Maternal Alcohol Consumption Biochemical and Haematological Findings During Pregnancy and Lactation Periods in the Offsprings of Albino Rats.

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ABSTRACT

Alcohol ingestion during pregnancy and lactation periods can cause some serious health challenges and may result in fetal disturbances in their off-springs. The experimental rats were grouped into three-A, B and C. Group A was the control while B and C served as test groups. Group B was administered with 1ml of 20% ethanol per kg weight up to the delivery time while group C was exposed with 1ml of 20% ethanol per kg weight up to the weaning period. A total of 120 off-springs from the three groups were used in the investigations. Eight off-springs were randomly selected every week from each group and 3 mls of blood was collected by ocular puncture for a period of five weeks. Vitros 360 chemistry analyzer was used for biochemical analyses while all haematological parameters were evaluated using Sysmex 2100 haematology analyzer. Mean values of the aspartate transaminase (AST), alkaline phosphatase (ALP) activities and bilirubin level were significantly increased (P<0.05) in all the weeks understudy 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 test group C. Furthermore, the mean value of cholesterol and creatinine levels were significantly increased in weeks 5 and 6 of the test groups while the mean value of glucose concentration were lower in the test groups compared with control. However, there was no significant changes (P>0.05) in mean value of urea in the test group compared with control. Also the mean leucocytes value was significantly lower (P<0.05) in the test groups B and C compared with control while no significant changes (P>0.05) in the mean value of haemoglobin levels of the off-springs compared with control. Furthermore while the mean value of haematocrit were significantly lower (P<0.05) test groups of weeks 3,5 and 6, the MCHC showed significant differences in weeks 5 and 6 of test groups compared with control. These investigations have therefore demonstrated that maternal alcohol consumption during prenatal and lactation periods has some deleterious effects on some biochemical and haematological parameters of their off-springs. Therefore, there is the need for controlled alcohol consumption during pregnancy and lactation periods.

Keywords: Pregnancy, alcohol, maternal, lactation.

INTRODUCTION

Alcoholism among pregnant women especially in riverine areas has been worrisome and observed to be socially acceptable amongst them. Incidence of liver diseases and reproductive function derangement had been high among this group. Acute and chronic alcohol abuses have wide spread direct and indirect effects on the haematological system which can mimic or obscure other disorders. Haemapoesis from stem cell in the bone marrow may be altered as well as leukocyte, erythrocyte, and thrombocyte functions [1].

Alcohol ingestion during pregnancy can result in fetal disturbances in their off-springs [2]. In experimental animal models the syndrome is characterized by

retardation of fetal life. Fetal alcohol syndrome is a pattern of mental and physical defect that can develop in fetus in association with high level of alcohol consumption during pregnancy [3]. Heavy alcohol consumption can cause damage to the central nervous system (CNS) and can affect the fetal brain structure and functions [4]. Besides damage to the CNS alcohol can result in disruption of hypothalamus pituitory gonadal axis which plays regulatory roles in reproduction. The pituitory on stimulation secrets luteinizing hormone and follicle stimulating hormone which are responsible for testosterone production and sperm cell maturation respectively. Deficiency of these hormones due to chronic alcohol intake could cause testicular, prostate and seminal vesicle atrophy [5], Investigations revealed that light to moderate drinking helps to improve cardiovascular health with decreased mortality rates and with decreased risk of cardiovascular disease[6]. Heavy drinking can increase the amount of triglycerides in the blood. This can cause heart disease, high blood pressure, obesity and type 1 diabetes mellitus. Many of the pathophysiological effects of alcohol ingestion are related to the pathway of ethanol metabolism [7]. Investigations reveal that heavy alcohol consumption affects some haematological patterns and several cell lines. It suppresses platelet production and causes thrombocytopenia, anaemia and many other blood disorders [8].

Alcohol may have direct or indirect impact in the haemopoetic system. The direct effects are primarily seen in the bone marrow and this involves the leucocytes, erythrocytes and the 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 [16]. 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

thrombocytes. The indirect effects are as a result of changes in the hepatic functions resulting in liver diseases [9]. Alcohol consumption may bring about changes that may alter the release of liver enzymes by disrupting the membranes of some specific organelles like the mitochondria. Once the liver is compromised, major liver diseases such as fatty liver, alcoholic hepatitis and cirrhosis may set in[10]. The metabolic alterations will equally result to some nutritional abnormalities, such as folate deficiency [11]. Alcoholics frequently have defect in the red blood cells that are destroyed prematurely possibly resulting in anaemia. The direct consequences of excess alcohol consumption according to other researchers include toxic effect on the bone marrow, the blood cell precursor, white blood cell and platelets [12]. Moderate consumption of alcohol can help improve cardiovascular health. This beneficial effect of moderate alcohol consumption might be explained by a rise 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 (Adams, 2011). 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 [14]. Also alcohol reduces functional hepatic nitrogen clearance and acutely down regulate urea synthesis in normal men [15].

organs and systems of adults [17]; [18], [19]. Also the effect of maternal alcohol consumption on different organs and systems of the developing fetus have been reported by [20]. A Large number of research had exposed the deleterious effect of alcohol consumption during pregnancy on both the mother and fetus [21], [22], [3] and [23]. Following reported increase in alcohol consumption among pregnant and breastfeeding women,this research is designed to investigate the

effects on some biochemical and haematological parameters of their offsprings.

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

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
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A total 150 male off-springs of the albino rats were used to carry out the work.30 rats died at various stages of the investigations. Only 120 male off-springs were finally used to carry out the

where bred by using1male 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

investigations. The tests were carried out every week for 5 weeks .In each week,24 male off-springs were randomly selected.8 from each group.

SAMPLE COLLECTION AND ANALYSES

About 3mls of blood samples were collected by ocular puncture using capillary tubes. 1ml was placed in tubes containing tripotassium ethylene diamine tetracetic acid salt (EDTA) and mixed thoroughly by invasion for haematological analyses.The remaining 2mls was allowed to clot,centrifuged at

3000g for10 minutes. The serum was separated into test tubes for biochemical analyses. All haematological parameters were investigated with Sysmex XE-2100 haematology automated analyzer while biochemical tests were carried out using Vitros 360 auto chemistry analyzer.

ESTIMATION OF HAEMATOLOGICAL PARAMETERS AND INDICES

All haematological parameters were determined with Sysmex XE-2100 haematological automated analyzer. This performs haematology analysis according to radio frequency/ direct current detection method. The size of blood cells was detected by direct current resistance

(DC) and the density of blood cell interior by changes in radio frequency (RF).Samples were analyzed for haemoglobin concentration,packed cell volume, total leucocyte count and mean cell haemoglobin concentration (MCHC).

ESTIMATION OF BIOCHEMICAL PARAMETERS

All biochemical analyses in this work was carried out with Vitros 360 auto analyzer according to manufacturer 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. 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.

STATISTICAL ANALYSIS

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

RESULTS

RESULT OF ASPARTATE TRANSAMINASE LEVELS OF OFFSPRINGS
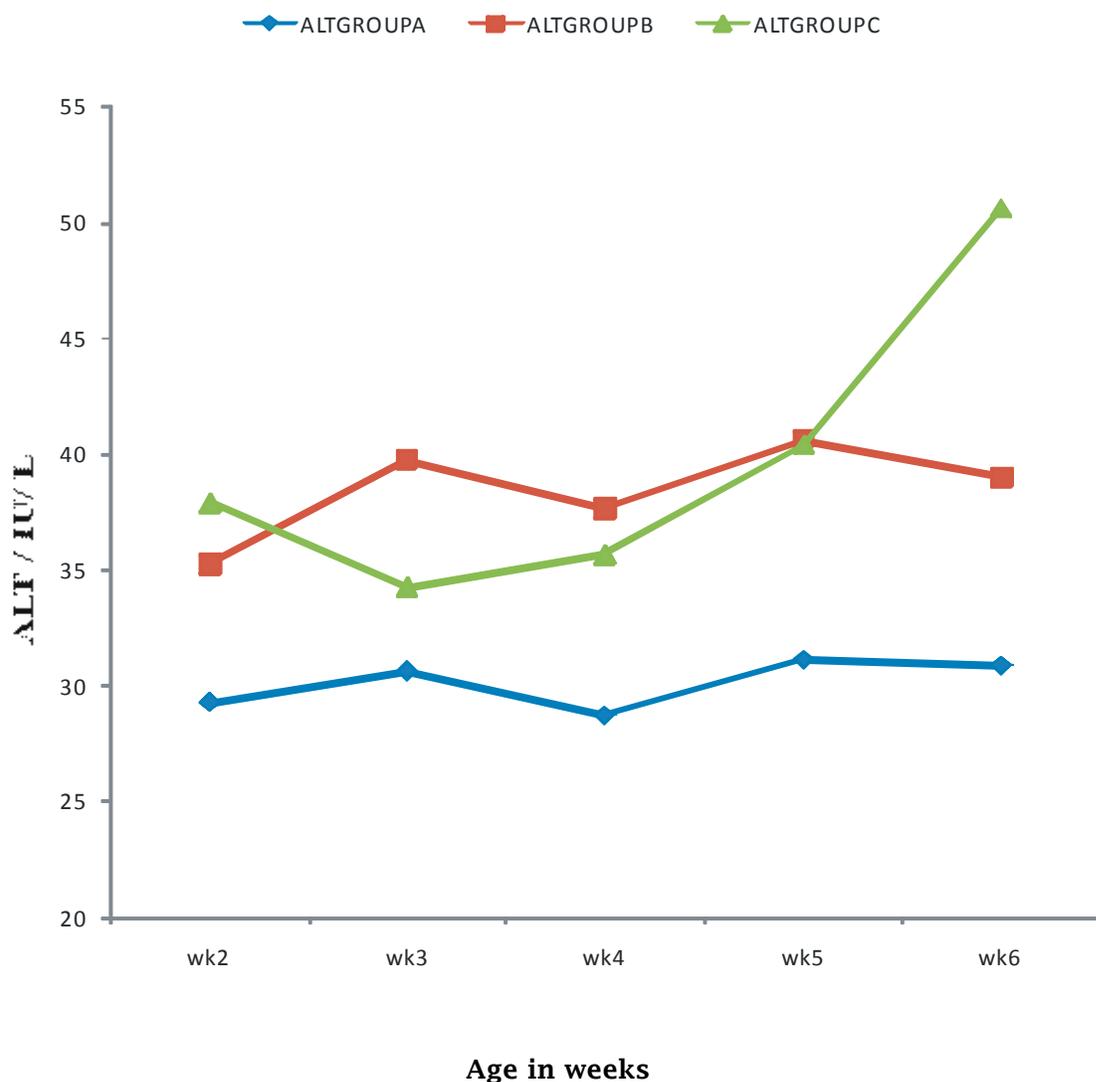

Age in weeks

Fig1. Effect of maternal alcohol consumption on AST activities of the

offsprings. AST activities were higher in alcohol exposed groups B and C than in control group A. Group C had higher AST levels in weeks 4 and 5 compared with groupB.
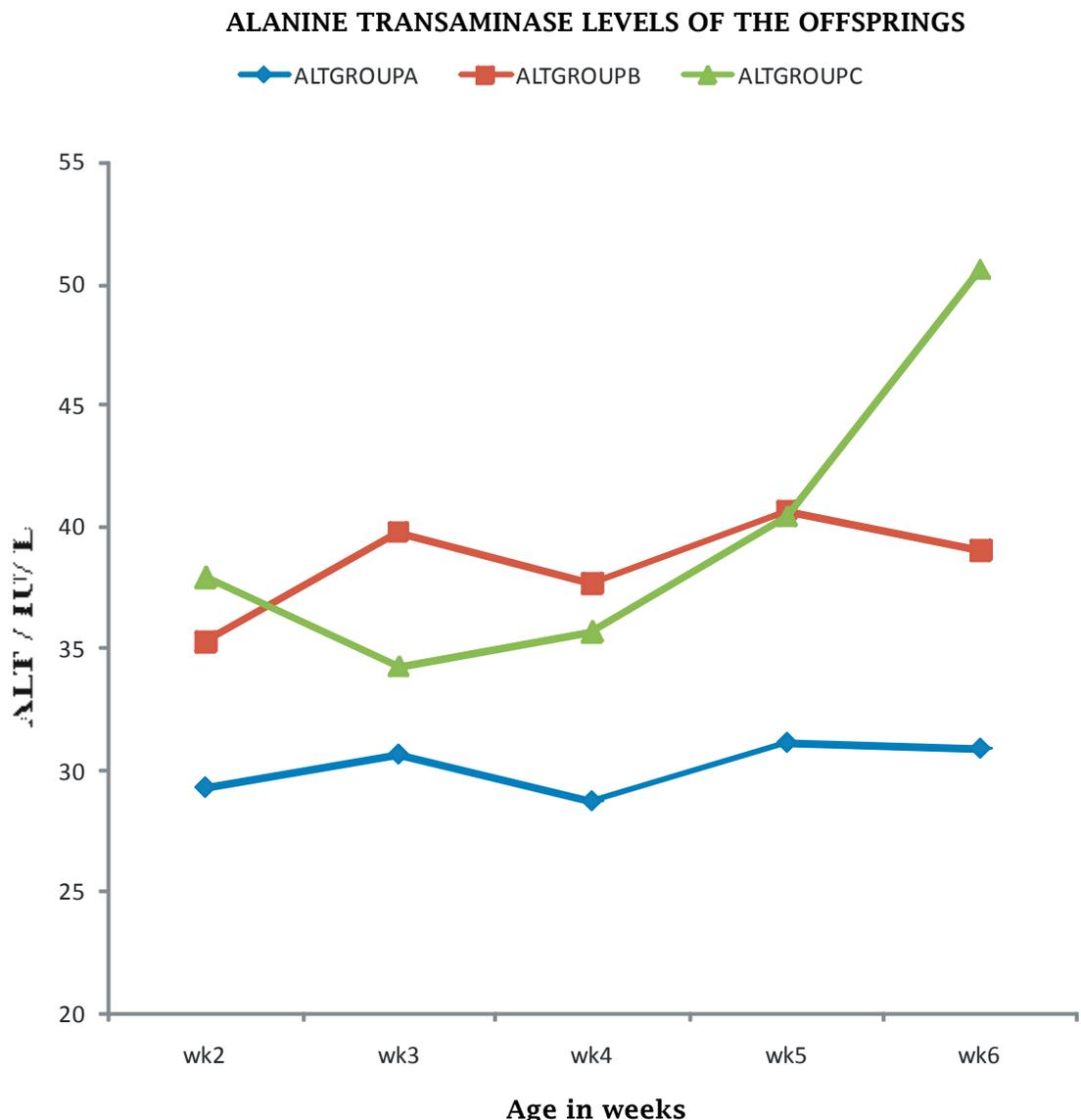
ALANINE TRANSAMINASE LEVELS OF THE OFFSPRINGS

Fig 2: Effect of maternal alcohol consumption on ALT levels of the off-springs. There were no significant differences on ALT levels from weeks 2 to 5 but in week 6 (P<0.05) significantly higher values were observed in group C.

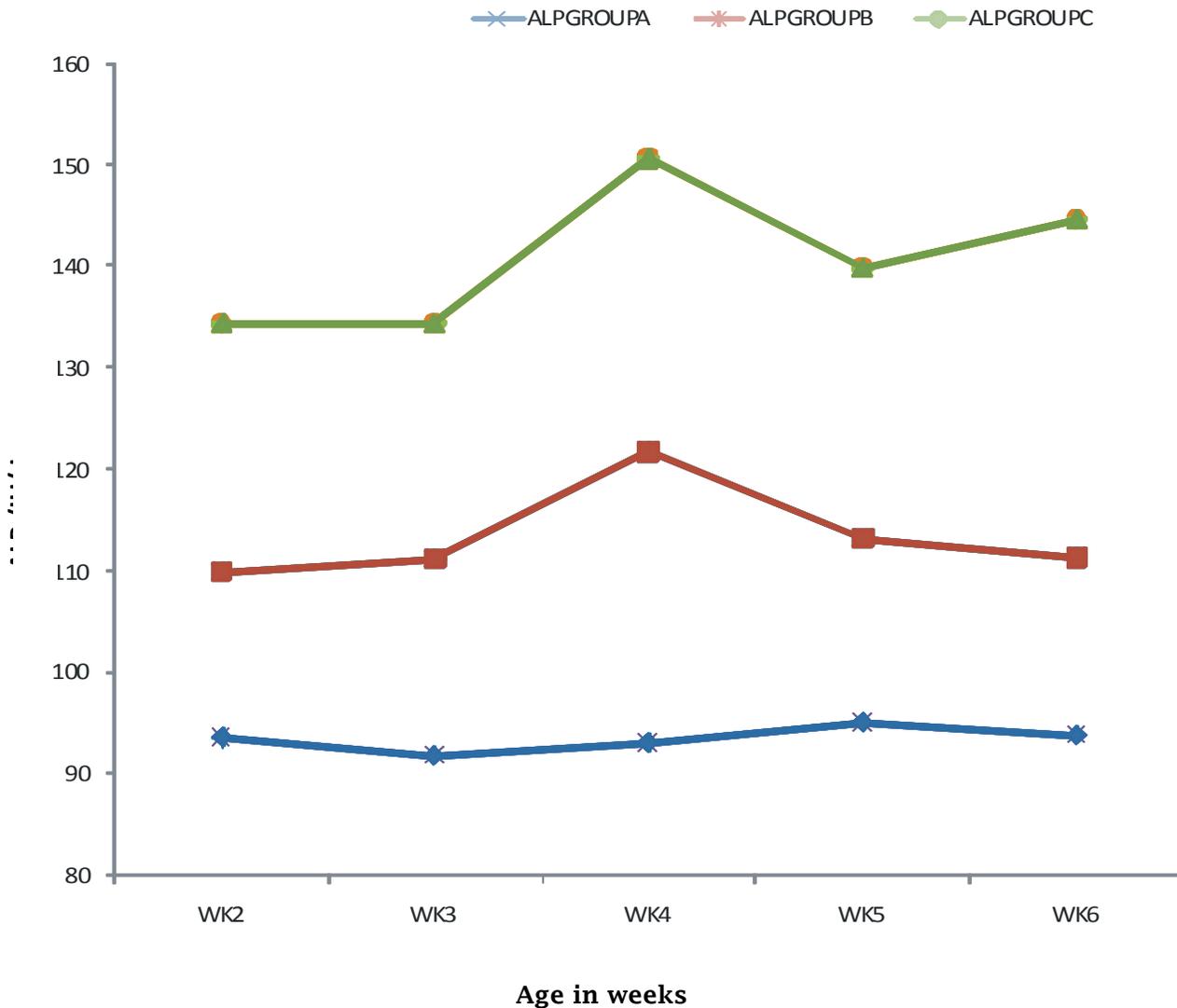**ALKALINE PHOSPHATASE (ALP) LEVELS OF THE OFF-SPRINGS**

Fig 3: Effect of maternal alcohol consumption on alkaline phosphatase levels of the offsprings. There were significantly high mean values of ALT in

with group B.

prenatal alcohol exposed group B and post natal exposed group C. However group C had higher mean values compared

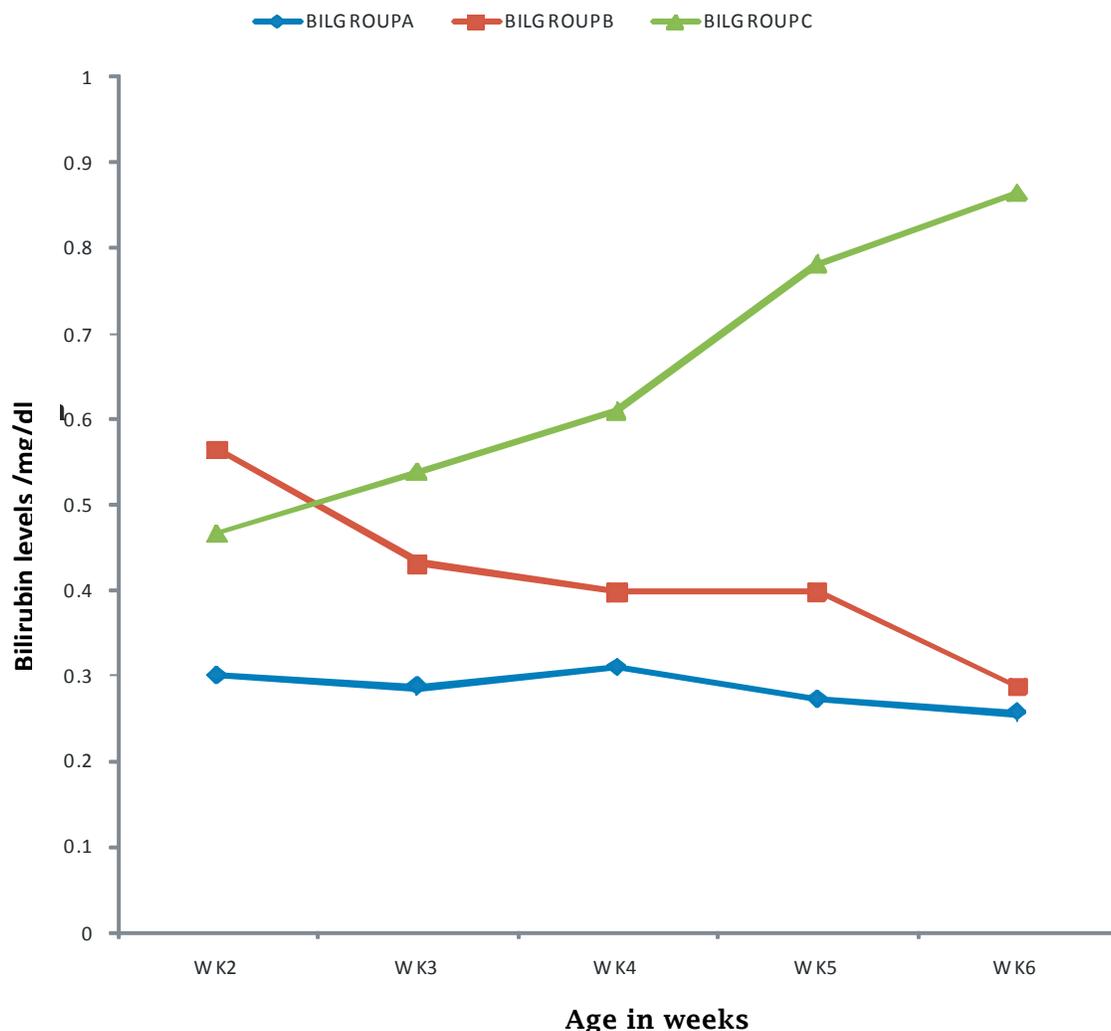**RESULT OF BILIRUBIN LEVELS OF THE OFF-SPRINGS**

Fig 4: Effect of maternal alcohol consumption on the bilirubin levels of the offsprings. There were significant differences (P<0.05 in mean values of pre natal group B and post natal alcohol exposed group C .
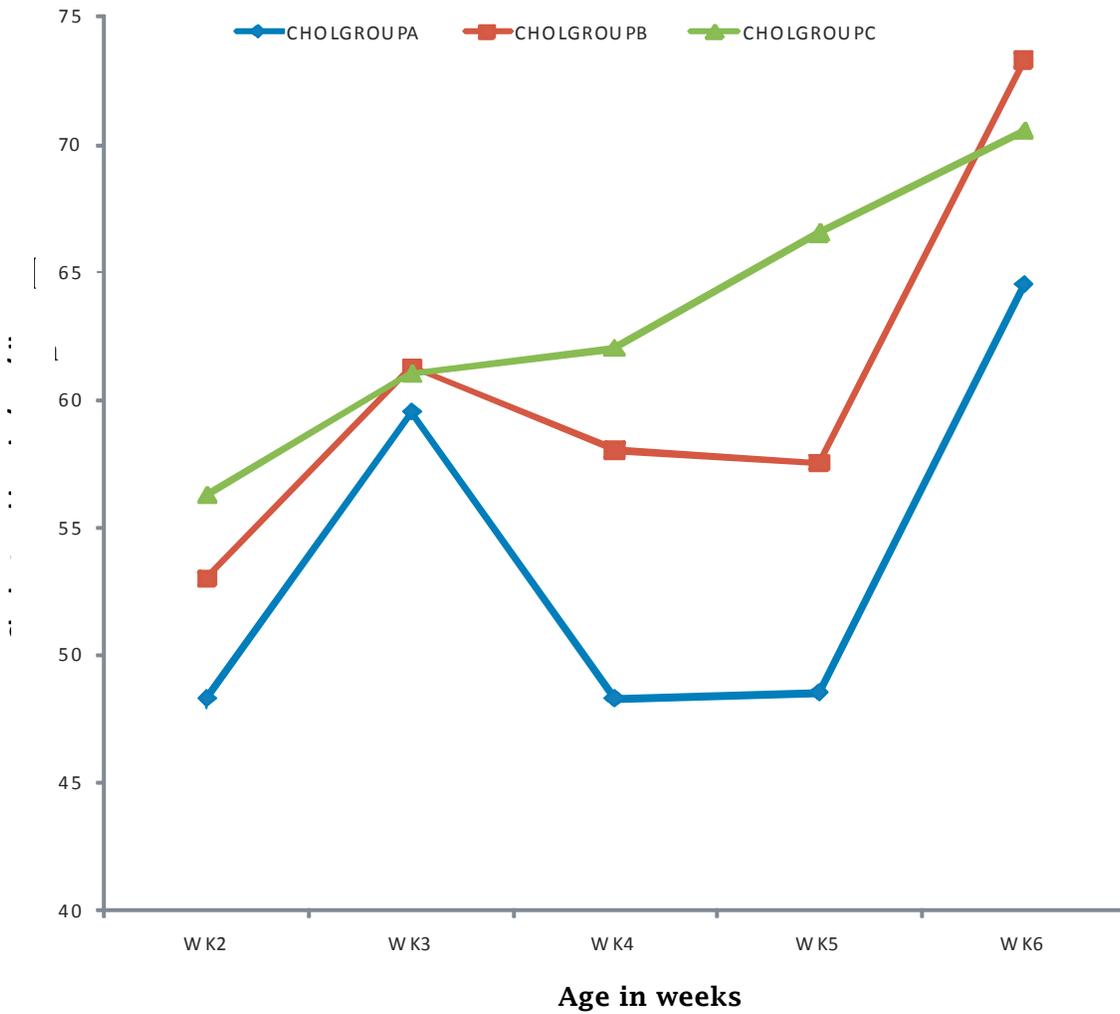
TOTAL CHOLESTEROL LEVEL OF OFF-SPRINGS.

Fig 5: Effect of maternal alcohol consumption on cholesterol levels of the offsprings. In weeks 4 and 5 group C had higher cholesterol mean value (P>0.05) than B.

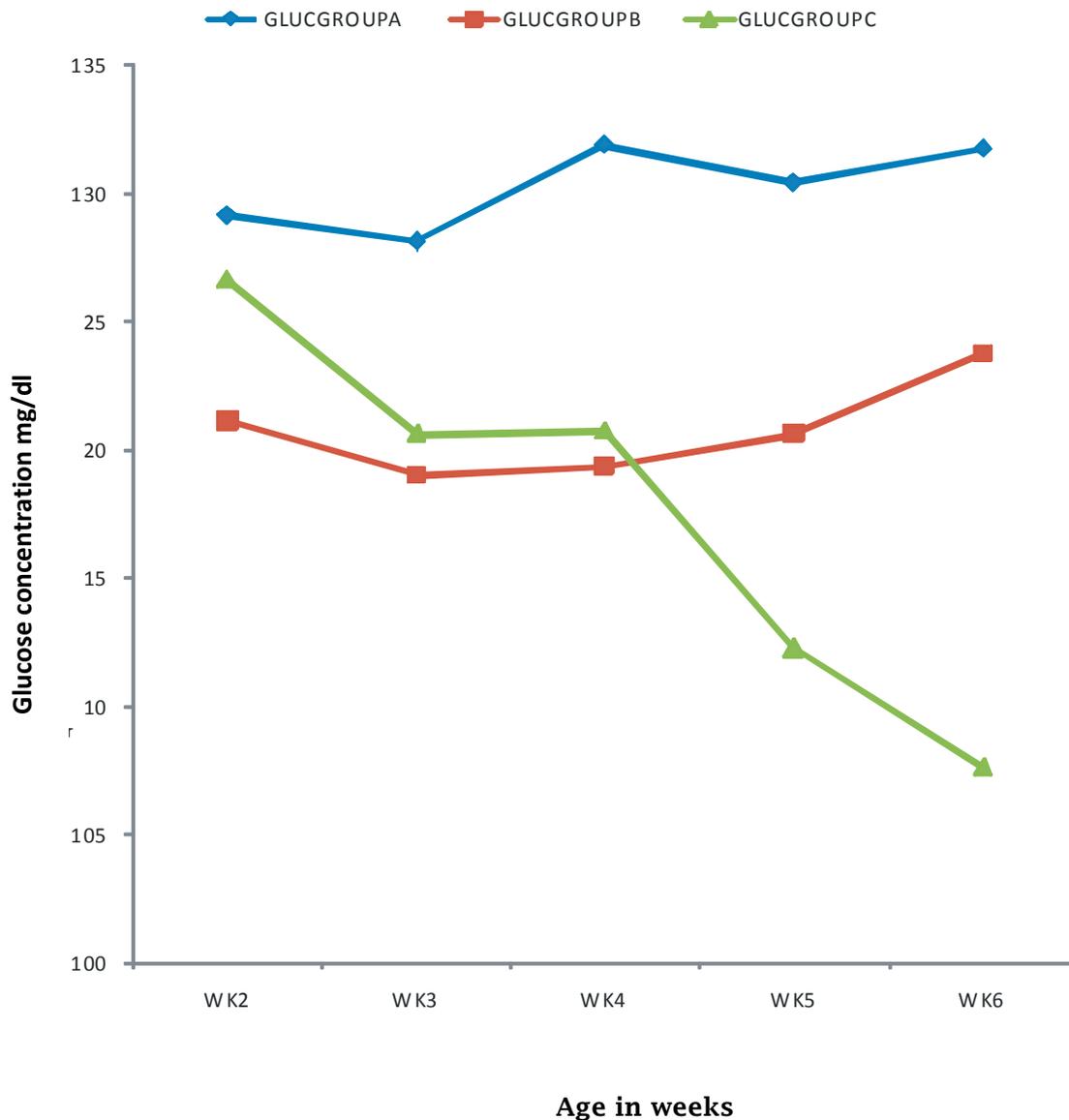**RESULT OF GLUCOSE LEVELS ON THE OFF-SPRINGS OF ALBINO RATS**

Fig 6: Effect of maternal alcohol consumption on the glucose levels of the offsprings compared with control group. Weeks 2 and 3 showed no significant mean values while week 6 had the lowest mean glucose level (P< 0.05).
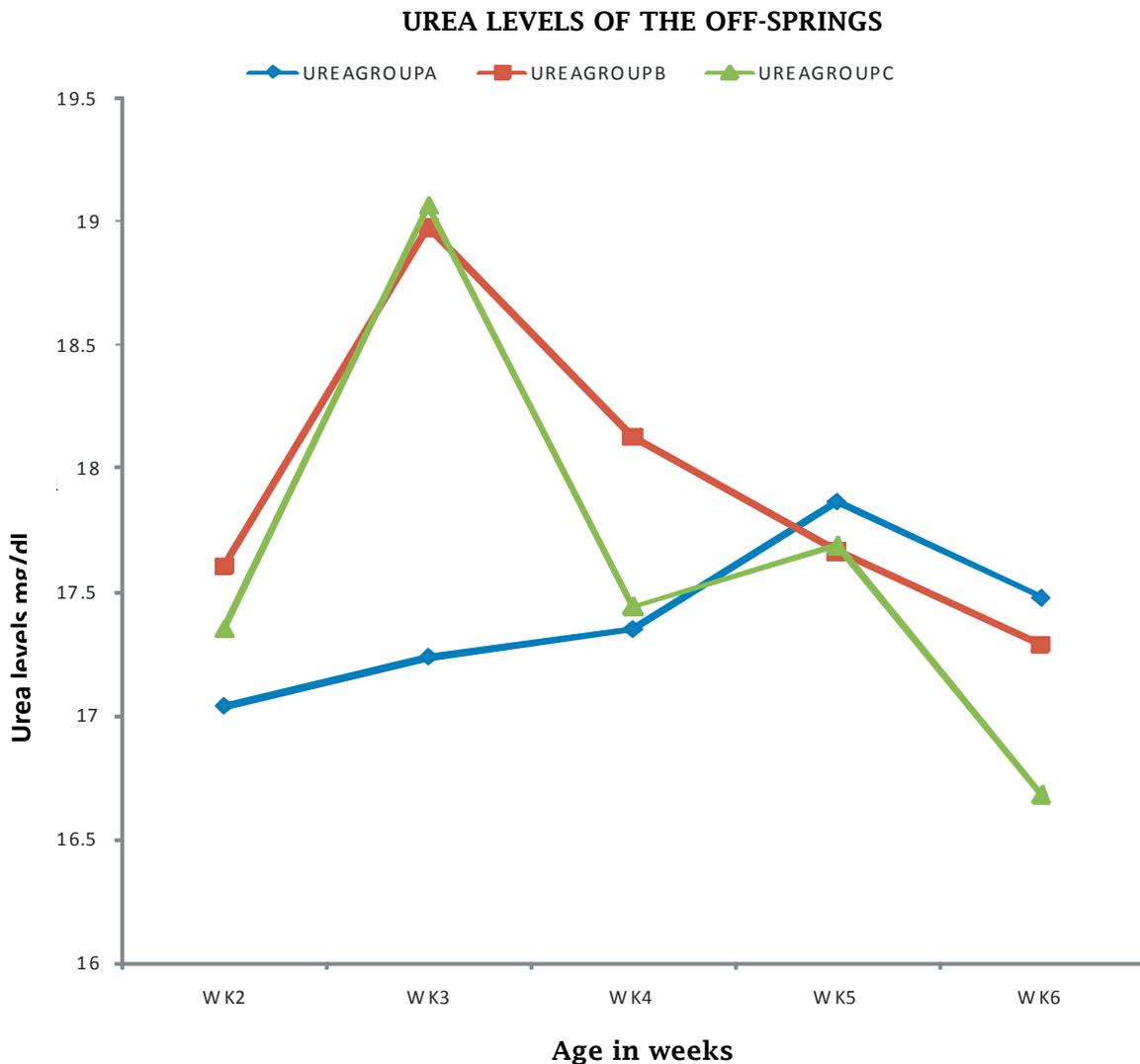
UREA LEVELS OF THE OFF-SPRINGS

Fig 7: Effect of maternal alcohol consumption on urea levels of the offsprings. There were no significant difference (P >0.05) in mean values of serum levels in weeks 2,4,5 and 6. Week 3 showed higher values (P<0.05) in groups B and C.

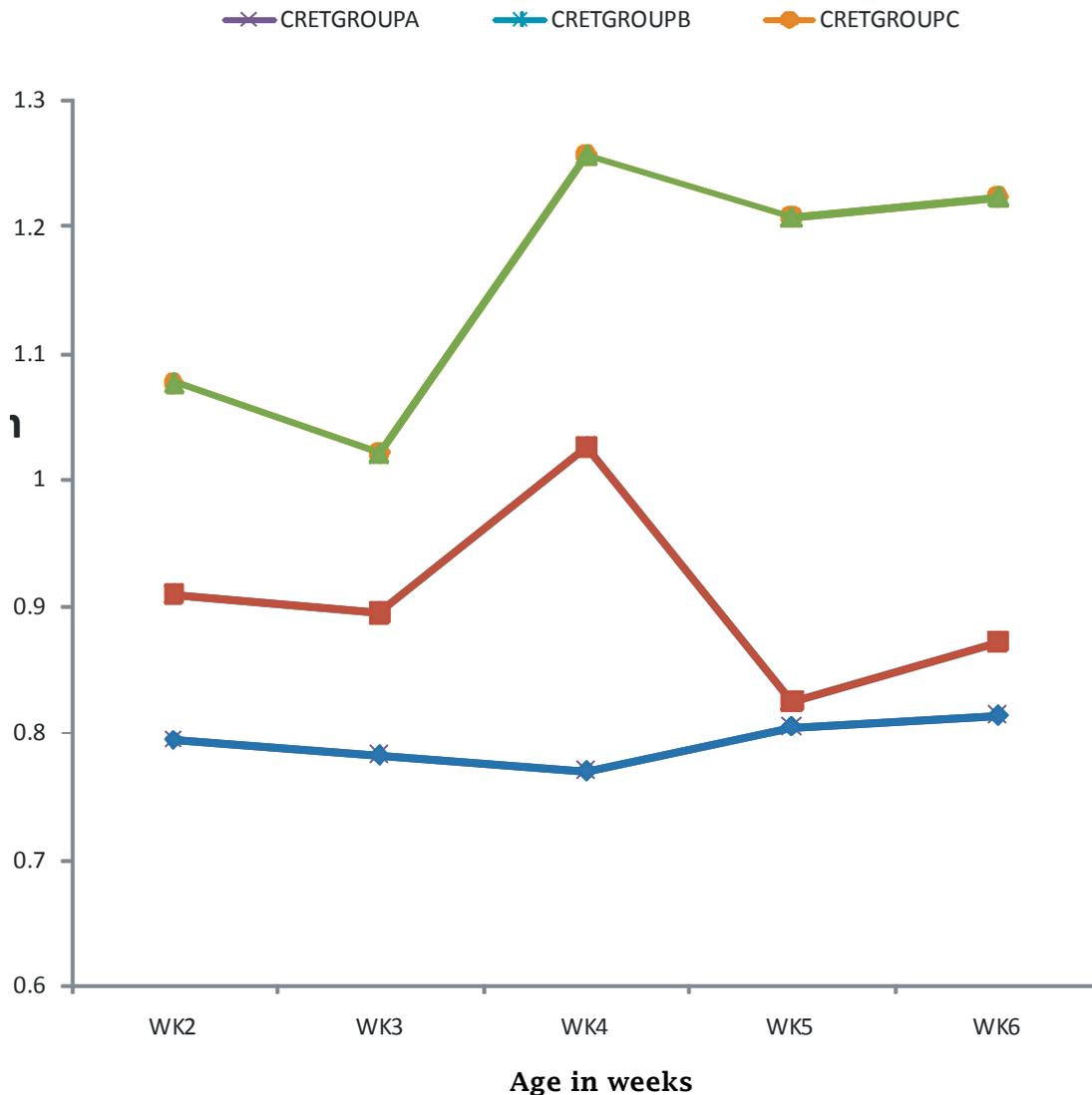**CREATININE VALUES ON THE OFF-SPRINGS OF ALBINO RATS.**

Fig 8: 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.

RESULTS OF HAEMOGLOBIN CONCENTRATION OF THE OFF-SPRINGS.
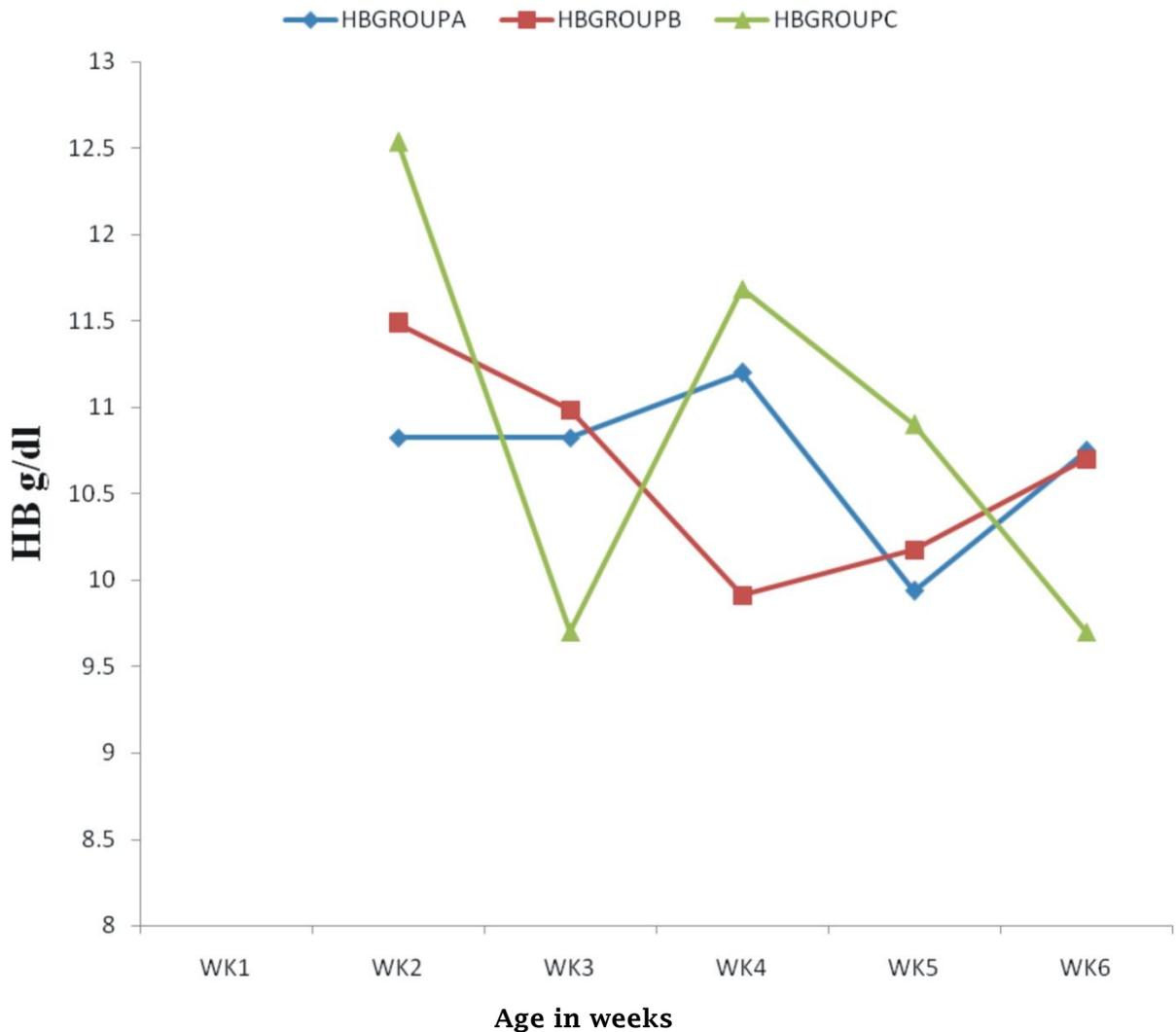
Fig 9: Effect of maternal alcohol consumption on the haemoglobin concentration of the off-springs. No
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significant differences (P>0.05) on the HB values of the control and the test groups

LEUCOCYTE COUNTS ON THE OFF-SPRINGS OF THE EXPERIMENTAL RATS
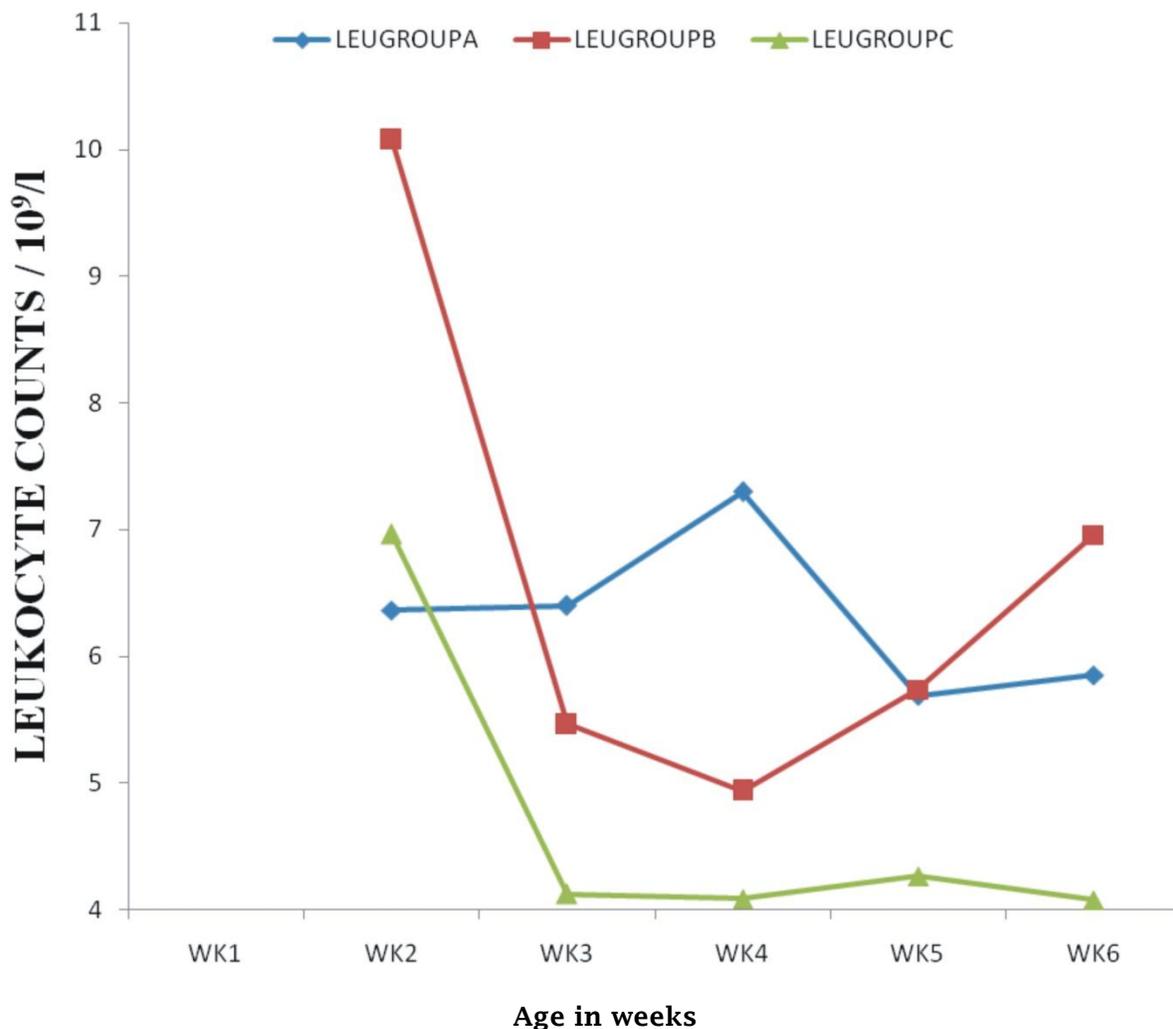

Fig 10: Effect of maternal alcohol consumption on the leucocyte count of the off-springs. Significant differences were indicated (P<0.05). Group C had lowest leucocyte count.

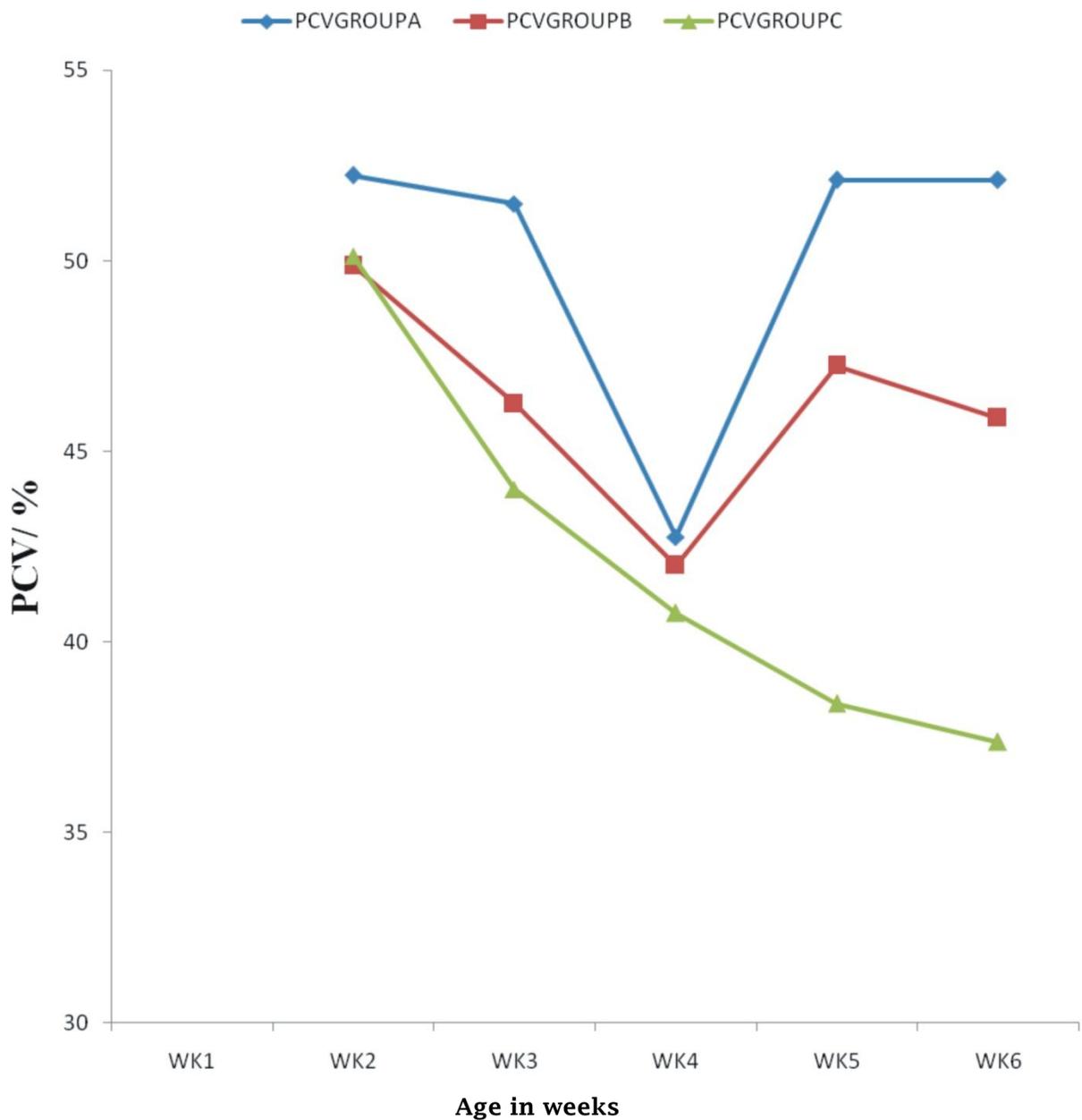**HAEMATOCRIT (PCV) VALUES ON THE OFF-SPRINGS OF EXPERIMENTAL RATS.**

Fig 11: Effect of maternal alcohol consumption on haematocrit (PCV) of the off-springs. There were significant differences in weeks 3, 5 and 6.

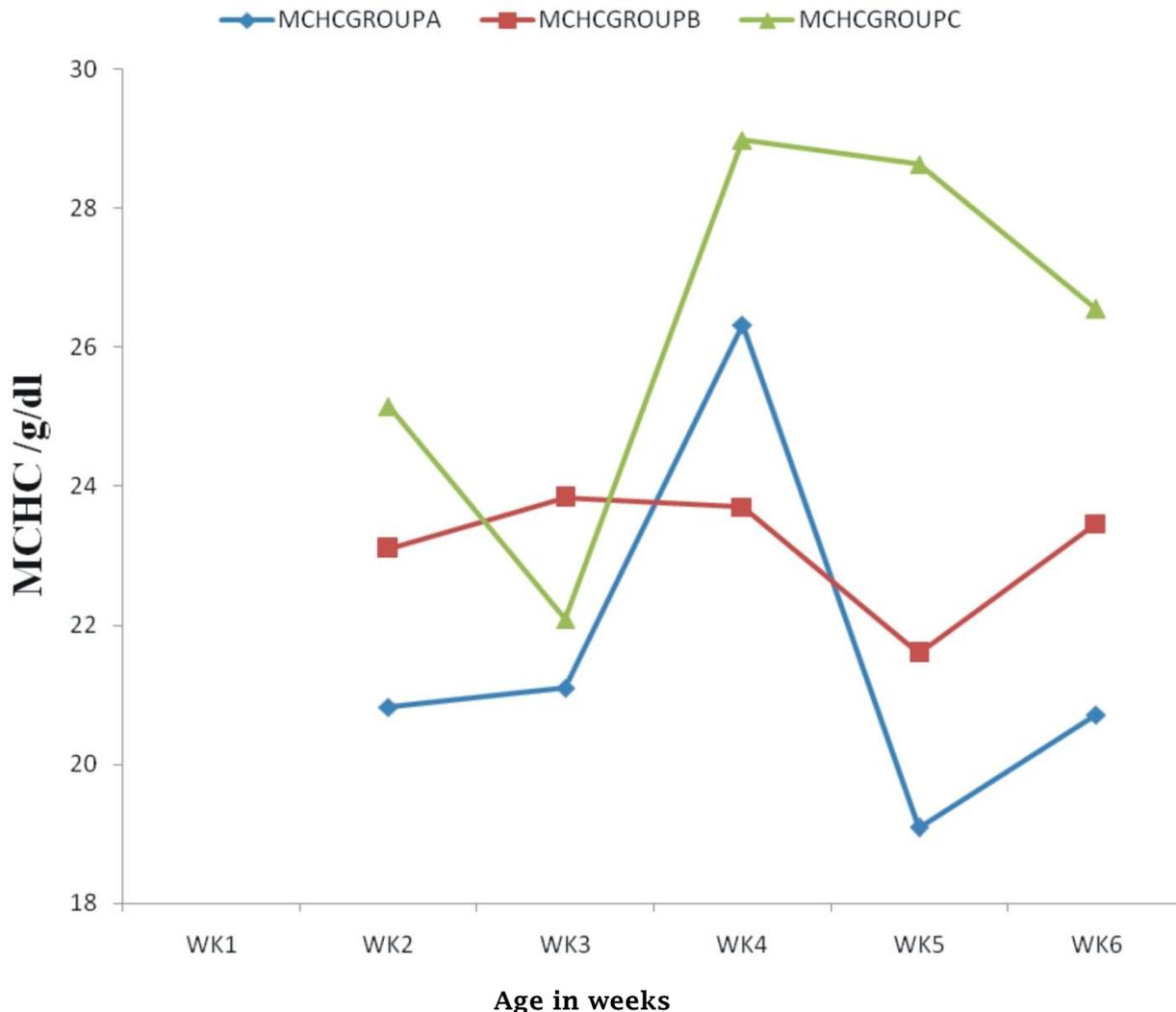**RESULT OF MCHC ON THE OFF-SPRINGS OF THE EXPERIMENTAL RATS**

Fig 12: Effect of maternal alcohol consumption on the mean cell haemoglobin concentration (MCHC) of the off-springs. The mean values showed significant changes in weeks 5 and 6 ($P<0.05$).

DISCUSSION

These investigations have apparently demonstrated that maternal alcohol consumption in albino rats can cause a lot of changes in the off-springs. As shown in Figs 1 to 12. 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 transminase and alkaline phosphatase activities in the offspring of mothers drinking alcohol during pregnancy and lactation periods. This was similar to a research carried out by [24]. [25] and [26]. 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 Following acute or chronic alcohol ingestion, the hepatocytes and mitochondria membrane permeability increase.Alcohol causes injury to a specific hepatocyte organelles 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' nudeotidase [26] [27]. 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 [9].

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 as seen in fig 4. It has been demonstrated that many diseases of the liver are accompanied by jaundice [4].The diseases of the liver include fatty liver, alcoholic hepatitis and cirrhosis [3].The rats used in this investigation were classified as heavy drinkers. They are groups that took alcohol consistently on daily basis [19]. 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 [5]. 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 5. Liver mitochondria can convert acetate to acetylCoA in a reaction requiring ATP and catalyzed by the enzyme thiokinase. However further processing of the acetyCoA 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 [9] 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 [11],[17] 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. [8] 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 hypoglyceamia and lactic acidosis [9].

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 [5]. 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 [20] 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 offsrpings 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 [20], 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 [27]. 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 off-springs in weeks 2, 3, 4 and 6. There was an elevation of creatinine levels in week 5of 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 [6]. 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 [13]. 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 [19].However in a study carried out by [16], it was concluded that alcohol intake has no effect in glomerular filteration rate and serum creatinine levels. [8] 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.

Haemoglobin estimation on the off-springs demonstrated no significant differences (P> 0.05) in test groups B and C compared with control group A as seen in fig 9.
Alcohol doesn't seem to affect the haemoglobin values of these groups compared with control groups. This is in contrast with earlier findings by some researchers. According to work carried out by [12] alcohol has adverse effects on blood cells and their functions. According to them, alcoholics frequently have defective red blood cells that are destroyed prematurely possibly resulting in anaemia. Alcohol exerts a direct effect to the bone marrow, the blood cell precusor, the white cell and platelets which may result to anaemia, leukaemia and thrombocytopenia [1] Alcohol consumption may result in liver diseases such as alcoholic hepatitis and cirrhosis and may indirectly affect haematological functions and metabolic derangement [13].
A study carried out by [1] recorded higher haemoglobin concentration and packed cell volume (PCV) in heavy alcohol drinkers. Our research work with off-springs of albino rats presented neither low haemoglobin levels which is an indication for anaemia nor high haemoglobin concentration as a result of dehydration. Therefore maternal alcohol consumption has no direct effect on the haemoglobin levels of their off-springs. The values obtained for haematological parameters showed a significant differences (P<0.05) in the packed cell volume (PCV) values of the off- spring.

(Fig 3). The values obtained from test groups B and C were lower compared with control group A. Those findings are in contrast with previous work carried out by [6] and [15], who recorded higher PCV values in alcoholic as a result of dehydration. The reason for this variation could be adduced to the fact that other researchers carried out their work to determine the effect of alcohol on the direct consumer while this work was designed to investigate the effect on the off-spring of mother indulging in alcohol consumption during pregnancy.

Furthermore, there were significant reduction (P<0.05) in mean leucocyte count of the test group B and C compared with control group (Fig2). Alcohol consumption by pregnant mothers can adversely affect the leucocyte count of their off-springs. This is comparable to previous research of [12] who reported that heavy alcohol consumption has direct consequences in blood cell precursor, mature red cells, luecocyte and thrombocyte but failed to agree with the work of [15] whose work showed high significant leucocyte values. Maternal alcohol consumption affects the white blood cell of the offspring thus exposing them to various infections. Many clinical observations support that alcohol adversely affect the production and function of virtually all types of blood cells [7], [19].Thus alcohol is toxic to the bone marrow which contains the precursor for all blood cells .
The investigation demonstrated that mean cell haemoglobin concentration (MCHC) in the off-springs of albino rats exposed to alcohol showed significant difference (p<0.05) in weeks 5 and 6 compared with control group (Fig4). Therefore the MCHC of the off-springs exposed to alcohol are affected by constant exposure to alcohol.

CONCLUSION

In this study alcohol consumption during pregnancy have been shown to have adverse effect on some biochemical and haematological parameters of the off-springs which could expose them to serious health challenges. Consistent consumption of excessive alcohol has deleterious effects on the off-springs.

Thus pregnant and breast- feeding mothers are advised to take little or no alcohol. More so health workers should be encouraged to carry out public enlightenment to inform the society of biochemical and haematological health risk of maternal alcohol consumption during pregnancy on their off-springs.

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