) Clinic, Akpajo, Rivers State, Nigeria while histology was carried out at Rivers State University, Port Harcourt, Rivers State.

2.4 Sample Analysis

Serum total protein and albumin concentrations were estimated quantitatively using Biuret Method and Bromocresol Green Method as modified by Randox Laboratories (United Kingdom). AST, ALT and ALP activities were also estimated quantitatively using Kinetic Method as specified by Randox Laboratories, total bilirubin and conjugated bilirubin concentrations were measured using colorimetric method as specified by Randox Laboratories. Estimation of TSH, T_3 and T_4 were carried out using Enzyme Linked Immunosorbent Assay Method (ELISA).

2.5 Histological Analysis

The liver was harvested for histological analysis and was fixed in 10% formal saline solution. The organ was dissected, and representative blocks were taken for histological processing each with identifying label in a tissue cassette. The fixed tissue blocks were dehydrated through ascending grades of alcohol, de-alcoholized in xylene, infiltrated and embedded in molten paraffin wax. Sections were cut at 3µm on a rotary microtome. Deparaffinized sections were then stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on the slide were examined and photomicrographs captured with X400 objective lens using the ScopeTek™ device and software v1.3.

2.6 Statistical Analysis

Data were expressed as mean ± SD. Statistical differences between groups were computed using Graph pad prism 7.0 version developed by Graph pad software, San Siago, Califonia, USA. Results were analyzed using analysis of variance (ANOVA) and significance between groups was taken at $p<.05$.

3. RESULTS AND DISCUSSION

Cyanide poisoning may result from a variety of exposures, including structural fires, industrial exposures, medical exposures such as sodium nitroprusside, and certain foods. In some developing countries, the most common cause of cyanide poisoning is domestic fires [6]. Cyanide is found to be highly poisonous to living organisms, primarily due to the formation of complexes with metal ions that are present as enzyme cofactors. The notable effect of cyanide occurs with Fe^{3+} ion in cytochrome, thereby obstructing cellular respiration and hence, oxidative phosphorylation [7]. Biochemical alterations are considered as sensitive indicators of toxicity before the expression of visible hazardous effects [7]. Biochemical parameters such as enzymes activities in tissues and body fluids play an important role in diseases investigation, diagnosis and liver toxicity [8].

Table 1. Mean ± SD of liver function parameters of rabbits that were given 0.05mg/kg NaCN orally for 30 Days of Treatment

S/N	Experimental groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin. (µmol/L)	Total Protein (g/dl)	Albumin (g/dL)
1	Control	46.84±0.58	45.76±0.49	38.10±0.65	1.05±0.03	0.94±0.03	63.34±0.70	35.45±1.77
2	Treated Group	61.71±0.45	50.76±0.51	57.90±0.60	7.99±0.48	1.50±0.16	53.42±1.89	32.84±1.17
3	T value	40.31	14.17	44.61	28.78	6.677	8.387	2.456
4	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0494

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline aminotransferase, p < 0.05 is significant.

Table 2: Mean ± SD of Liver Function Parameters of Rabbits that were given 0.05 mg/kg NaCN orally for 60 Days of Treatment

S/N	Experimental Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin. (µmol/L)	Total Protein (g/dl)	Albumin (g/dL)
1	Control	46.89±0.79	45.35±0.54	37.82±0.49	1.12±0.08	0.91±0.03	63.87±1.02	35.13±2.98
2	Treated Group	64.07±0.40	54.21±0.36	61.86±0.46	10.08±0.31	1.92±0.04	43.14±1.72	26.98±1.34
3	T value	38.79	27.44	71.36	56.92	37.3	20.71	7.247
4	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline aminotransferase, p < 0.05 is significant.

Table 3. Mean ± SD of Liver Function Parameters of Rabbits that were given 0.05 mg/kg NaCN orally for 90 Days of Treatment

S/N	Experimental Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin. (µmol/L)	Total Protein (g/dl)	Albumin (g/dL)
1	Control	47.21±0.71	45.48±0.38	37.85±0.37	1.14±0.08	0.88±0.09	64.07±1.11	34.81±1.90
2	Treated Group	69.15±0.38	57.15±0.20	66.11±0.40	12.49±0.39	2.73±0.18	33.02±1.73	16.12±1.45
3	T value	54.34	52.84	99.98	56.88	18.51	30.19	11.63
4	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline aminotransferase, p < 0.05 is significant.

The biochemical parameters (Tables 1, 2 and 3) showed decrease *(p <.05)* of total protein and albumin concentrations and increase *(p<.05)* of bilirubin concentration and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in 30, 60 and 90 days compared to control group (untreated). Significant changes in plasma AST and ALT activities indicate that the sodium cyanide caused mild changes in the liver. ALT is a cytoplasmic enzyme and an increase in plasma is an indication of mild injuries caused by chemicals to the liver. Liver injury is characterized as hepatocellular when there is predominant elevation of the ALT, while AST is a mitochondrial enzyme whose increased activity in plasma reflects severe tissue injuries [9].

Further studies as reported by [8] have shown that subtle membrane changes are sufficient to allow passage of intracellular enzyme to extracellular space. Therefore the sodium cyanide induced elevation in serum ALT and AST levels in this study may be attributed to damages structural integrity of the liver, because these enzymes are normally located in the cytoplasm of hepatocytes and are released into circulation after cellular damage.

The thyroid function parameters (Tables 4, 5 and 6) showed significant *(p<.05)* decrease in thyroxine (T_4), triiodothyronine (T_3) and increase in thyroid stimulating hormone levels. The observed decrease in T_3 and T_4 could be attributed to the effect of cyanide on the uptake of iodine by thyroid gland. Consequently, the decreased T_3 and T_4 induced the increased TSH secretion [10]. Toxicity effects observed might be attributed to the long term exposure to 0.05 mg/kg sodium cyanide that could cause cyanide poisoning with related symptoms of headache, weakness, nausea, vomiting, dizziness, stomach pains, exacerbates goiter, diarrhea and death [11].

Table 4. Mean ± SD of Thyroid Hormones of Rabbits that were given 0.05 mg/kg NaCN orally for 30 Days of Treatment

S/N	Experimental Groups	Triiodothyronine (pg/mL)	Thyroxine (ngdL)	Thyroid stimulating Hormone (mIU/L)
1	Control	2.76±0.17	7.42±0.33	1.53±0.17
2	Treated Group	2.40±0.07	6.73±0.21	1.94±0.05
3	T value	3.893	3.496	4.657
4	P value	0.0080	0.0129	0.0035

p < 0.05 is significant.

Table 5. Mean ± SD of Thyroid Hormones of Rabbits that were given 0.05 mg/kg NaCN orally for 60 Days of Treatment

S/N	Experimental Groups	Triiodothyronine (pg/mL)	Thyroxine (ng/dL)	Thyroid stimulating Hormone (mIU/L)
1	Control	2.79±0.13	7.36±0.25	1.62±0.10
2	Treated Group	2.13±0.10	5.21±0.10	2.21±0.08
3	T value	8.146	16.3	9.265
4	P value	0.0002	<0.0001	<0.0001

p < 0.05 is significant.

Table 6. Mean ± SD of Thyroid Hormones of Rabbits that were given 0.05 mg/kg NaCN orally for 90 Days of Treatment

S/N	Experimental Groups	Triiodothyronine (pg/mL)	Thyroxine (ng/dL)	Thyroid stimulating Hormone (mIU/L)
1	Control	2.76±0.08	7.44±0.08	1.62±0.06
2	Treated Group	1.76±0.07	4.49±0.13	2.44
3	T value	18.8	39.75	17.04
4	P value	<0.0001	<0.0001	<0.0001

p < 0.05 is significant.

Photomicrographs showing the histological findings of the liver tissues harvested from the experimental animal, rabbit, from various groups are shown in Fig. 1 to Fig. 4. The control slide shown in Fig. 1 represent rabbits that were not exposed to sodium cyanide , while Fig. 2, Fig. 3 and Fig. 4 are rabbits exposed to sodium cyanide for thirty, sixty and ninety days respectively. Histological examination revealed significant changes on thirty, sixty and ninety days respectively. The different rabbits exhibited different characteristics, such as hepatocyte microvesicular steatosis, some with glycogen accumulation, liver steatosis and liver necrosis.

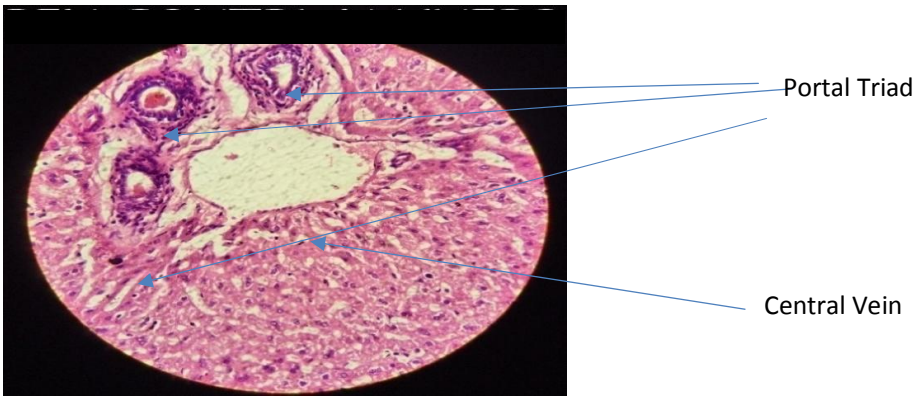


Fig. 1. Photomicrograph of Normal Liver Tissue showing normal hepatic morphology and vasculature. H & E x400

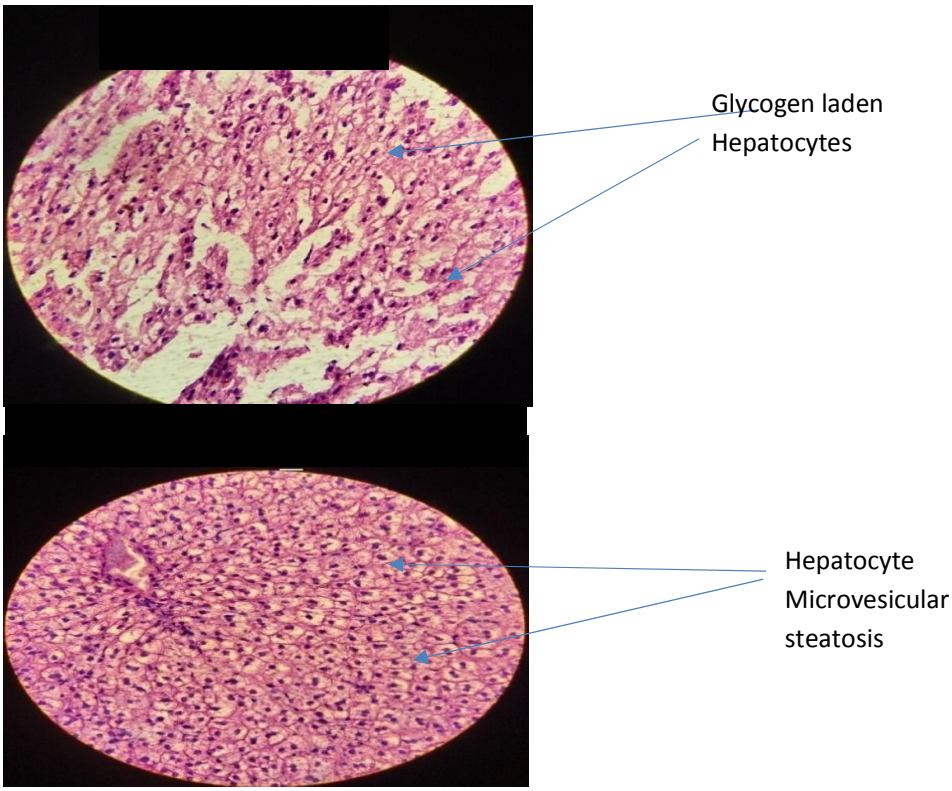


Fig. 2. Photomicrograph of Liver Tissue exposed to sodium cyanide for thirty days Showing hepatocytes with glycogen laden and hepatocyte microvesicular steatosis. H & E x400

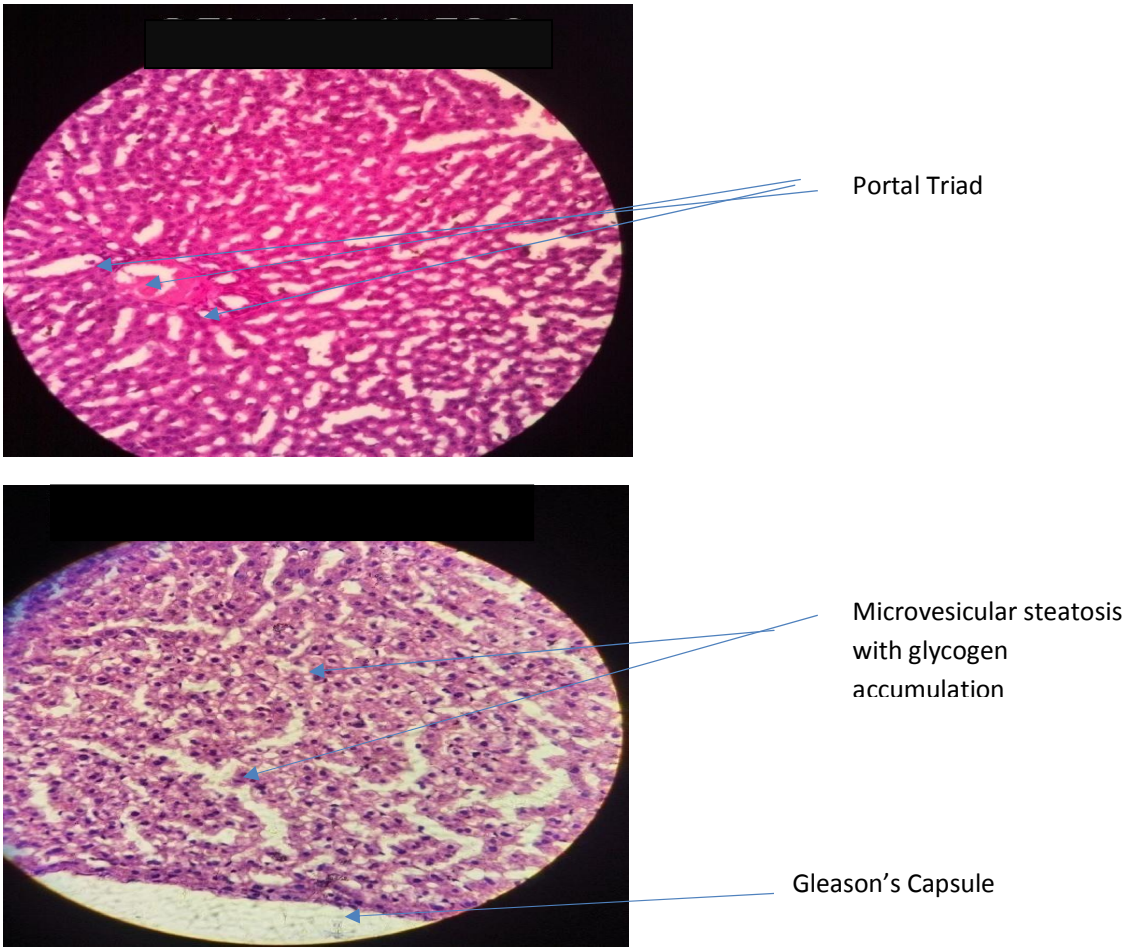
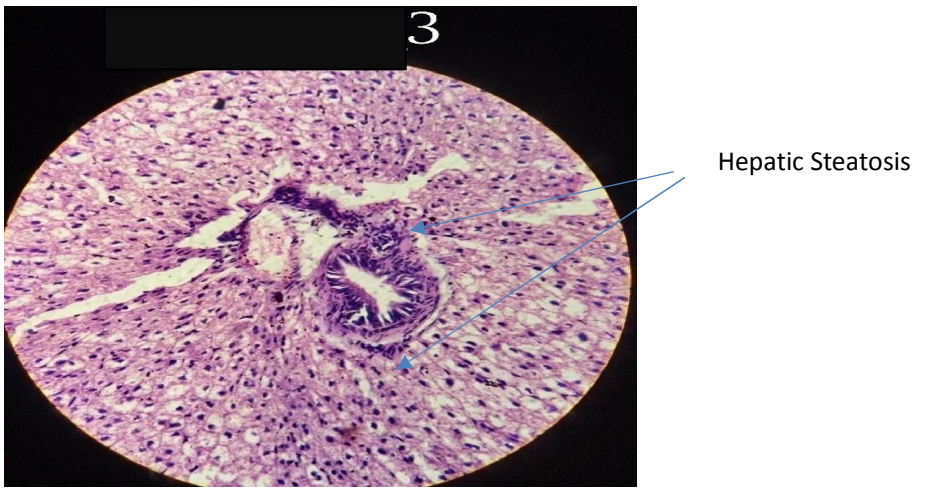


Fig. 3. Photomicrograph of Liver Tissue exposed to sodium cyanide for sixty days showing microvesicular steatosis with glycogen accumulation. H & E x400



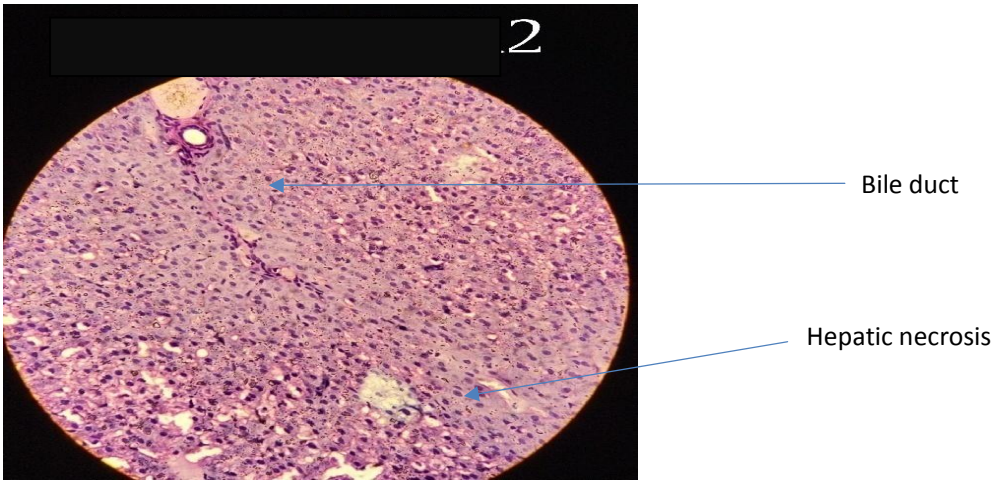


Fig. 4. Photomicrograph of Liver Tissue exposed to sodium cyanide for ninety days showing areas of hepatic steatosis and hepatic necrosis. H & E x400

4. CONCLUSION

It can be concluded that long term exposure to 0.05 mg/kg sodium cyanide may have harmful effect on biochemical and parameters due to damages done to organs such as liver and the thyroid gland.

CONSENT

It's not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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