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The Effects of Chronic Carbon Monoxide Intoxication on some liver Biochemical Parameters in Rabbits

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ABSTRACT

Carbon monoxide (CO) is a toxic gas that escapes easy detection due to its euphoric nature. It utilizes hypoxia in causing mortality or morbidity. The study was aimed at evaluating the effect of chronic CO concentration (>200 ppm) in some liver biochemical parameters utilizing rabbit as a choice animal model. A total twenty (20) apparently health albino rabbits constituted the sample size as validated by mead's equation. The animals were divided into four groups of five rabbits each. The first group constituted the controls, the remaining groups constituted rabbits exposed thirty minutes daily to CO for 10^{th}, 20^{th} and 30^{th} respectively. Blood samples were extracted from the hearts of the animals for the analysis of liver enzymes, proteins and bilirubin. One way anova (pos hoc-LSD) was used for the statistical analysis with level of significant considered at > 0.05. The study showed that serum aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), total and conjugated bilirubin and albumin/glibulin ratio increased significantly (p>0.05) across the durations of exposure. On the contrary, serum total protein and globulin decreased significantly (p>0.05). The findings revealed that chronic inhalation of CO could have a long term negative consequence on the liver.

INTRODUCTION

Carbon monoxide (CO) is a poisonous non-irritant gas produced as a result of incomplete combustion of organic materials due to insufficient supply of oxygen. It displaces oxygen from binding to haemoglobin in the circulatory system, hence resulting to asphyxic and subsequent hypoxia that usually result to the collapse of the respiratory system. Carbon monoxide not only decreases the oxygen content of blood, but also decreases oxygen availability to tissues, thereby producing a greater degree of tissue hypoxia than equivalent reduction in oxyhaemoglobin caused by hypoxia.[1] Carbon monoxide also binds to other heme proteins, such as myoglobin and mitochondrial cytochrome oxidase a_3, which limits oxygen use when tissue partial oxygen pressure (PO_2) is very low. Organs with high oxygen demand, such as the heart and brain, are most sensitive to hypoxia and account for the major clinical sequelae of carbon monoxide poisoning.[2] Others less impacted are lungs, liver and spleen.[2] If concentration of CO concentration exceeds 100 ppm, it can be dangerous for humans. Symptoms of CO poisoning may include headache, sweating, dizziness, dim vision, tremor and loss of consciousness.[3] Rabbit was the choice animal model for the research work. The suitability of rabbit as a choice animal for this study is attributed to its anatomical

and physiological similarities to human.[4,5] The liver carries out several important functions that involve excretory, synthetic or detoxifying mechanisms. Liver function tests are groups of blood tests that give information about the state of the liver.[6] A liver function test used for this study includes total protein, albumin, globulin, aspartate and alanine aminotransferases, bilirubin and alkaline phosphatase. These biochemical parameters give a clearer picture of the hepatocellular and biliary axis of the liver status. Distortions in these biochemical parameter's concentrations and activities point to a compromised liver. Nigerians are exposed to varying degrees of CO due to the high demand for CO-producing machines and equipment. A deliberate study of the effect of chronic exposure to CO on liver biochemistry could possibly open up unknown pathophysiology of some idiopathic diseases. The products of the findings could be useful in policy shaping in combating chronic diseases, preventing avoidable epidemics and enhancing Medicare. The effects of CO on a lot of organs, tissues and cells have been studied by handful of researchers with findings implicating CO.[7,8,9,10,11,12] Carbon monoxide mechanism of action is primarily through starvation of organs of oxygen. Most organs survival is basically depended on the regular supply of oxygen; hence any denial could be deleterious. Liver is the center of metabolic processes and usually need regular supply of oxygen. This study is aimed basically to reveal the possible effect of chronic CO intoxication on the biochemical parameters that is used to evaluate the integrity and status of the liver.

MATERIALS AND METHODS

Study Area

The CO intoxication aspect of the study was carried out at the fringe of Epie Creek section of Igbogene Epie in Bayelsa State Nigeria. Similarly, the Chemical Pathology Laboratory of the Niger Delta University Teaching Hospital Okolobiri, Bayelsa State served the biochemical analysis.

Study Population

The strength of the sample size of the study was derived from Mead's resource equation.[13] A total of twenty (20) albino rabbits constituted the sample size. The study involved a chronic exposure of study animals to daily thirty minutes of mild concentration of carbon monoxide for a minimum of ten days and maximum of 30 days. The rabbits were divided into four groups. The first group constituted the controls which were not exposed to CO prior to mechanical sacrifice. The remaining three groups (10^{th}, 20^{th} and 30^{th}) were exposed to CO for 30 minutes daily for ten days, twenty days and thirty days respectively. The daily exposure of CO concentration was pegged not more than 200 ppm as defined by Golden,[14] and Struttmann ***et al***.[15] for chronic CO intoxication study. The carbon monoxide gas was obtained from a portal Sumac generating set.

Ethical Approval

The ethical clearance and experimental protocol were approved by the Ethics Committee of the Bayelsa State Ministry of Health. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to.[16]

Selection Criteria

Rabbits used were apparently healthy and active as confirmed and approved by a veterinary doctor. Rabbits showing signs or symptoms of illness were excluded from the research. Lysed blood samples were also rejected. The research utilized only male albino rabbits of same age and weight. The age range was between six to eight months. The weight brackets were 1.5-2kg.

Collection of Blood Samples

Blood samples were collected from the heart using the method postulated by Ness.[17] Blood was withdrawn slowly into the appropriate containers to prevent the heart from collapsing. The blood samples collected were dispensed into plain containers, allowed to clot and then separated for the biochemical analysis.

Laboratory Analysis

Serum total protein and albumin concentrations were estimated quantitatively using Biuret and Bromocresol (BCG) methods respectively as modified by Randox Laboratories (United Kingdom). Serum globulin concentration and albumin/globulin (a/g) ratio were derived mathematically.[18]

Total Protein = Albumin + Globulin

Hence, Globulin = Total Protein – Albumin.

A/G ratio= Albumin/Globulin

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Aspartate aminotransaminase (AST), alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) activities were assayed using ELITech Clinical Systems with the aid Selectra proM.. Malloy-Evelyn modified end point method was used for the estimation of total and conjugated bilirubin.[18] The unconjugated bilirubin was estimated mathematically by subtracting conjugated bilirubin from total bilirubin.

Statistical Analyses

Data were analyzed with Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21). One-way ANOVA (Post Hoc- LSD) was used in comparing the means of the liver biochemical parameters of the various chronic CO intoxication groups of the study.

DISCUSSION

The pulsatile inhalation of CO by Nigerians is routine due to the massive use of equipment that releases CO and the huge gap in power supply. Chronic carbon monoxide poisoning is the inhalation of low quantity of CO over a long duration. This study revealed a significant decrease ($p < 0.05$) in concentrations of serum total protein and

RESULTS

Table 1: A Multiple Comparison of Serum Protein Profile on the Basis of Duration of Chronic CO Intoxication

Parameters	Control	Duration of CO Exposure				
		Day 10	Day 20	Day 30	f-value	p-value
TP (g/L)	47.75 ± 6.24	36.75 ± 4.03 [a]	33.50 ± 3.11 [a]	33.50 ± 3.70 [a]	8.021	0.003
ALB (g/L)	30.25 ± 2.36	28.25 ± 4.92	26.75 ± 3.86	29.25 ± 2.99	0.665	0.590
GLO (g/L)	16.50 ± 6.56	8.50 ± 1.73 [a]	8.25 ± 4.99 [a]	4.25 ± 0.96 [a]	5.870	0.010
A/G Ratio	2.20 ± 1.23	3.50 ± 1.16	5.00. ± 4.14	7.10 ± 1.49[a]	3.194	0.063

Legend: TP- Total Protein; ALB-Albumin; GLO- Globulin; A/G- Albumin/Globulin.
Symbols- a: $P < 0.05$ vs control, b: $P < 0.05$ vs Day 10, c: $P < 0.05$ vs Day 20
Data are expressed as mean ± SD; Significant at 0.05 Confidence ($p < 0.05$)
Concentration of acute CO intoxication= ≤ 200 pm

Table 2: A Multiple Comparison of Serum Liver Function Tests Parameters on the Basis of Duration of Chronic CO Intoxication

Parameters	Control	Duration of CO Exposure				
		DAY 10	DAY 20	DAY 30	f-vale	DAY 30
AST (U/L)	14.75 ± 2.06	19.00 ± 4.69	38.50 ± 6.35 [ab]	44.00 ± 4.32 [ab]	38.68	0.00
ALT (U/L)	13.75 ± 4.79	42.75 ± 3.10 [a]	85.50 ± 4.80 [ab]	115.75 ± 13.07 [abc]	144.07	0.00
AST/ALT	0.88 ± 0.25	0.45 ± 0.09 [a]	0.46 ± 0.09 [a]	0.38± 0.03 [a]	10.83	0.00
ALP (U/L)	18.75 ± 6.99	38.50 ± 5.80 [a]	96.50 ± 11.82 [ab]	169.25 ± 19.96 [abc]	117.84	0.00
TB (µmol/L)	2.13 ± 0.22	2.38 ± 0.44	3.80 ± 0.48 [ab]	4.20 ± 0.88 [ab]	135.23	0.00
CB (µmol/L)	0.93 ± 0.15	0.98 ± 0.15	2.30 ± 0.42 [ab]	2.50 ± 0.50 [ab]	24.01	0.00
UB (µmol/L)	1.20 ± 0.14	1.40 ± 0.47	1.50 ± 0.79	1.70 ± 0.55	0.598	0.00

Legend: AST- Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALP-Alkaline Phosphatase; TB-Total Bilirubin; CB- Conjugated Bilirubin; UB- Unconjugated Bilirubin.
Symbols- a: $P < 0.05$ vs control, b: $P < 0.05$ vs Day 10, c: $P < 0.05$ vs Day 20
Data are expressed as mean ± SD; Significant at 0.05 Confidence ($p < 0.05$). Concentration of acute CO intoxication= ≤ 200 pm

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globulin concentrations across the chronic CO intoxication groups (Table 1). The reduction in serum total proteins and globulins concentrations could be attributed to immune-suppression. Globulins are immune proteins produced in response to infection and inflammation. The consistent exposure to CO resulted to the decrease in concentration of serum globulin which is indicative of depression of the immune system. Serum total protein concentration decrease as observed in this study is due to the fall in globulin concentration as further elucidated by the increase in A/G ratio. An increase in A/G ratio is attributable to immunodepression and compromise[19]. A depressed immune system is a path usually utilized by a lot of diseases. The depression could lead to an ease acquisition of a lot of diseases that could be idiopathic origin. Furthermore the results of this study revealed a significant increase ($p < 0.05$) in concentrations and activities of some of the liver biochemical function parameters (Table 2) as the duration of CO intoxication increased. The increased activities of AST and ALT are proof of the toxicity of CO on the hepatocytes and liver parenchymal cells. The deterioration of the liver could be due to the hypoxic action of the offending agent (CO). The liver requires continuous availability of oxygen for its wide range of functions. This finding agreed partly with Nanji ***et al.,*** [20] that showed a necrotic effect on the liver by the synergistic effect of CO and ethanol. This study also showed that serum ALP activity increased markedly as the duration of CO exposure increased. The increase showed that CO hypoxic effect also extended to the biliary tree and surrounding cells. This report contrasted the work reported by Niebró ***et al.,***[21] that showed a decline in concentration of ALP in guinea pigs after 7 days exposure with 4-5 percent of CO in the air. Moreover, this study showed a steady increase in total and conjugated bilirubin as the duration of CO exposures increase. The elevation of conjugated bilirubin could be attributed to the effect of CO on the blood cells, hepatocellular insufficiency or the biliary tree reflux. Stefan ***et al.,***[22] showed that at a low concentration of carbon monoxide, bilirubin exerts an anti-oxidative function, hence protecting cells and tissues from injuries and that low concentration of CO serve as a protective shield of various body organs. Unconjugated bilirubin exhibited no significant difference across the study duration. This has further shown that CO induced hyperbilirubinaemia cause be reversible as the liver cells were still conjugating and not have completely collapsed

CONCLUSION

The aim of the study was to assess the effect of chronic CO poisoning on biochemical parameters commonly used to evaluate the integrity and status of the liver. The finding showed that chronic inhalation of CO could cause long liver damage resulting from the consistent starvation of the liver of oxygen. Furthermore, the hypoxia characteristics could instigate immune-suppression that may make the body vulnerable to arrays of diseases.

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