

Effects of Maternal Alcohol Consumption on the Biochemical Parameters in the Pups of Albino Rats

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ABSTRACT

Alcohol ingestion during pregnancy can result in fetal disturbances in the offsprings. In this work, the effects of maternal alcohol consumption on the biochemical parameters in the pups of albino rats were carried out. Three groups of the animals, A, B and C were used in this study. Group A was the control group while groups B and C served as test groups. Group B (Prenatal group) was exposed to 1ml of 20% ethanol per kg weight up to the delivery time while group C (postnatal group) was administered with 1ml of 20% ethanol per kg weight up to the weaning period. A total of 120 pups from the three groups were used in the investigations. Eight pups were randomly selected every week from each group and 3mls of blood was collected by ocular puncture for a period of five weeks. Vitros360 chemistry autoanalyzer was used for biochemical assay. Mean values of the aspartate transaminase (AST), alkaline phosphatase (ALP) activities and bilirubin were significantly elevated ($p < 0.05$) in test groups (B & C) from 2nd to 6th week of age compared with control while the mean value of alanine transaminase (ALT) activities was only significantly increased ($p < 0.05$) in the 6th week of age in the test group C compared with control. Furthermore, while the mean value of glucose concentrations were significantly increased ($p < 0.05$) in weeks 5 and 6, mean values of cholesterol and creatinine levels were only significantly increased in week 5 in the test groups compared with control ($P < 0.05$). However, there was no significant elevation ($p > 0.05$) in mean value of urea in the test groups in all the weeks under study. These investigations have therefore demonstrated that maternal alcohol consumption during prenatal and postnatal periods has some deleterious and toxic effect on some biochemical parameters and has revealed potential risks in the offsprings. Therefore, there is the need for controlled maternal alcohol consumption during pregnancy and before weaning periods.

Keywords: Alcohol, pregnancy, pulp, offsprings.

INTRODUCTION

Alcoholism amongst pregnant women has become a serious socioeconomic and health problems. Acute and chronic alcohol misuse have been shown to cause reproductive function derangements in human and experimental animals [1]. Alcohol ingestion during pregnancy can

result in fetal disturbances in their offsprings [2]. In experimental animal models the syndrome is characterized by retardation of fetal life. Fetal alcohol syndrome is a pattern of mental and physical defects that can develop in fetus in association with high levels of alcohol

consumption during pregnancy [3]. Damage to the central nervous system (CNS) has emerged as one of the most serious consequences of fetal alcohol syndrome [4]. Alcohol crosses the placental barriers and can stunt fetal growth or weight, create distinctive facial stigmata, damage neurons and brain structures which can result in physiological or behavioral problems and causes other physical damage [2]

Studies in human and primates on brain structure and function now strongly suggest that maternal alcohol consumption can affect fetal brain structure and functions [4]. The main effect of fetal alcohol syndrome is permanent central neurons system damage especially to the brain. Developing brain cells and structures can be malformed. Maternal alcohol exposure can create cognitive and functional disabilities including poor memory, attention deficits and impulsive behaviour [5]. This could cause the disruption of hypothalamic pituitary gonadotropin axis which plays a regulatory role in reproduction. Gonadotropin stimulation starts from release of leutenising hormone releasing hormone (LHRH) from hypothalamus and is released to the pituitary. In response the pituitary produces luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH stimulates testosterone production and FSH plays role in sperm maturation [6].

Fetal gonadotropins provide the stimulus for the maturation of Fetal Leydig cells and onset of testosterone secretion [7]. In adults, alcohol is known to disturb many of the rhythms of neuroendocrine functions, probably through its actions on the hypothalamus [2]. This could be as a result of disruption of the hypothalamic pituitary gonadal axis.

In chronic alcohol groups with male rats it was observed that testicular, prostate and seminal vesicle atrophy occurs in addition to lowered plasma testosterone. Some biochemical studies revealed among others that chronic alcohol groups produce increase in β endorphin,

prolactin and produces decrease in LH hormone in male [8]. Heavy alcohol intake had been associated with a significant increase of all-cause and non-cardiovascular mortality rates especially by cirrhosis, cancer and violent deaths [9].

Alcohol consumption has been found to have considerable effect in the liver [10]. This has aroused serious medical interest. Alcohol has been found to injure the nervous system by inhibiting growth processes. It can attack the brain function and may have metabolic effects on the liver function enzymes-the liver being one of the most important organs in drug metabolism [11]. Alcohol consumption may bring about changes that may alter the release of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Heavy drinking is associated with major liver diseases such as fatty liver, alcoholic hepatitis and cirrhosis [12]. Moderate consumption of alcohol can help improve cardiovascular health. This beneficial effect of moderate alcohol consumption might be explained by arise of high density lipoprotein cholesterol (HDL-c) induced by alcohol consumption [13]. Heavy drinking can increase the amount of triglycerides in the blood. This can increase the risk of heart diseases, high blood pressure, obesity and type 2 diabetes mellitus [14]. Studies have shown that subjects with alcohol consumption has high level of serum triglycerides, high density lipoprotein cholesterol (HDL), uric acid, estimated creatinine clearance rate (CCr) and glomerular filterate rate (GFR) values than none drinkers [15]. Also alcohol reduces functional hepatic nitrogen clearance and acutely down regulate urea synthesis in normal men [16].

One of the pharmacological actions of alcohol is to lower blood glucose. While moderate amounts of alcohol can cause blood glucose to rise, excess alcohol can actually decrease blood sugars [17]. Alcohol has direct effect on the glucose levels of diabetics. Alcohols like beer and sweet wine contain carbohydrate and may raise the glucose level of a diabetic.

Acute or chronic alcohol consumption causes degeneration in different internal organs and systems of adults [18]; [19],[20]. Also the effect of maternal alcohol consumption on different organs and systems of the developing fetus have been reported by [21] and [4]. A Large number of research had exposed the

deleterious effect of alcohol consumption during pregnancy on both the mother and fetus [7], [2], [3] and [1].

Following reported increase in alcohol consumption among pregnant women, this research is designed to investigate the effects on some biochemical parameters of their off-springs.

MATERIAL AND METHODS

Animal Selection and Grouping

The animals (albino rats) were randomly selected at the weaning age of twenty one (21) days from a colony of inbred rats

maintained for research in the animal house of university of Nigeria. Thirty virgin female and 15 immature male rats were used.

RESEARCH DESIGN

selected.8 from each group.

Preparation and Grouping of Test Animals

The female albino rats were grouped into three (A, B, & C) with 10 animals in each group. Group A served as control while groups B and C were used as test groups. The animals were allowed to acclimatize for 3 weeks. All the groups were fed with water and commercial rat diets. The investigations commenced at the end of six weeks which is the age of sexual maturation. During investigation, the control group A was fed with water and commercial rat diets while groups B and C where fed with 1ml of 20% ethanol per kg weight for 3 weeks. The three groups where bred by using 1 male per cage of 2 females. Day 1 of pregnancy was presumed after observation of vaginal plug.

Following the diagnosis of pregnancy, groups B and C continued to receive 1ml of 20% alcohol until delivery. After delivery alcohol for group B was replaced with water. This group is called prenatal alcohol exposed, while group C continued to receive 1ml of 20% alcohol till weaning their off-springs at 21 days. This group is called post natal alcohol exposed.

A total 150 off-springs of the albino rats were used to carry out the work.30 rats died at various stages of the investigations. Only 120 off-springs were finally used to carry out the investigations. The tests were carried out every week for 5 weeks .In each week,24 male off-springs were randomly

Sample Collection and Analysis

About 3mls of blood samples were collected by ocular puncture and placed into plain tubes. The samples were allowed to clot and centrifuged at 3000 g for 10 minutes. The serum was separated into plain test tubes for biochemical analyses. The samples were analyzed for aspartate transaminase (AST), Alanine transaminase (ALT),Alkaline phosphatase (ALP) activities. Also bilirubin, cholesterol, glucose,urea and creatinine levels were estimated using Vitros auto analyzer.

Estimation of Biochemical Parameters

All biochemical analyses in this work was carried out with Vitros 360 auto analyzer according to manufacturers instructions for use. This system makes use of thin film analyzer that uses dry reagent spread in extremely thin layer on a plastic slide to which the sample is added. When light is incident on the slide, light passes through different layers of the slide, namely the spreading layer, reagent layer, indicator layer and support layer. The amount of light that enters the slide is different from the amount that leaves the slide due to absorption of light at the reagent layer. The difference in light intensity is directly proportional to quantity of analyte present in the sample and is used to compute the value of the analyte. Each biochemical parameter uses a different slide with the apparatus.

STATISTICAL ANALYSIS

Data collected were subjected to statistical analysis using the analysis of variance (ANOVA). Values were deemed significant if $p < 0.05$.

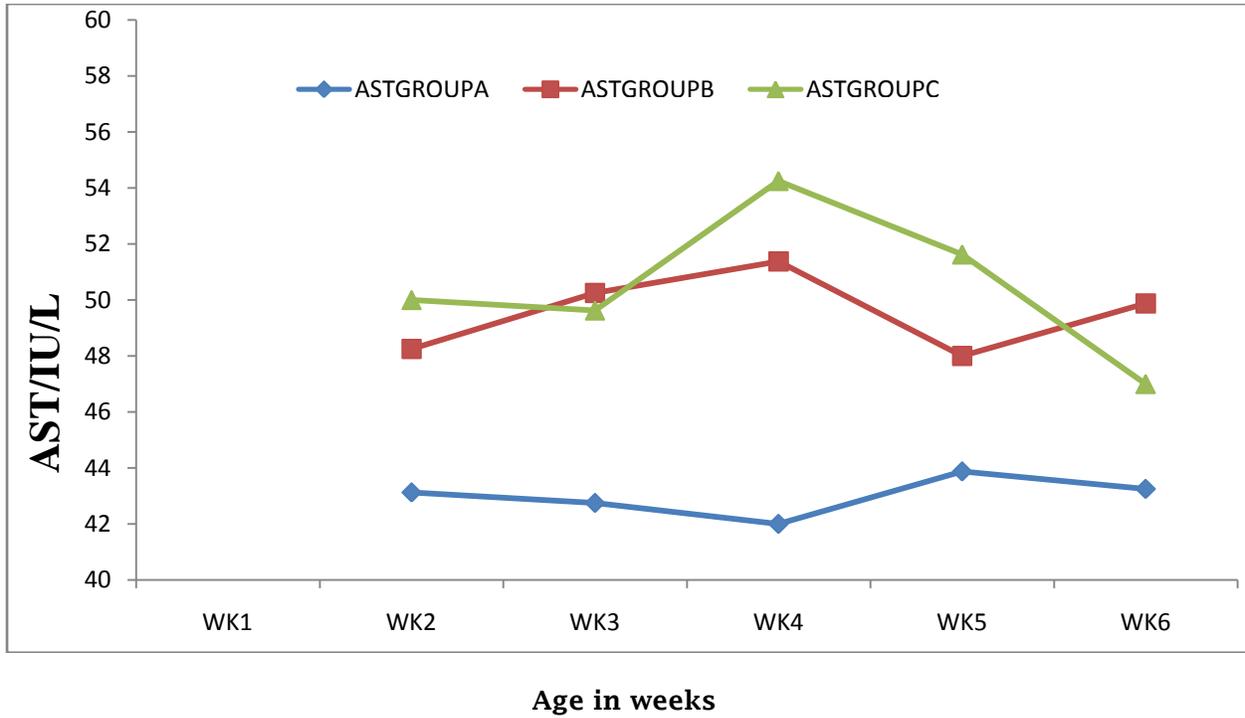


Fig 1: RESULT OF ASPARTATE TRANSAMINASE LEVELS OF PUP

Group C had higher AST levels in weeks 4 and 5 compared with group B ($P < 0.05$).

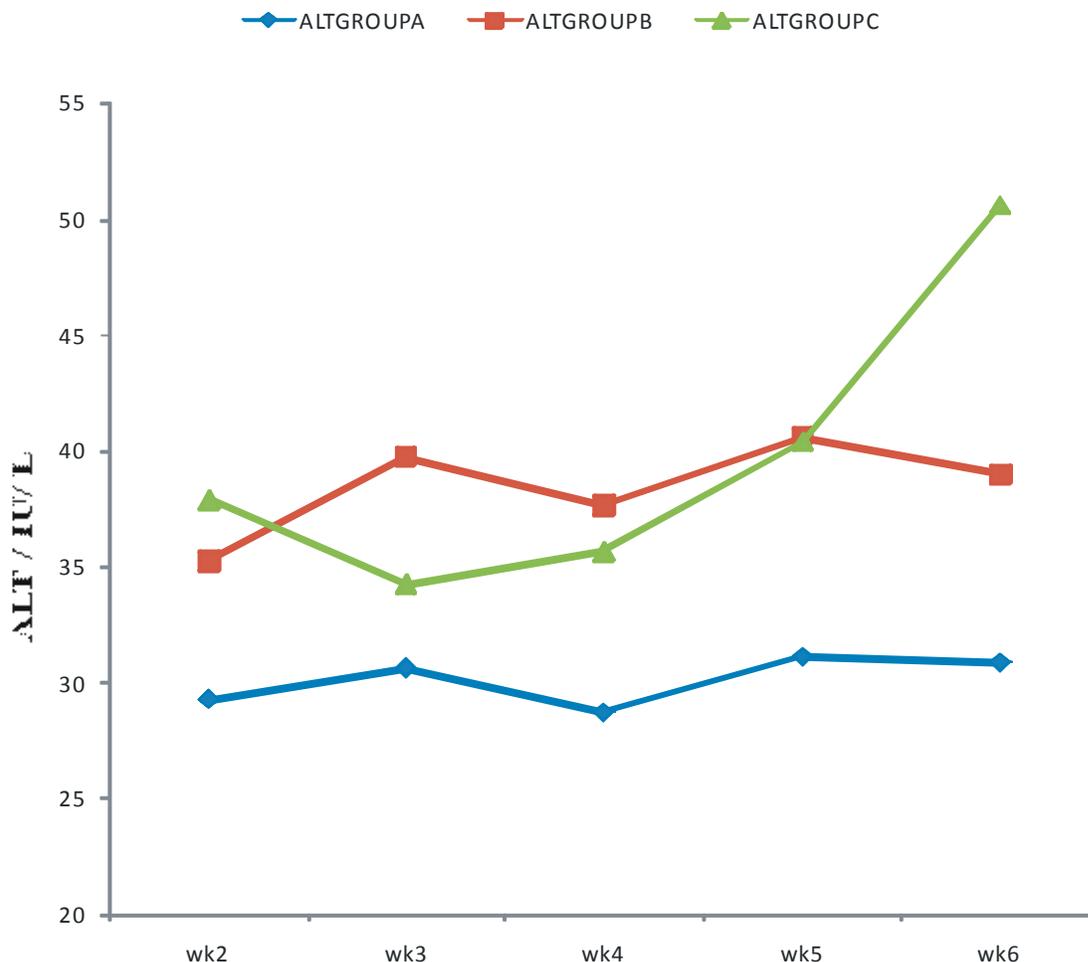


Fig 2: Alanine Transaminase Levels of The Pup.

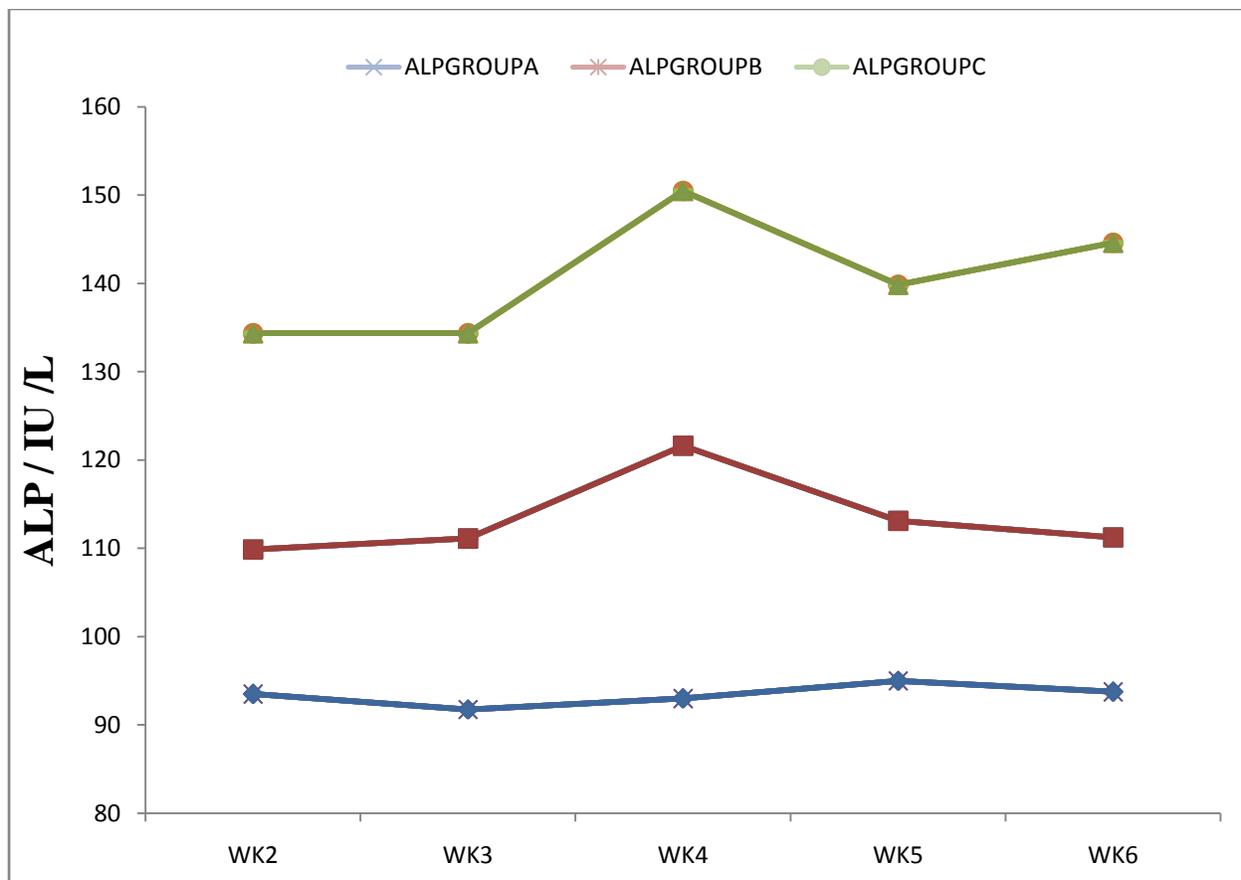
Comparison of mean values of alanine transaminase (ALT) Activities showed no significant differences in weeks 2, 3, 4 and 5. Significant difference in mean for groups A,B and C.

ALT activities was indicated in week 6 of group C with mean values of 30.88±4.88, 39.00±6.97, 50.625±18.52

ALKALINE PHOSPHATASE (ALP) LEVELS OF THE OFFSPRINGS

Activities of Alkaline phosphatase was compared in the three groups. The mean ALP values differ significantly in weeks 2, 3, 4, 5 and 6 (P<0.05). In week 2,group C,(the pre & post natal alcohol exposed group) had higher mean value of

134.37±34.51 compared with the control group A with mean value of 93.50±22.81,while group B (prenatal alcohol exposed group) had mean value of 109.87±31.32 (Fig.6).



Age in weeks

Fig 3: Effect of maternal alcohol consumption on Alkaline phosphatase levels of the Pup. There were significantly high mean values of ALT in prenatal B.

alcohol exposed group B and pre & post natal exposed group C. However group C had higher mean values compared with group

RESULT OF BILIRUBIN LEVELS OF THE PUP.

Comparism of mean values of bilirubin estimated indicated significant differences in weeks 2,3,4,5 and 6 in the three groups (P<0.05).

The highest mean bilirubin value was observed in week 6 of group C. (Fig 7)

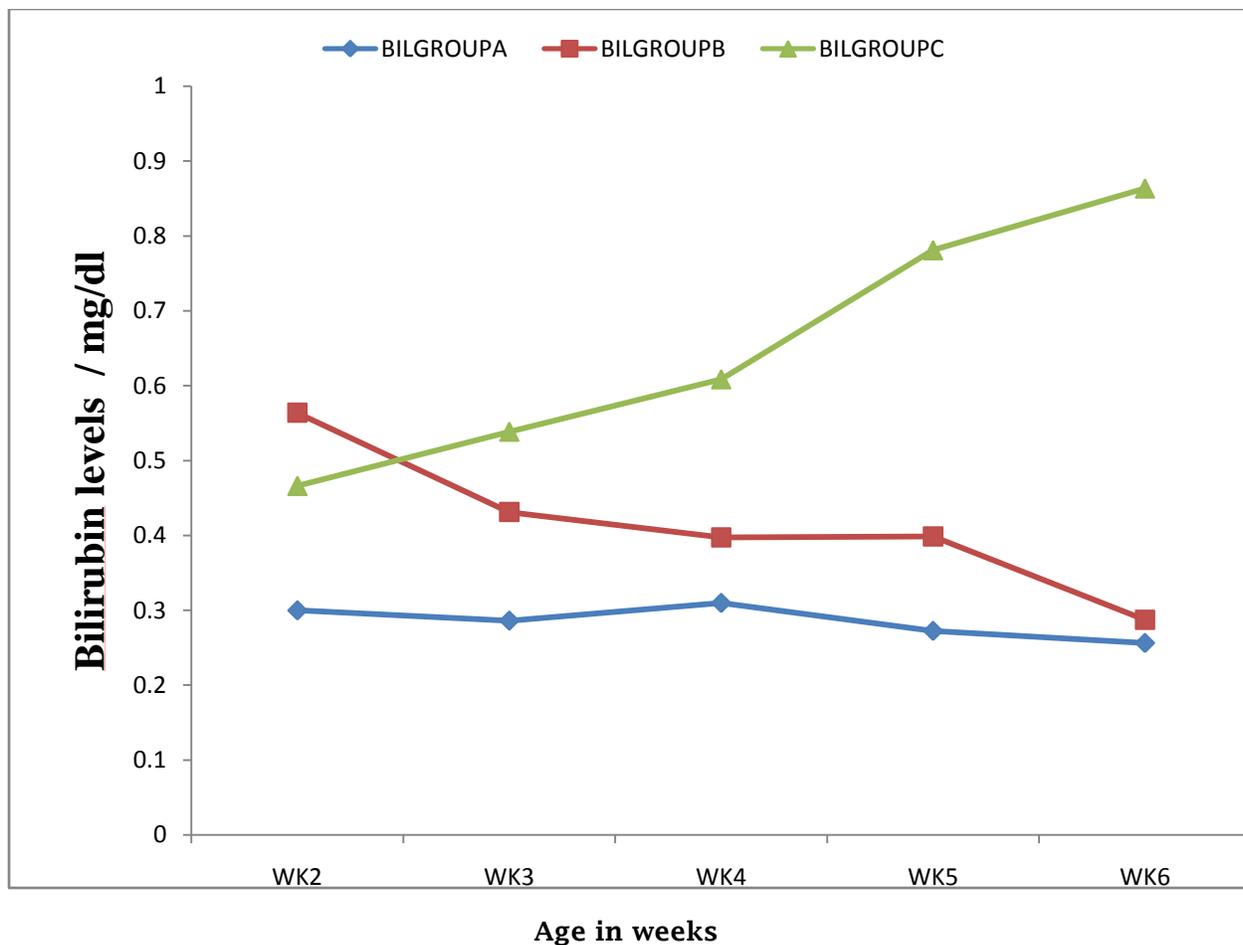


Fig 4: Effect of maternal alcohol consumption on the bilirubin levels of the Pup. There were significant differences in mean values of pre natal group B and pre

& post natal alcohol exposed group C (P<0.05).

TOTAL CHOLESTEROL LEVEL OF OFFSPRINGS

Comparative mean values of serum total cholesterol levels carried out in the three groups showed no significant difference in weeks 2, 3, 4 and 6. (P<0.05) But in

week 5 there was significant difference. Group C had higher cholesterol mean values than group B in weeks 4 & 5. (Fig 8)

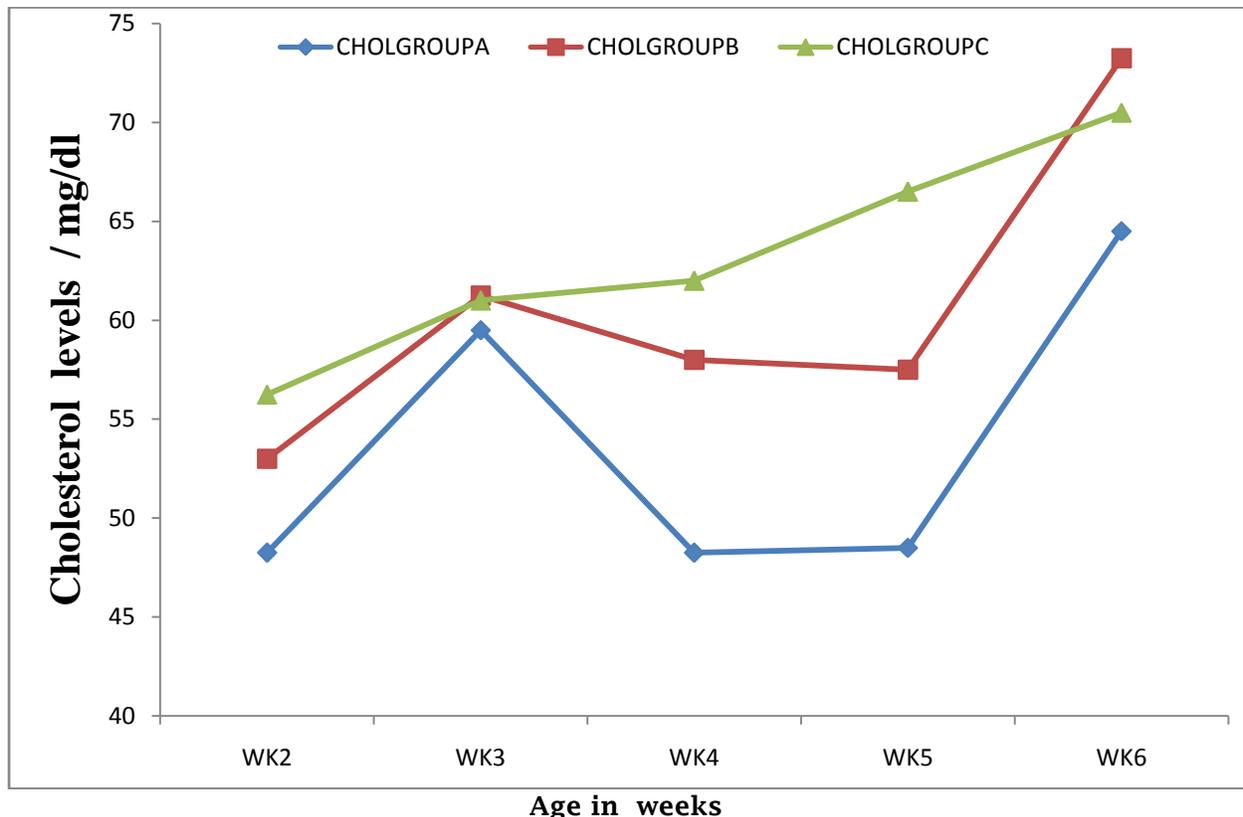
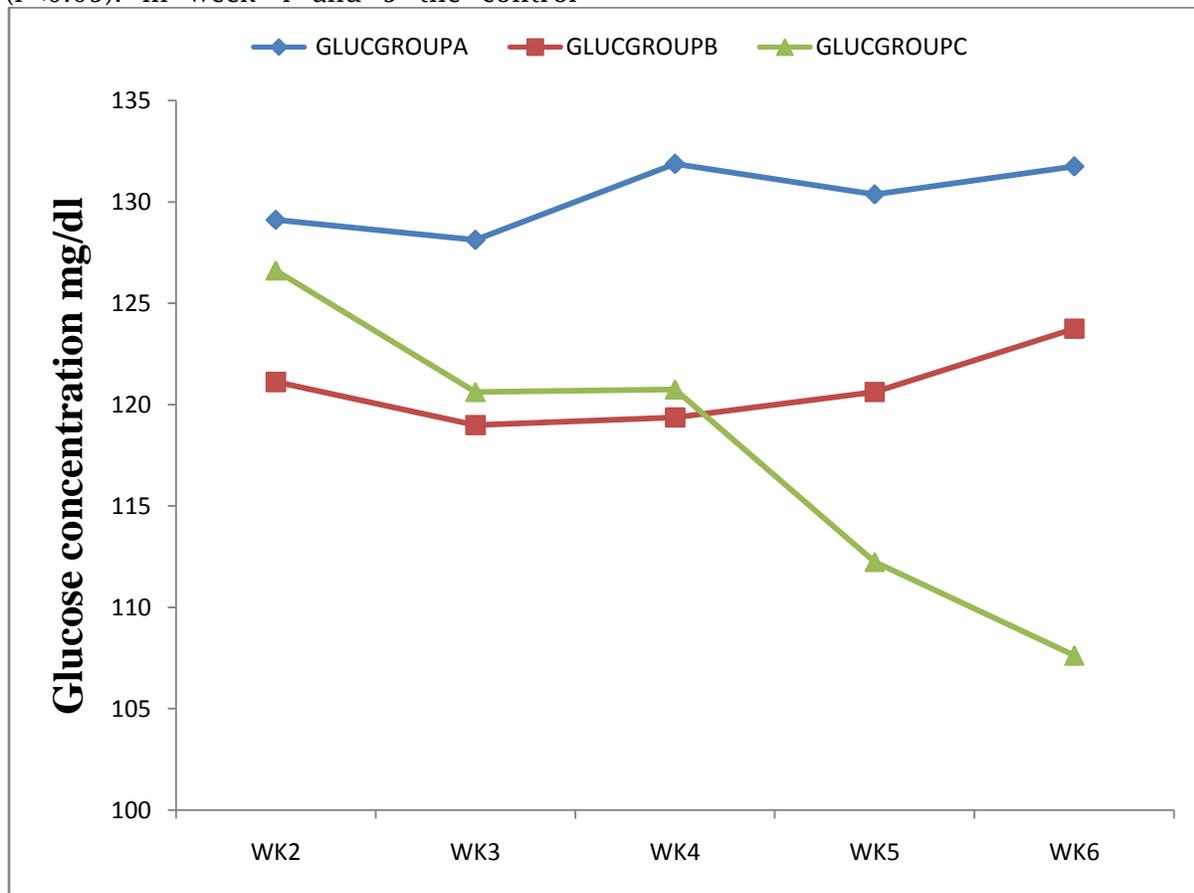


Fig 8: Effect of maternal alcohol consumption on cholesterol levels of the offsprings. In weeks 4 and 5 group C had higher cholesterol mean value than B compared with control (p<0.05).

RESULT OF GLUCOSE LEVELS ON THE OFFSPRINGS OF ALBINO RATS

The mean glucose levels in groups A, B and C showed no significant differences in weeks 2 and 3 but there were significant differences in weeks 4, 5 and 6 ($P < 0.05$). In week 4 and 5 the control

groups had significantly higher mean value than group B and C. (see figure 9). Group C had the lowest mean glucose levels in week 6.



Age in weeks

Fig 9: Effect of maternal alcohol consumption on the glucose levels of the offsprings compared with control groups.

Weeks 2 and 3 showed no significant changes while week 6 had the lowest mean glucose level ($P < 0.05$).

UREA LEVELS OF THE OFF-SPRINGS

The mean urea levels in groups A, B and C showed no significant differences in weeks 2, 4, 5 and 6. In week 3, the

control group A mean value is different from test groups B and C.

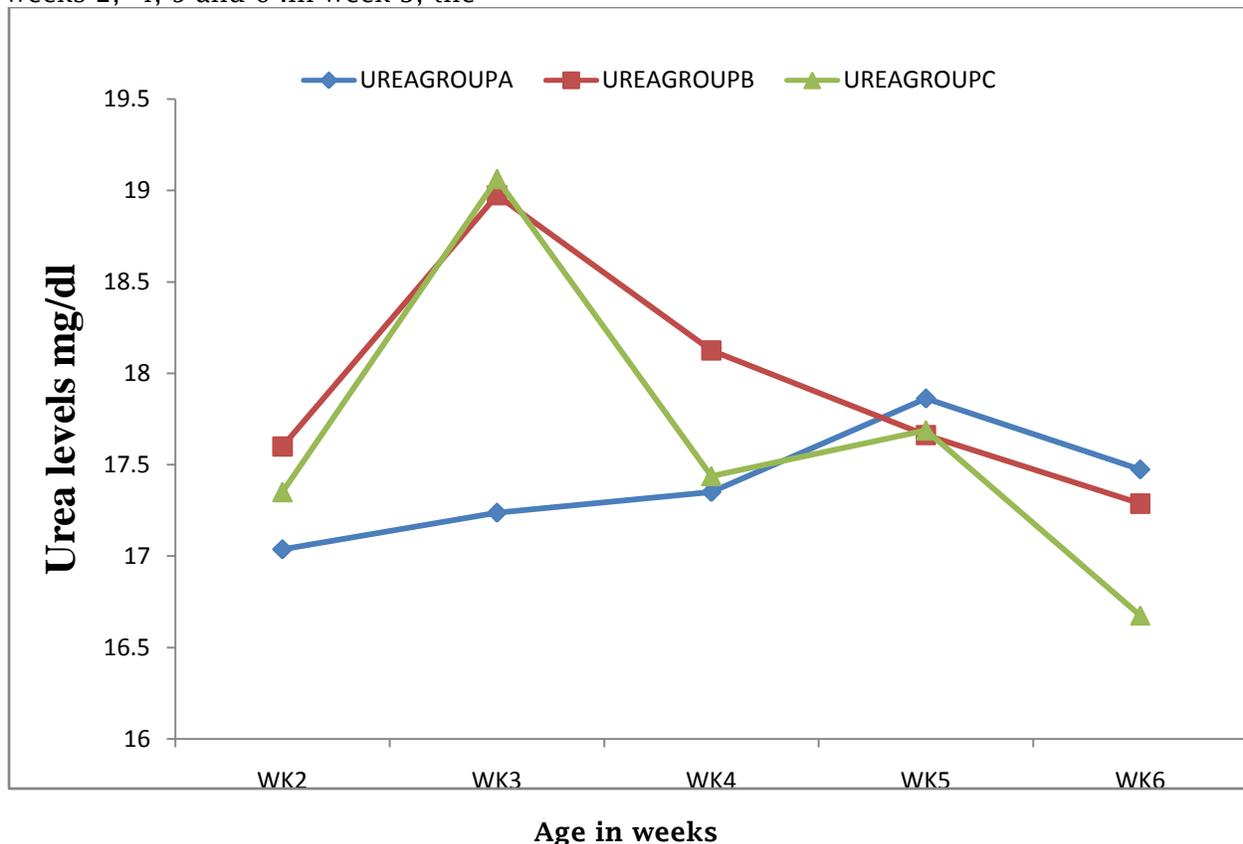


Fig 10: Effect of maternal alcohol consumption on urea levels of the offsprings. There were no significant differences in mean values of serum

levels in weeks 2,4,5 and 6 ($P < 0.05$). Week 3 showed higher values in group B and C.

CREATININE VALUES ON THE OFFSPRINGS OF ALBINO RATS.

Mean creatinine values of the three groups showed no significant differences in weeks 2, 3, 4 and 6. However a significant difference in week 5 was observed with mean values of $0.8038 \pm .2$, $0.8250 \pm .35$ and $1.2063 \pm .42$ for groups A,

B and C respectively. The pre & postnatal exposed group C had a significant higher mean vlaue than group B compared with the control group A. See (Fig.11)

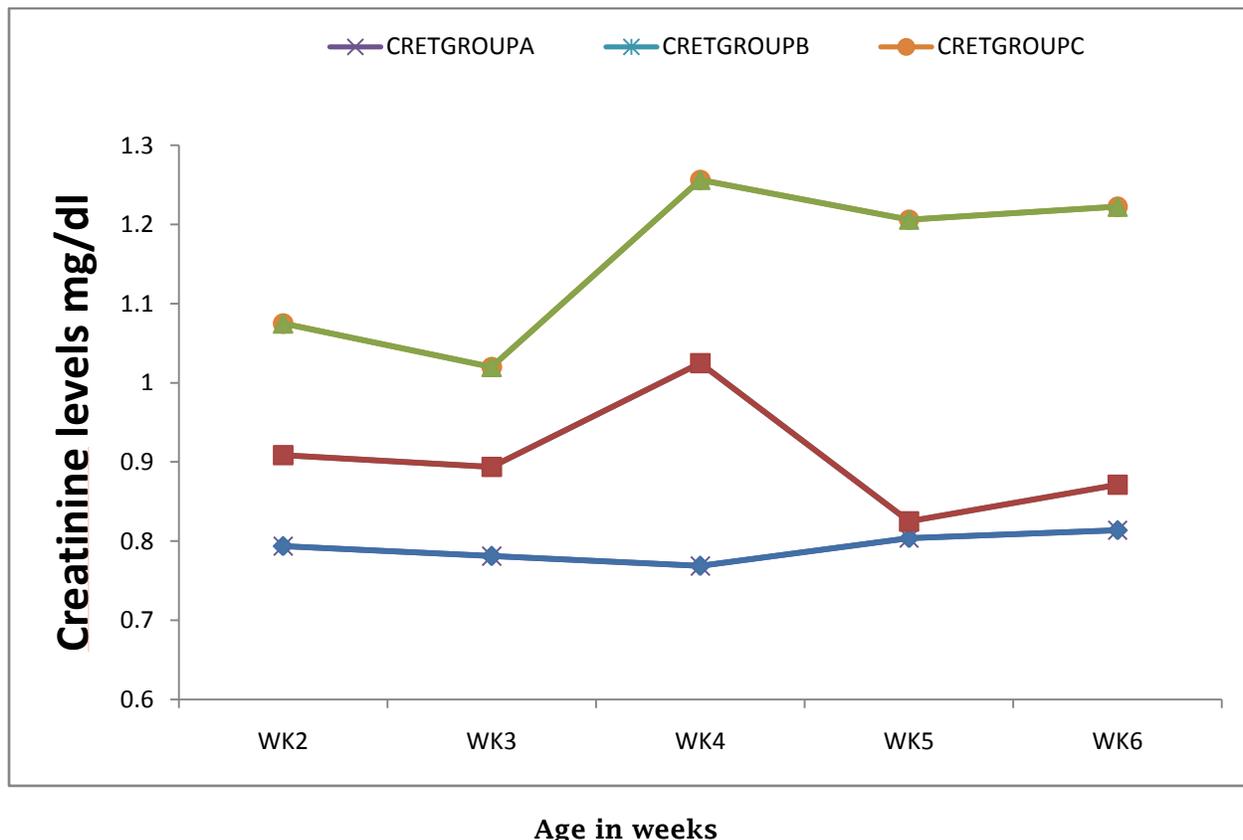


Fig 11: Effect of maternal alcohol consumption on the creatinine levels of the off-springs. There were no significant

differences in the mean values of the off-springs except in week 5 with significant high mean value in ($P < 0.05$) in group C.

DISCUSSION

These investigations have apparently demonstrated that maternal alcohol consumption in albino rats can cause a lot of changes in the offsprings. In liver enzymes, maternal alcohol consumption during prenatal and postnatal periods have demonstrated elevation of aspartate transaminase both in prenatal alcohol exposed albino rats (Group B) and in postnatal alcohol exposed albino rats (Group C). Alcohol consumption up to weaning period further increases the aspartate levels in group C as seen in fig1. Similarly the study demonstrated significant increase in alanine transaminase and alkaline phosphatase activities in the offspring of mothers drinking alcohol during pregnancy and breast feeding. This was similar to a research carried out by [5], [18] and [6]. In the process of alcohol metabolism, acetaldehyde and some free radicals are released. Acetaldehyde though toxic is quickly converted to Acetate while free radicals can cause damage to the liver. A damage to the liver will cause the liver enzymes to leak into the blood hence high level of liver enzymes in alcohol exposed groups. However, prolonged post natal exposure gave significant elevation in alanine transaminase as demonstrated in the sixth week of age in fig.2

Since Aspartate transaminase is not a specific marker for liver damage because it can be found in cardiac and skeletal muscles, alanine and aspartate ratio must be used to determine liver damage [14]. Alcohol ingestion causes direct pattern of liver damage. Following acute or chronic alcohol ingestion, the hepatocytes and mitochondria membrane permeability increase.

Alcohol causes injury to a specific hepatocyte organelles [8]. Following alcohol ingestion, predominantly mitochondria damage may result from acetaldehyde a toxic metabolic product from ethanol. The hepatocyte and mitochondria membrane permeability increased, as a result cytosolic and mitochondria isoenzymes of the liver spill into sinusoids and then into the

peripheral blood resulting in elevated aspartate and alanine transaminases, alkaline phosphatase and 5' nucleotidase [13].

Also the microsomal ethanol oxidizing system, (MEOS) a cytochrome P450 dependent pathway also generates acetaldehyde which may have similar effect on the liver. However MEOS oxidizes NADPH to NADP thereby generating free radicals that damage the tissues and elevates the liver enzymes levels in the blood. Moreover, because the system consumes NADPH, the antioxidant glutathione cannot be generated thereby increasing the oxidative stress [3].

Bilirubin estimation which is an important parameter in hepatitis and other liver diseases was investigated. In this work it was observed that there was an elevation of bilirubin ($P < 0.05$) on the offsprings in all the weeks under study for both pre and postnatal alcohol exposed albino rats. It was observed that bilirubin levels differ in both prenatal group B and post natal group C in week 2 compared with control. It has been demonstrated that many diseases of the liver are accompanied by jaundice [7]. The diseases of the liver include fatty liver, alcoholic hepatitis and cirrhosis [9].

The rats used in this investigation were classified as heavy drinkers. They are groups that took alcohol consistently on daily basis [14]. From the pattern of results the bilirubin levels of offsprings of rats exposed to alcohol during pregnancy appeared significantly lower when compared with rats exposed to alcohol during pregnancy and also at lactation periods. It has been demonstrated that not all heavy drinkers develop alcohol hepatitis or cirrhosis [6]. These findings suggest that other factors ranging from hereditary to environmental may affect bilirubin level.

Cholesterol mean values were elevated in offsprings of rats exposed to alcohol prenatally and during lactation periods as seen in fig 4. Liver mitochondria can

convert acetate to acetylCoA in a reaction requiring ATP and catalyzed by the enzyme thiokinase. However further processing of the acetylCoA by the citric acid cycle is blocked, because NADH inhibits two important regulatory enzymes isocitrate dehydrogenase and α -Ketoglutarate dehydrogenase. This results in accumulation of acetylCoA, a precursor in the cholesterol synthesis. Chronic alcohol ingestion will result in accumulation of acetylCoA and increases cholesterol synthesis [11].

Findings in week 5 agree with earlier studies that moderate consumption of alcohol increases high density lipoprotein cholesterol (HDL) by as much as 4mg/dl within 24 hours. According to Adam, [20], HDL protects against arteriosclerosis and heart attack. It has been established that excessive alcohol consumption increases the amount of triglycerides which can increase low density lipoprotein.LDL increases the risk of cardiovascular disease, high blood pressure and obesity [12],[4]

Considering the fact the albino rats were exposed to heavy alcohol, one expected that the cholesterol level should be high in all the test groups as carried out by other researchers but the results in this investigation was on the contrary except in weeks 4 and 5 of group C where high cholesterol values were recorded. The reason for this picture is not understood. [14] reported that maternal alcohol consumption at a level that does not affect calorie intake increases cholesterol concentration.

The glucose levels of the offsprings of alcohol exposed groups in weeks 2 and 3 did not reveal any significant change when compared with the control group. But alcohol affected the glucose levels in weeks 4, 5 and 6. (Fig.6).

Ethanol is metabolized primarily in the Liver. This metabolism occurs by two pathways.

The first pathway comprises catalysis of ethanol to acetaldehyde by alcohol dehydrogenase. Acetaldehyde is further metabolized by aldehyde dehydrogenase to acetate.

The two steps lead to accumulation of NADH as a result of continuous alcohol consumption. This high concentration of NADH inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate. The high concentration of NADH will lead to accumulation lactate and the consequences may be hypoglycemia and lactic acidosis [8].

Previous works revealed that alcohol consumption has effects on glucose levels of rats exposed directly to alcohol and this depends on the type of alcohol consumed and also affects the sugar levels in diabetics [11]. The liver performs the function of glycogenesis and glucogenolysis. This result is similar to those of Michele, [2]. Alcohol therefore reduces glucose levels on the offsprings of both prenatal and pre and post natal alcohol exposed. This finding suggests that alcohol could be diabetogenic. Studies carried out by [3] revealed that consumption of alcohol in diabetic rats decreases body weights. Though this work is not specifically on diabetic rats but has demonstrated that alcohol consumption both prenatally and pre & postnatally can be deleterious on the offsprings due to possible inhibition of gluconeogenesis.

In this study urea levels of the offsprings in alcohol exposed rats both prenatally and pre and postnatally showed no differences when compared with control group. (Fig7).

According to [16], alcohol consumption acutely down regulates urea synthesis in healthy volunteers, favouring nitrogen preservation.

These findings did not differ from our results because all our findings from pre natal alcohol exposed and postnatal alcohol exposed rats had no differences ($P>0.05$) with the control groups. This is so because only the mother and not the offsprings are exposed to alcohol. High urea levels are implicated in renal failure, dehydration, diabetes and diet. This suggests that alcohol may affect urea level only when it may cause some physiological changes like renal failure, dehydration and stress related situations

[9]. This investigation cannot be reconciled with reported nitrogen wasting of chronic alcoholics.

The research carried out observed that alcohol exposure of albino rats did not statistically affect the creatinine levels on the offsprings in weeks 2, 3, 4 and 6. There was an elevation of creatinine levels in week 5 of postnatal alcohol exposed (Group C) as seen in figure 8.

These findings did not suggest reason for the change in week 5. However there is strong suggestion that factors other than alcohol can influence such changes. Such factors are likely to be dehydration, renal impairment of some rats in that particular group and probably genetic. The association between alcohol consumption and renal function is poorly understood [5]. There was an evidence that chronic alcohol consumption may cause direct damage to the kidneys [8]. It may also indirectly alter renal function by elevating blood pressure.

Alcohol consumption was independently and significantly associated with a higher level of estimated Ccr and GFR as well as

serum urea [5]. This assertion is in line with the pattern presented in week 5 of figure 8, where we noticed significant changes in post natal alcohol exposed against control group. This implies that constant exposure to alcohol may affect creatinine levels as a result of blood pressure which may cause renal dysfunction [17].

However in a study carried out by [16], it was concluded that alcohol intake has no effect in glomerular filtration rate and serum creatinine levels. [7] in a prospective study showed that alcohol intake has no long term adverse effect on renal function as assessed by calculating creatinine clearance rate Ccr and glomerular filtrate rate (GFR) and may in fact have a renoprotective effect in women with hypertension. This study is in line with our findings in weeks 2, 3, 4 and 6 where alcohol intake in both pre natal and post natal groups show no significant increase on the creatinine levels of the offsprings when compared with control group

CONCLUSION

Maternal alcohol consumption have been shown to affect some biochemical parameters of the offsprings. This could expose them to serious health challenges. It has been demonstrated that acute or chronic alcohol consumption during pregnancy and lactation periods can

affect the liver enzymes, bilirubin levels and most biochemical activities. Therefore, pregnant and nursing mothers are advised to abstain from alcohol consumption.

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