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Original Article

Determination of Risk of Birth Defects Associated with three herbal bitter Formulations Administered during Organogenesis in female Albino rats

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ABSTRACT

The use of herbal bitters gained its stay since ages and they are prone to abuse by the general public including pregnant women. In this study we investigate possible birth defects associated with the use of herbal bitters, three popular bitter formulations in Nigeria herberceutical market were studied during organogenesis stage in female rats. Forty Female rats weighing (187.5g ± 41.48) were divided into four groups of ten in each group: Group A, for Control group; B for Bitters B; C for Bitters C and D for Bitters D, administered from 6-17th day of their gestation period. The administration of bitters was carried out within 11-12days. Five female rats in each group were randomly selected and sacrificed on the 20th day prior to 21st day of gestation. The fetuses were harvested through abdominal incision for morphological examination, biochemical and haematological analysis. The liver, kidney and brain of these 1st filial rats sacrificed were subjected to further histopathological examination. The results obtained in this study at ($p \leq 0.05$) level of significance revealed significant difference in the morphological parameters examined. There was also statistically significant increase in the RBC, WBC and platelet at ($p \leq 0.05$) concentrations as compared with control group. . The biochemical examination (ALT and AST) for the treatment groups revealed a significant elevation ($p \leq 0.05$).Oral ingestion of these bitters were teratogenic to the growing fetus. Thus, recommended that they should be avoided at all stages in pregnancy.

Keywords: *Bitters, Organogenesis, Birth defect, Teratogenesis*

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INTRODUCTION

Birth defect simply means a case of deformity. It is a problem that emanates while a baby is developing in the fallopian tube. A birth defect may affect how the body looks, works or both. Some birth defects like cleft palate lip or neural tube defects are structural problems that can be easily seen. Birth defects can vary from mild to severe depending on the stage of exposure to the teratogen. There has been extensive research into the structure and working of the foetus. In humans, the foetal stage of prenatal development may be defined as beginning at the eleventh week in gestational age, which is the ninth week after fertilization (Klossner, 2005). In biological terms however, prenatal development is a continuum, with no clear defining feature distinguishing an embryo from a foetus.

The use of the term "foetus" generally implies that a mammalian embryo has developed to the point of being recognizable as belonging to its own species, and this is usually taken to be the 9th week after fertilization. A foetus is also developed and functional, and may not all be situated in their final anatomical location. Evidence has revealed that a lot of birth defect in human are caused by indiscriminate use of drugs that are injurious to health. (Awodele*et al,* 2013)

Herbal medicine is a practice that is as old as mankind; every human culture on every continent of the earth has practiced herbal medicine of one form or another. Herbal bitters are much sought after for their health benefits. They have become common medicines in many Nigerian homes. But recent research has raised concerns that indiscriminate use of packaged herbal bitters may have a toxic effect on the spleen, pancreas, and heart. Also it may possibly have a toxic effect on the growing foetus in pregnant women. Plant extracts, now popularized as 'herbal medicines', have been shown to prevent, treat, manage and cure several diseases from cough to cancer. (Agarwal and Bajpai, 2010)

This proven efficacy has resulted in great patronage for any product that comes with the name 'herbal'. Most companies rely on this window to rip in more profits. However, the manufacturers in an aggressive marketing drive claim they are recipes for indigestion, weight loss, youthfulness, and strength among others, claimed and proved by the leaflets in the packaged bitters. Medicinal, herbal bitters contain blended ingredients in a water or alcohol (tincture) base. Originally sold as a digestive aids because of their ability to increase the production of saliva and digestive juices, bitters became popular in Europe in the 1600s. This was due in part to the opening of trade routes with China, where the origin of bitters can be traced back more than 5,000 years. (Peter *et al.*, 2005).Today, herbal bitters are used primarily as digestive stimulants, detoxifiers and antibacterial agents.

Phytochemical analysis shows that bitters contain complex carbohydrates, alkaloids, vitamins and minerals that have antioxidant, antiviral and antispasmodic properties. It has been shown that these ingredients work together to reduce inflammation, control pain, relax muscles and improve digestion and elimination.(Hope, 2009) Bitters can also be effective as appetite stimulants in some people. Research works have been done on the effectiveness of these Bitters with their toxic effect on the body been neglected to the background. Therefore, the purpose of this research work was to investigate the teratogenic tendency of bitters on the foetus of female albino rats. There have been paucity of information relating to the use of bitters in pregnant women hence, this study was carried out to create health care awareness in the use of bitters.

MATERIALS AND METHOD

Materials: Three bitters formulations were obtained from the manufacturers' vendors and are labeled B, C & D. The B bitter contains Aloe-vera, *Acinosarventis, Citrus aurantifolia, Chenopodiummurale and Cinamomumaromtic* while the C bitter contains Aloe-vera, Senne Leaves, Rhubarb roots, Zedoary roots, Manna, Theriacvenez, S. opio, Angelica roots, Myrrh, Carline thistle, Camphor saffron and alcohol base(%unknown).The D bitter contains Erythrocarpus, Lecaniodiscus, Dialiumguineese, *Treculia Africana, Khayaivorensis* with 42% alcohol. The other materials include Cages, Feed (growers mash), Oral cannula, Weighing balance, Meter rule, Flat wood for dissection, Dissecting set, Marker, Swab stick, Cover slip, Slide, Microscope, Plastic bowl for feeding, Gloves, Wood shavings for bedding, Conical flask, Beaker, Measuring cylinder, Paper tape, Lithium Heparinized bottles, EDTA bottle (Ethylenediaminetetracetic acid), Needle and syringe (1ml), incubator, sysmex 2000i.

Reagents: Diethyl ether for anesthesia, Formalin for preserving the fetus, 0.9% of normal saline, distilled water, Phosphate buffer 100 mmol/L, pH 7.4, L-alanine 100 mmol/L,α-oxoglutarate 2.0 mmol/L, 2,4-dinitrophenylhydrazine 2.0 mmol/L, Sodium hydroxide 0.4 mol/L

Animal: Forty matured female albino rats with an average weight of 150g were obtained from Animal house, Olabisi Onabanjo University teaching

hospital, Sagamu, Ogun state. The animals were gotten directly from department of clinical pharmacy and biopharmacy, Faculty of Pharmacy Animal care unit and were acclimatized for two weeks before the experiment was commenced. They were fed on growers mash, animal feed and water. They were properly fed and the wood shavings for beddings were changed regularly to prevent infestations by insects and maggots.

Study Duration: The duration for this study was 10 weeks (2weeks for acclimatization and 8 weeks for the experiment). During the 10-week period, the animals were fed and monitored between the hours of 8:00am – 12:00 noon to monitor and record any form of behavioral and observational changes.

Cycle Determination (Detection of Pregnancy): Prior to administration of the bitters, it was ensured that the rats were pregnant, this was done by taking a smear from the rats vaginal with the use of swab stick from an already pregnant rat and a non-pregnant rat which was spotted on a slide and viewed under the microscope for early detection of pregnancy in rats. They were viewed under the microscope and a non-pregnant rat showed varying spotting in a dispersed phase which is the pro-estrus stage and it's the receptive state according to Marcondes*et al*, 2002, while for the pregnant rat the spotting's formed a straight line like shape which is known as the sperm plug. The detection of the sperm plug denotes the onset of pregnancy in rats (Marcondes*et al*, 2002).

To the four groups of ten albino rats each, four male rats of equivalent weights were introduced to each group. The vaginal smear was taking from rats in each group on a daily bases until the sperm plug was seen in all the female albino rats, and then the male rats were withdrawn.

Treatment Groups and Administration: The gestational period of an average non-lactating rat is 21-23 days depending on the numbers of lithers, as proposed by Helen (1913). Period of drug exposure which is the stage of organogenesis occurs within 6-17 days of pregnancy.

The therapeutic doses of the drug administration was done to simulate the treatment pattern of the bitters

Group A (control) received 0.9ml of distilled water; B received 0.1ml of the bitters B; C received 0.5mls of the bitters C and D received 0.5ml of Bitters D formulation. All administration was through the oral route with the use of oral cannula.

Morphological and Histopathological examination: After the stipulated period of administration three rats from each group were removed and dissected, the fetuses harvested with the aid of the dissecting set and the following morphological parameters were taken; the tail length, head crump, weight of the foetus, length of umbilical cord, the overall weight of fetus and placenta. The harvested fetuses were further subjected to gross histopathological analysis at the Morbid Anatomy Department of Olabisi Onabanjo University Teaching Hospital.

Biochemical and Haematological Examination: The other animals in each group were left to lither, the lithers after a month were sacrificed with appropriate apparatus. Blood samples were collected from the animals for biochemical and hematological examination. The biochemical parameters carried out include Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity which was determined following the principle described by Reitman and Frankel (1957) as described by Odewabi*et al*, (2013). The haematological parameters were carried out with the use of Sysmex 2000i machine. Parameters observed include the determination of the white blood cells (WBC), red blood cells (RBC), haemoglobin, haematocrit (PCV), platelet, lymphocyte count and percentage, neutrophil percentage, mean red cell volume, mean cell haemoglobin concentration(MCHC), and mean haemoglobin (MCH

Histopathological Analysis: The liver, kidney and the brain are the organs harvested from the sacrificed lithers, fixed in a labelled bottle containing 10% formaldehyde to preserve the tissues and were subjected to histopathological analysis undertaken in the histopathology laboratory of University of Ibadan with the use of hematoxylin and eosin staining technique which was then viewed under the electron microscope.

Statistical Analysis: The results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by F distribution test table were used to analyze the results with $p \leq 0.05$ considered significant.

RESULTS

Table 1: Physical observation of control and experimented rats during treatment with three bitter formulations

Physical observations	Control A	B bitters	C bitters	Dbitters
Skin Changes	-	-	-	-
Fur Changes	-	-	+	-
Eyes	-	-	-	-
Behavior pattern	-	+	+	+
Blood from vaginal (induced abortion)	-	-	-	+
Tremors	-	-	-	-
Salivation	-	-	-	-
Diarrhea	-	+	-	-
Urination	-	-	+	+
Death	-	-	+	+

Observations done before sacrifice.
Key: -Absent, + Present

Table 2: Mean average of live fetuses in treated animals and the number of resorptions

Groups	Stage of Drug exposure	Percentage (%) of live fetuses	Average number of live fetuses	Number of resorptions
A	Organogenesis	100	6.0	0
B	Organogenesis	100	6.6	0
C	Organogenesis	100	7.0	0
D	Organogenesis	100	3.0	0

Table 3: Incidence of growth retardation and size abnormality in fetuses of treated rats

Groups	TL (cm) (mean ± SEM)	UCL (cm) (mean ±SEM)	HD (cm) (mean ±SEM)	TW (g) (mean ±SEM)
A	0.95±0.09	1.81 ± 0.20	0.92 ± 0.10	2.41 ± 0.73
B	1.01 ± 0.13*	1.89 ± 0.77*	1.11 ± 0.29*	2.27 ± 0.56*
C	1.00 ± 0.12*	1.96 ± 0.76*	0.99 ±0.16*	3.21 ± 1.37*
D	0.75 ± 2.08*	2.40 ± 0.36*	1.37 ± 0.12*	6.50 ± 0.35*

Results are presented as Mean ± SEM (n = 10).
*represent significant difference in the tested group at $p \leq 0.05$ using ANOVA method of analysis.
Key: TL is tail length; HD is the head length; UCL is umbilical cord length; TW is total weight of fetusand placenta; SEM is standard error of mean

Table 4: Incidence of gross malformations in fetuses treated with bitters and control

Groups	Total number of fetuses studied	Formation of digital ray	Neural tube defects	Cleft palate abnormalities	Comments
A	18	0/18	0/18	0/18	Normal
B	20	0/20	0/20	0/20	Normal
C	21	0/21	0/21	0/21	Normal
D	3	0/3	0/3	0/3	Normal
The table shows the incidence of gross malformations in fetuses following herbal bitters exposure. Fetuses were grossly examined for formation of digital rays, neural-tube defects, and cleft palate abnormalities.					

Table 5: Biochemical parameters

Groups	AST	ALT
A	18.67 ± 0.52	17.33 ± 2.73
B	22.0 ± 3.58*	21.0 ± 2.37*
C	21.5 ±1.22*	19.17 ± 0.98*
D	21.67 ± 3.72*	20.0 ± 0.89*

Results are presented as Mean ± SEM (n = 6).
* represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1)
AST is Aspartate Transaminase; ALT is Alanine Transaminase; SEM is standard error of mean.

Table 6: The Effect of in utero Bitters exposure on hematological parameters at first filial stage.
HEMATOLOGICAL PARAMETERS

Parameters	A(Control)	B(Bitters)	C(Bitters)	D(Bitters)
WBC(10^3 /µL)	4133.33±287.52	5100±709.93*	6250±273.86*	4766.67±608.82*
HB (g/dL)	9.6±2.09	12.4±0.32*	9.85±0.27*	12.33±1.30*
PCV (%)	29.0±6.20	36.67±1.03*	27.5±2.74*	38.0 ±2.36*
MCV (fl)	41.71 ± 5.93	61.56±14.09*	41.0 ± 0.11*	44.3 ± 6.36*
MCH (pg)	14.47±1.79	18.6 ± 3.09*	13.15 ± 0.71*	15.1 ± 1.93*
MCHC(g/dL)	33.2±5.93	33.4±0.05*	33.25±0.05*	33.5±0.21*
PLT(10^4/ µL)	155±1182.16	218.3±21134.45*	175±5477.23*	229.3±44599*
NEU(%)	40±0.89	41.3±2.88*	39.5±0.55	37±0*
LYMPH(%)	59.33±1.03	61.56±14.09*	61±1.10*	59.33±3.61*
RBC(10^3/µL)	6.9±0.46	6.57±1.37*	7.9±0.66*	8.3±0.63*

*—P< 0.05
**—P< 0.01

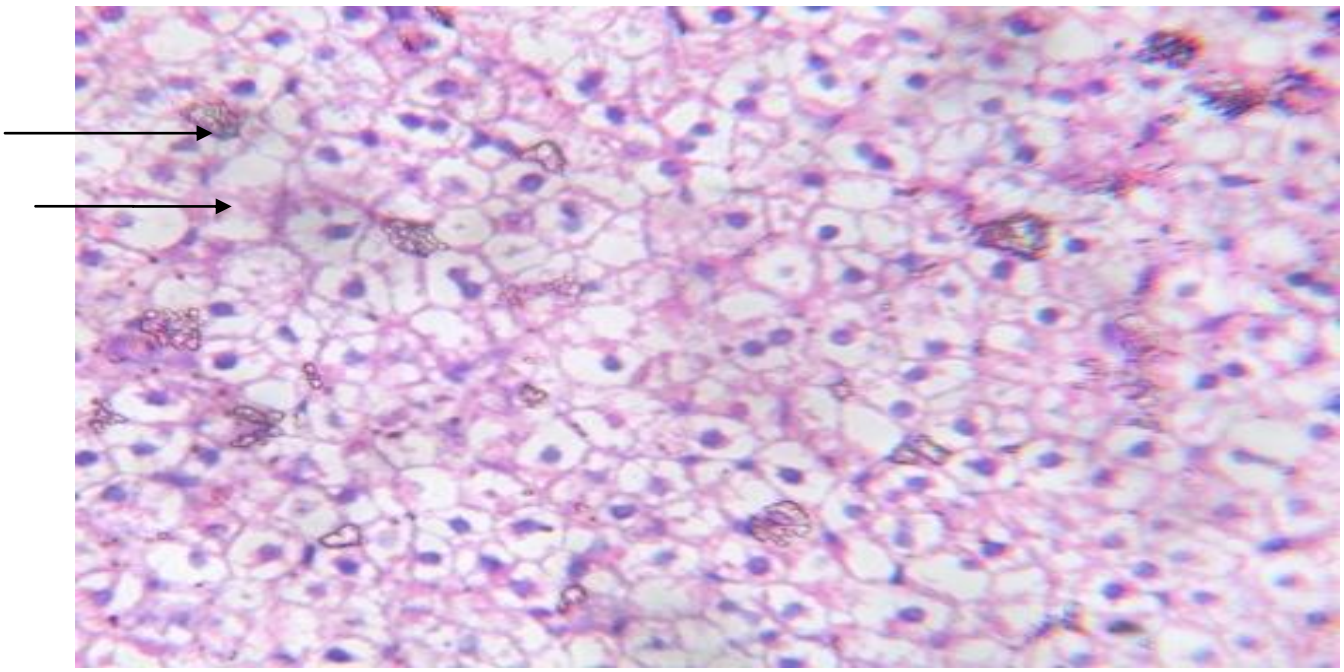

FIG I: LIVER. Section shows no significant lesions with control group (A)

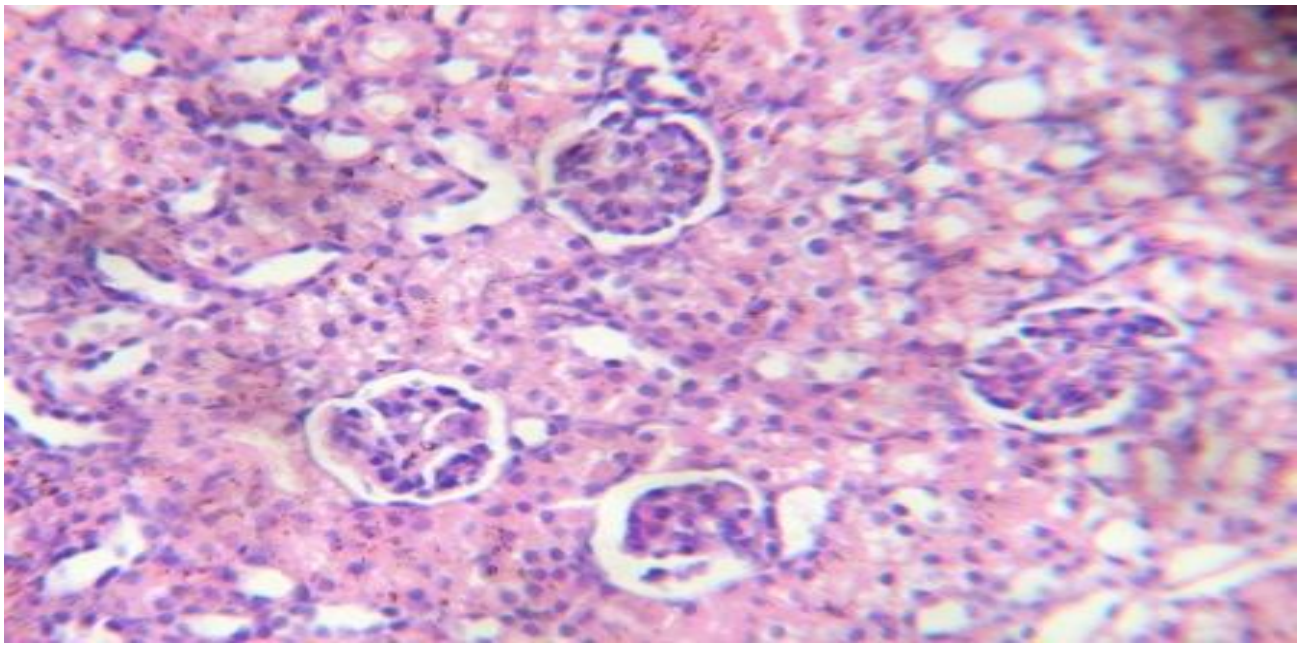

FIG II: KIDNEY. Section shows no significant lesion with control group (A)

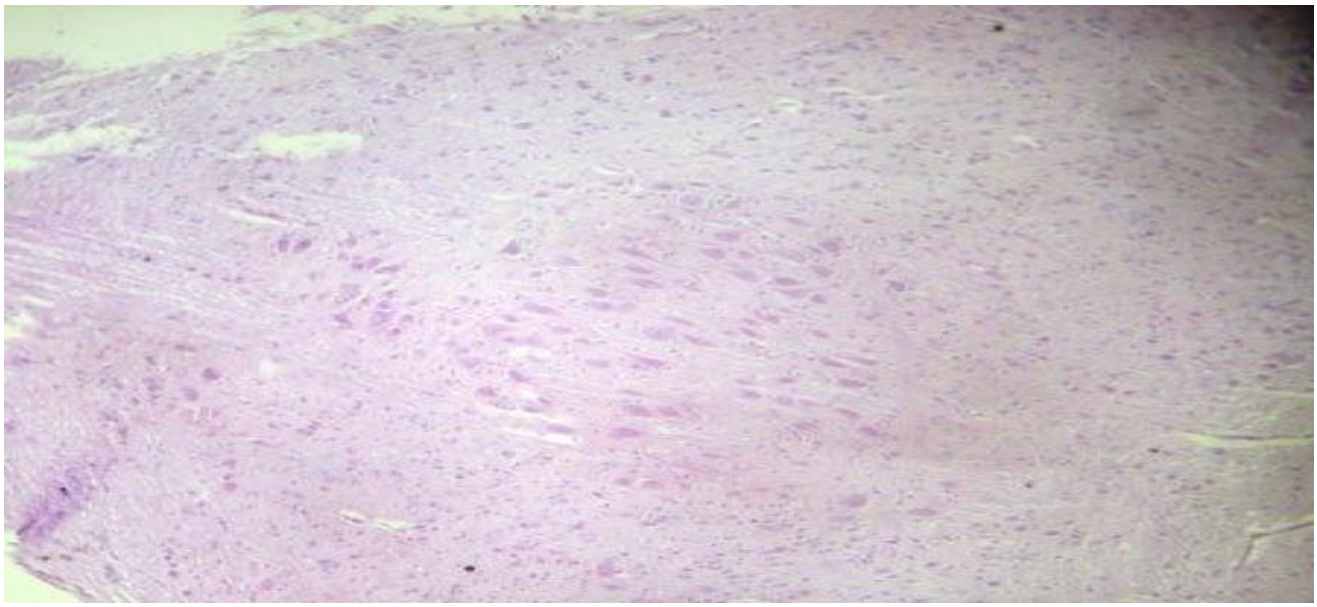

FIG III: BRAIN. Section shows no significant lesion with the control group (A)

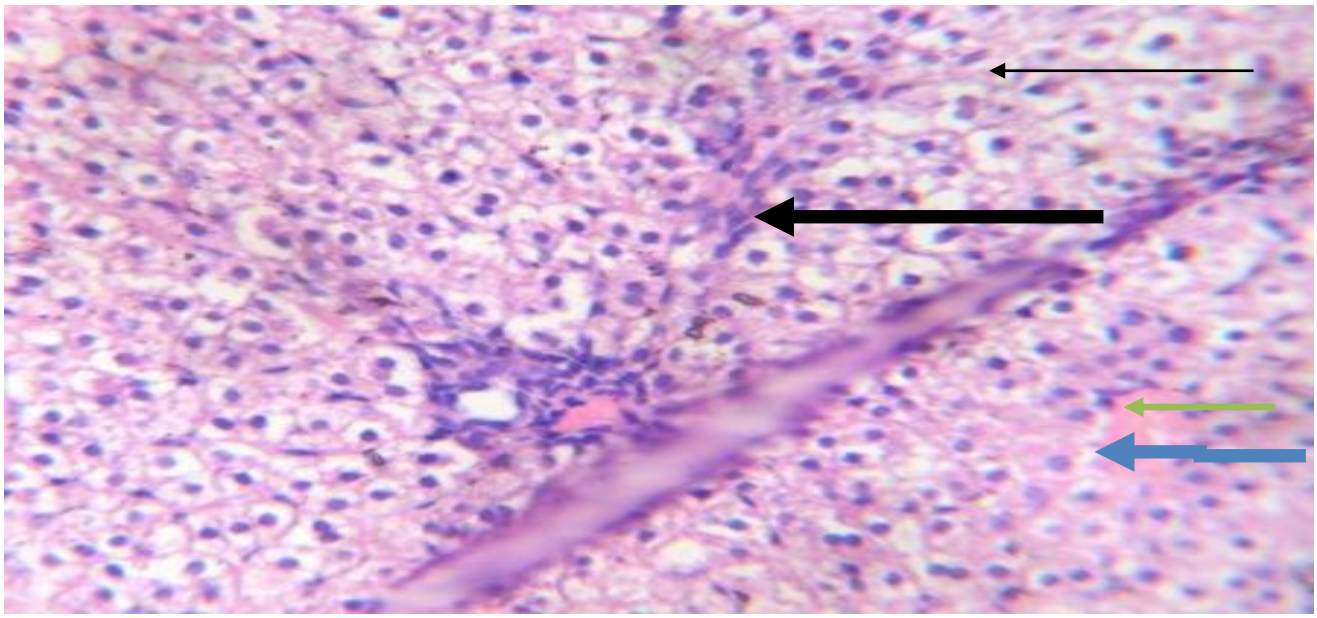

FIG IV: LIVER. Sections show disseminated severe macrovesicularsteatosis (thin arrow) of hepatocytes and mild periportal inflammation (black arrow). There is mild congestion of blood vessels (green arrow) and haemorrhage (blue arrow) in group **(B-Bitters)**

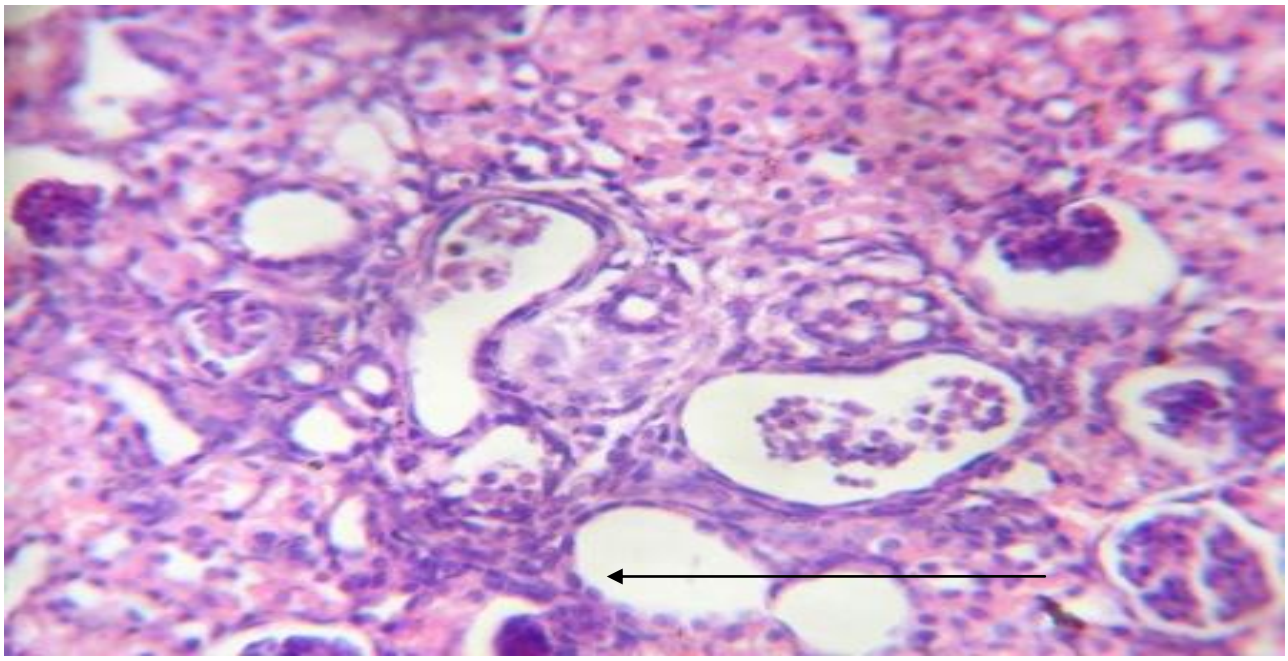

FIG V: KIDNEY. Sections show mild infiltration of inflammatory cells (thin arrow) in group **(B-Bitters)**

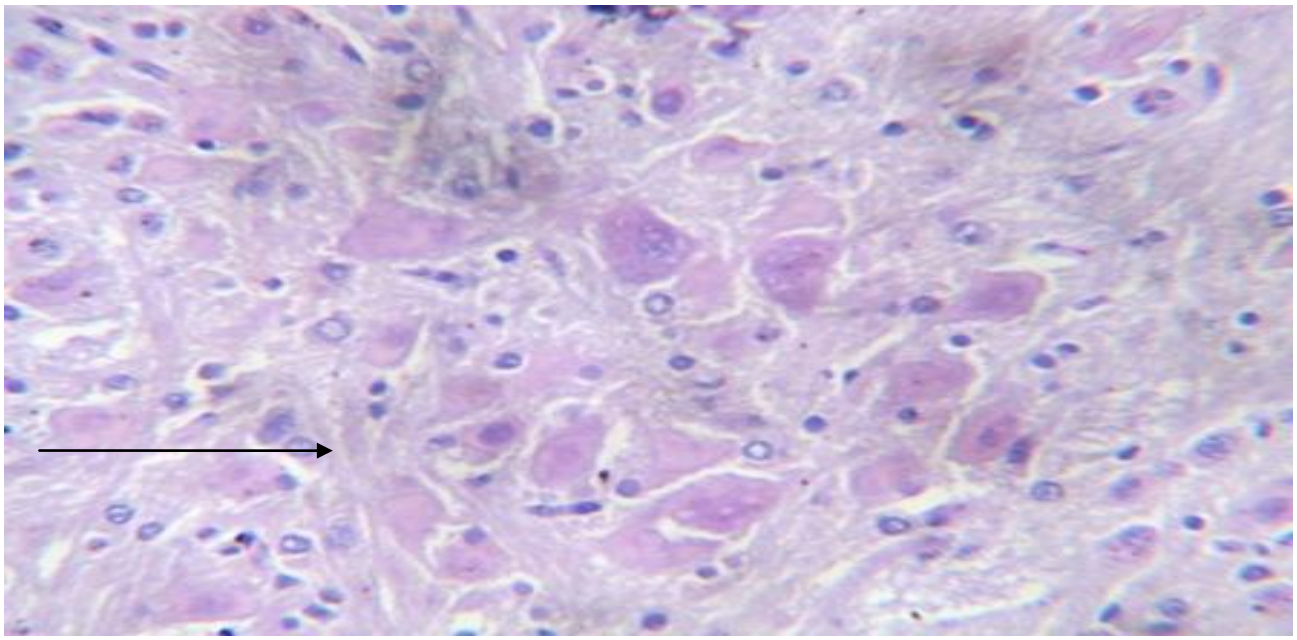

FIG VI: BRAIN. Sections show moderate presence of red neurons in group **(B-Bitters)**

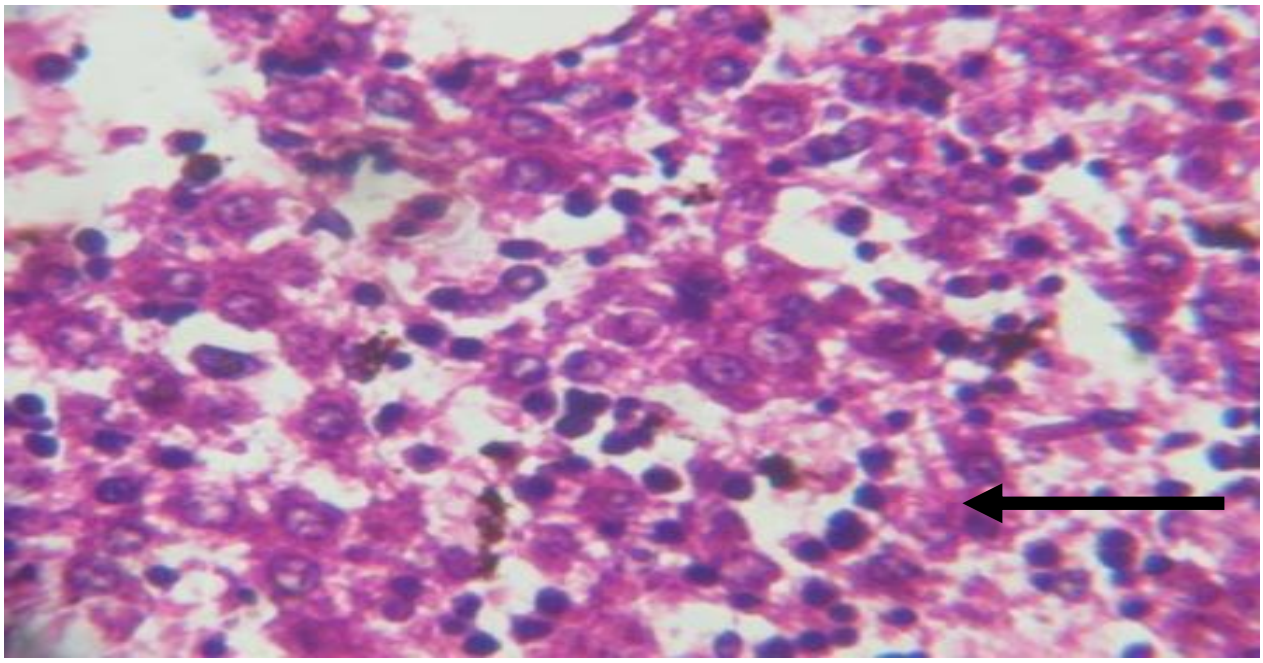

FIG VII: LIVER. Sections show disseminated inflammation of all the zones of the liver (black arrow) in group (**C-Bitters)**

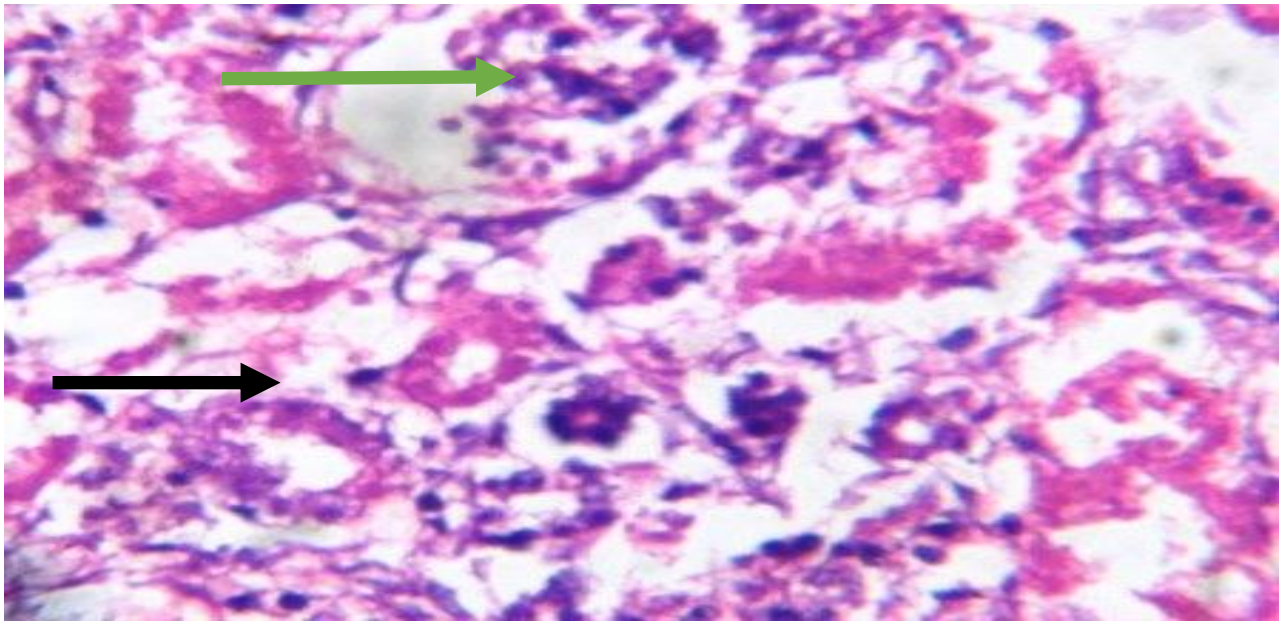

FIG VIII: KIDNEY. Sections show necrotic tubules (black arrows), the glomeruli are not well differentiated (green arrow) in group (**C-Bitters).**

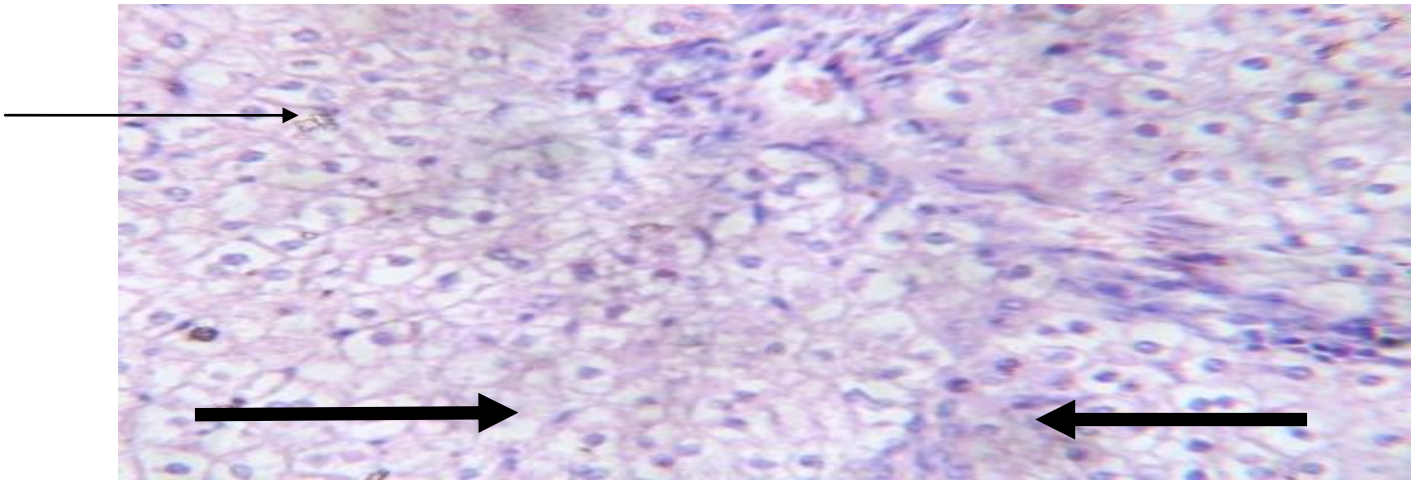

FIG IX: LIVER. Sections show disseminated severe macrovesicularsteatosis (thin arrow) of hepatocytes. There are presences of binucleation (black arrow) of hepatocytes in group (**D-Bitters)**

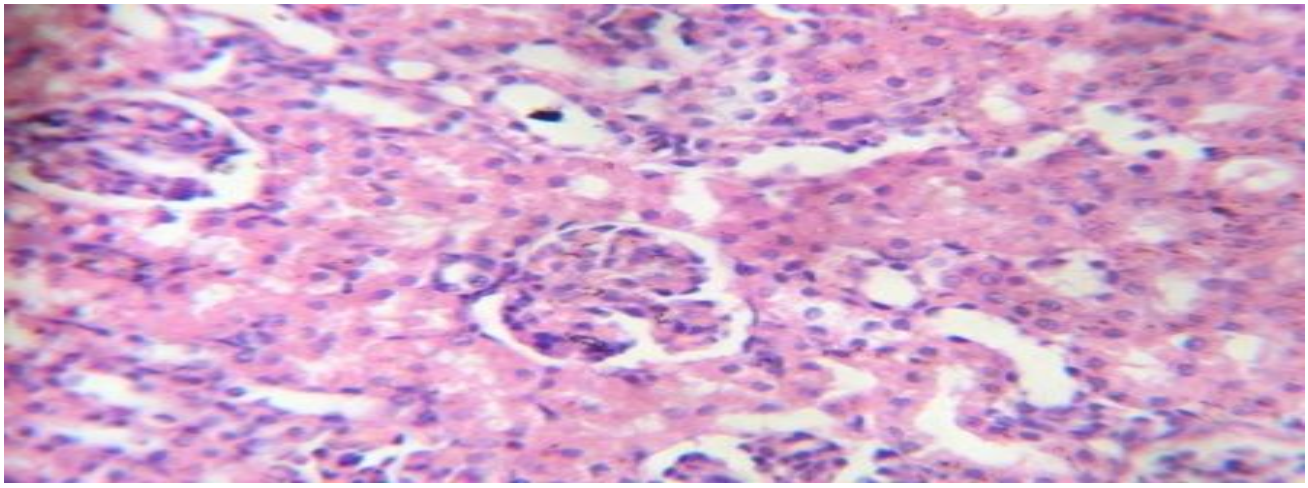

FIG X: KIDNEY. Sections show no significant lesion in group (**D-Bitters)**

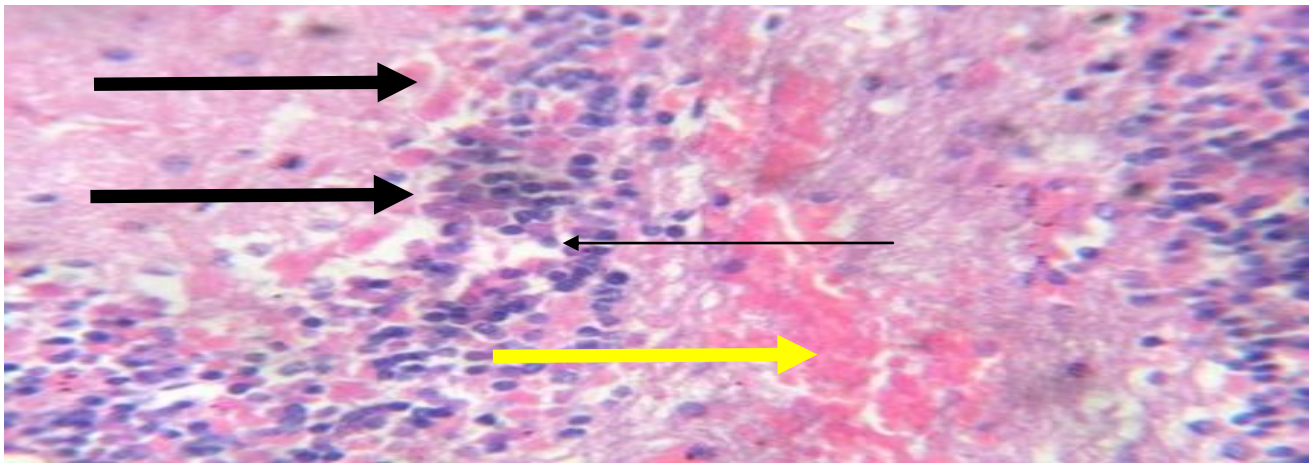

FIG XI: BRAIN. Sections show marked presence of red neurons (black arrow), slight inflammation (thin arrow) and congestion of cerebellum (yellow arrow) in group **(D-Bitters.)**

DISCUSSION

Pregnancy state is the most delicate state in a woman's life because varying factors could serve as a teratogen to the growing fetus depending on the mode of exposure. In general, drug administration during pregnancy should be done with great supervision by the physician in order to avoid any form of fetal damage since most drugs in pregnancy are considered as beingteratogenic at varying fetal stage and in relation to the dose administered (Hope, 2009). The tendency for these agents to inflict deleterious effects on growing fetuses have not been considered. However, the use of laxatives in pregnancy has been suggested to have possible adverse effect on the growing fetus but there is no sufficient data to justify its use, (Moriarty *et al*, 1985; West *et al*, 1992; &Tack *et al, 2011*). *Senna* leaf, an ingredient of B bitters is said to possess stimulant and laxative effect. It has diuretic property and can cause dehydration and problems related to the heart. The inclusion of this ingredient makes the tonic dangerous in large doses due to the stimulant effect (Hope, 2009). However, pregnancy predisposes women to developing constipation owing to physiologic and anatomic changes in the gastrointestinal tract, hence the use of a mild laxative is employed (West *et al*, 1992). Oral ingestion of *Aloe vera* may also cause diarrhea which in turn can lead to electrolyte imbalance, kidney dysfunction, dry mouth, nausea, and headache which should be avoided during pregnancy (Boudreau *et al*, 2006). Severe abdominal cramping is a common side effect of rhubarb root. This problem can often be alleviated by reducing the dose and also due to the possible loss of potassium, rhubarb root should not be taken in combination with cardiac medications, diuretics, other laxatives or cathartics, or steroids. Loss of potassium from the system can be decreased by combining the rhubarb root with licorice root (Grieve *et al*, 1992). Myrrh which is also one of the

components of B bitters is said to have rejuvenative property and also serve as a source of tonic. However, it is contraindicated when renal dysfunction or gastrointestinal disorder is apparent, or for women who are pregnant or have excessive uterine bleeding (Gibson, 2011). While efficacy and safety data for some of the imported herbal medicines are available in the literature to guide their use, such data are lacking for the locally produced herbal medicines.There were increase in the foetal weight plus placental weight in C and D bitters as compared to the control group with maximum reduction in B bitters (2.27 ± 0.56g).(Table 3)Earlier studies have shown that children with low birth weight (LBW) have an increased risk of developing diabetes, obesity and reduced intelligence later in life (Rich *et al,* 1990; Matte *et al,* 2001; Gillman *et al,* 2003 & Singhal *et al,* 2003).The heads of the fetuses that received the bitters as compared to the control is in accordance with previous studies.

The umbilical cord length as found in this study, were slightly increased when compared with the control, statistical ($p \leq 0.05$). Reductions in the foetal size/weight is an indication of growth retardation (stunted growth) and have clinical implications for threatened abortion (Reljic, 2007) and the risk of spontaneous abortion in assisted conceptions (Choong*et al,* 2003). Pre-eclampsia, in turn, is associated with LBW and preterm birth (Vatten*et al,* 2004). Studies have suggested that individuals who were born with low birth weight may be at increased risk for certain chronic conditions in adulthood. These conditions include high blood pressure, type-2 (adult-onset) diabetes and heart disease. One study found that men who weighed less than 3,000g at birth were 10 times more likely to have metabolic syndrome than the men who weighed more than 4,390g at birth (Valsmakis, 2006).On the other hand, there were significant increase in the weight of the litters from the D bitters which has the highest of four lither. This is in variance with the report by Helen (1913) that the average number of lither is six in rats. Considering the D bitters lithers drastic increased weight, it could be linked to the amount of food intake by the mother. Since, body weight could be assessed by the rate of food intake in normal adult (Graham *et al,* 2011). This may suggest for further complications. Hence, further investigation need to be carried out to know the underlying factor for the increased weight and reduced number of lithers.

The pathological results as seen in this study show that there is presence of severe macrovesicular steatosis in all groups including the control but more severity in other groups as it is seen in the B bitters group that the liver section showed mild periportal inflammation, mild congestion of blood vessels and haemorrhage, D bitters showed presence of binucleation and C bitters showed disseminated inflammation of all the zones of the liver respectively. The risk factors associated with steatosis are varied, and include diabetes mellitus (Araya, 2006), protein malnutrition (Conde, 1993), hypertension (Brookes, 2007), cell toxins, obesity (Saadeh, 2007), anoxia (Cotran, 1998) and sleep apnea (Ahmed, 2010). As the liver is the primary organ of lipid metabolism it is most often associated with steatosis; however, it may occur in any organ, commonly the kidneys, heart, and muscle (Cotran, 1998). Nutrient malnutrition may also cause the mobilisation of fat from adipocytes and create a local oversupply in the liver where lipid metabolism occurs. Excess alcohol over a long period of time can induce steatosis which could be related to the ethanol content of in D bitters group. The breakdown of large amounts of ethanol in alcoholic drinks produces large amounts of chemical energy, in the form of NADH, signalling to the cell to inhibit the breakdown of fatty acids (which also produces energy) and simultaneously increase the synthesis of fatty acids. This "false sense of energy" results in more lipid being created than is needed. Grossly, steatosis causes organ enlargement and lightening in colour (Cotran, 1998) which was observed in the organs harvested from the D bitters group. This observed effects simply indicate that administration of bitters in pregnancy could cause severe liver damage, including liver failure and fatty liver disease.

The effect associated with the kidney of animals treated with C bitters could be as a result of its composition as its been reported in previous study that “Oral ingestion of *Aloe vera* may also cause diarrhea which in turn can lead to electrolyte imbalance, kidney dysfunction, dry mouth, nausea, and headache”(Boudreau *et al,* 2006). The observed effects occurred only in the D bitters group which is suggestive of the effect of alcohol in the brain. It has been postulated that alcohol consumption during pregnancy leads to fetal alcohol syndrome which is a pattern that can develop in the fetus. Alcohol readily crosses the placental barrier and can stunt fetal growth or weight, create distinctive facial stigma, damage neurons and brain structure which can result in intellectual disability and other psychological or behavioural problems, and also cause other physical damages (Ethen *et al,* 2008).

Aspartate Transaminase (AST) values were significantly higher ($p \leq 0.05$) in all the treated groups with more significance in the B bitters group compared to the control group. The results for ALT show a significant increase ($p \leq 0.05$) in the three groups with more significant elevation in B bitters group when compared with control. This observed result

implies a high risk of liver disease associated with B bitters due to increase in the liver enzyme level in the lithers. Hematological results from this study showed a statistically significant ($p \leq 0.05$) increase in White Blood Cell counts (leukocytosis) in all the groups with a higher significance in C bitters group when compared with control. There is increased susceptibility to diseases during utero exposure to these bitters at the stage of organogenesis in the treated groups. Neutrophilia (increase in Neutrophils) was observed in B bitters group as compared to control. Neutrophilia describes a high number of neutrophil granulocytes in the blood and it is mainly as a result of acute bacterial infections, inflammation, tissue necrosis e.g. heart attack and burns (Mitchel *et al*, 2007). Platelet counts were increased (thrombocytosis) in all the groups with more significant increase in the D and B bitters group which could lead to hemorrhage as observed in the result of the pathology of the liver of animals treated with B bitters. An abnormality of the platelets called a thrombocytopathy, which can be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis) proposed by Maton*et al*, 1993. Thrombocytosis which could be due to congenital asplenia (Chanet*et al*, 2000), iron deficiency anemia or hemorrhage and it is also a potential cause of thrombophilia (Mitchelet *al*, 2007).

Elevated hemoglobin level was more significant in the Alomo bitters group compared to the control group. High hemoglobin may be caused by an underlying lung disease or problems with the bone marrow. Red cell counts were increased (polycythemia) in all the groups with a distinctive increase in D bitters group compared to the control. Erythrocytes that have a normal size or volume (normal MCV) are called normocytic, when the MCV is high as seen to be more significantly elevated in B bitters group compared to control, is called macrocytic, when the MCV is low, it is termed microcytic**.** Erythrocytes containing the normal amount of hemoglobin (normal MCHC) are called normochromic, when the MCHC is abnormally low they are called hypochromic and when the MCHC is abnormally high they are called hyperchromic as seen in treated groups when compared to control. All these parameters are used together to describe different forms of anaemia. Previous studies proved that in a normal and healthy adult the effects of these bitters are not permanent if it's withdrawn but none confirmed its effect on a growing foetus as it can cause damage to growing cells temporarily or permanently when used in pregnancy.

CONCLUSION

The present investigation has established adverse effects of the administered bitters on the growing fetus by exhibiting significant deleterious effects on the organs of the lithers which were harvested after a month. Inference can be drawn that the experimented bitters possess deleterious effects on the growing fetus. Liver diseases as seen in B and C bitters, ataxia as seen in D bitters, renal disorder and leukocytosis as seen more prominently in C bitters, thrombocytosis in B bitters, polycythemia in D bitters and other forms of anemia in all bitters treated groups. Thus, the use of bitters formulation should be avoided at all stages of pregnancy as it can cause temporal to permanent damage to the growing fetus.

Note: For the purpose of public interest and health awareness, the brand names of the studied bitters are as follows: B-Bitters **(Yoyo Bitters),** C-Bitters **(Swedish Bitters) and** D-Bitters **(Alomo bitters).**

RECOMMENDATION

Public health information about the use of bitters particularly in pregnancy should be discouraged. A misleading information in manufacturer's information leaflet on the use of C bitters in pregnancy should be vehemently resisted as we have demonstrated from our laboratory it severe deleterious effects on vital organs of the lithers hence, may require complete avoidance in pregnancy.

DELARATION

The research involved the use of animal hence, ethical clearance was sought from the Faculty of Pharmacy, Olabisi Onabanjo University ethical committee and in accordance with animal protection law of the country.

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