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IMPACTS OF HEAVY METAL CONCENTRATIONS IN ASA RIVER ON HAEMATOLOGY, BIOCHEMICAL AND HISTOLOGICAL INDICES OF RATTUSNOVERGICUS FROM A TOXICOLOGICAL PERSPECTIVES

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Abstract

The toxicity of heavy metals when their concentration exceeds allowable limits has made their presence in our environment of great concern. These metals are released into the environment through a range of sources, including industrial processes into nearby rivers thereby polluting the river for domesticated usage. Thirty-six male, healthy rats (*Rattus novergicus*) were purchased from the Animal House and given water samples from the Asa River in different concentrations (20, 40, 60, 80, and 100%). Rats from the experimental and control groups were sacrificed and the liver and kidney were removed, washed in 10% formal saline, and prepared for histomorphological examinations. Rats' blood was also taken for haematological and biochemical indices during the animal sacrifice process. In this study, Alanine amino transferase (ALT) and Aspartate amino transferase (AST) levels at higher concentrations were significantly noted when compared to the control group resulting to alteration of the liver and kidney organs at higher concentrations with mild periportal cellular infiltration and mild congestion of the renal interstitium and severe congestion and hemorrhage at the renal interstitium, respectively. Rats' haematological and biochemical indices did not differ significantly from the control group.

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Introduction:-

Heavy metals are dangerous compounds that pose a major threat to ecosystems and organisms' health because of their extreme toxicity and environmental endurance (Abbas, 2002).

However, the majority of these sectors have significantly increased the degree of contamination in our ground and surface water supplies. Indeed, the introduction of certain pollutants and toxins has led to the extinction of numerous aquatic creatures (Ogunleye and Izuagie, 2013).

One of the things that pollutes running rivers is industrial wastewater produced by the industry. A significant disease burden is linked to contaminated air, soil, and water caused by industrial effluents (WHO, 2002). It contains large

concentrations of heavy metals that, if released into the environment, might pollute it. Included in this group are Cr, Cu, Zn, Cd, and Fe. Among the most harmful type of water contaminants are these. At least 20 metals are toxic, and around half of them are released into the environment at amounts that are harmful to both the ecosystem and human health, according to Khaled *et al.* (2008). In recent years, heavy metals have become a major environmental concern. Their toxicity, bioaccumulation, and bioconcentration in living things are the causes of this. Additionally, their non-biodegradable nature and environmental persistence have increased their concern (Yoon *et al.*, 2006).

Materials and Methods

Thirty-six male rats (*Rattus norvegicus*) weighing 140 ± 20 g and in good health were purchased from Animal House in Ogbomosho, Oyo State. Before the experiment started, the rats were acclimated for two weeks. The hybrid agrosop supplied the growers' mash of hybrid feed, which the rats were fed for the entire duration of the research. Water and feed were supplied ad libitum.

For two weeks, the rats were housed in six plastic cages with adequate ventilation to allow them to acclimate. The concentrations led to the animals being divided into six groups. Water samples from the Asa River were given to the rats in these four groups in varying concentrations based on the steps taken. Every rat received 0.5 ml of the water via gavage twice daily for 30 days at 12-hour intervals.

Following the experiment, the rats in the research groups and the rats in the control group were both anesthetized in a chamber saturated with chloroform. Five milliliters of blood were then drawn from each of the rats via heart puncture. To ensure homogeneity and prevent blood clots, the blood samples were carefully mixed and placed into lithium heparin anti-coagulated bottles. A Gulfex Medfield Equipment and Scientific Limited Macro-centrifuge, model number 800D, was then used to spin each blood sample for 10 minutes at 2500 revolutions per minute (rpm).

The biochemical parameters were analyzed using the collected plasma. Following this, the *Rattus norvegicus* rats were sacrificed and the liver and kidney were removed by cervical dislocation from the experimental and control groups, respectively. They were then preserved in 10% formal saline and prepared for histomorphological analysis.

Haematological Analysis

A sterile disposable 2 ml needle and syringe were used for collecting blood from five rats in each group. The blood was then stored in vials containing the anticoagulant Ethylene Diamine Tetra Acetic (EDTA) for the assessment of haematological parameters, specifically Packed Cell Volume (PCV), White blood cells count (WBC), red blood cells count (RBC), and haemoglobin concentration (Hb) with their indices Mean Corpuscle Volume (MCV), Mean Corpuscle Haemoglobin (MCH), Mean Corpuscle Haemoglobin Concentration (MCHC), were estimated by the standard methods as described (Dacie and Lewis, 1977; Lee *et al.*, 1999).

Evaluation of Biochemical Parameters

Blood samples were taken and allowed to coagulate in EDTA-free, transparent, dry centrifuge tubes. They were then centrifuged for 15 minutes at 3500 rpm. Trinder's (1969) enzymatic colorimetric approach was immediately applied to a piece of the clear supernatant serum in order to determine the amount of glucose present.

The remaining serum was frozen at -200°C for further investigation, such as estimating the activity of the enzymes aspartate amino transferase (AST) and alanine amino transferase (ALT) using the methodology outlined by Varley (1969) and George *et al.* (2014). We used the Belfield and Golderg (1971) method to measure serum alkaline phosphate.

The serum's total cholesterol levels were measured in accordance with Allain *et al.* (1974). The techniques outlined by Doumas (1975, Doumas *et al.*, 1971) were used to estimate the levels of albumin and serum total protein. A calculation of serum globulin was made. Serum and creatinine levels were calculated to include urea (Emmanuel *et al.*, 2017).

Histopathological Examination of kidney and liver

In order to ascertain the impact of the adsorbent on organ tissues, pre-processing preparations and processing were performed on each tissue to check for any disruptions in the tissue architecture. The treated rats had surgery to remove their kidney and liver. To detect any pathological changes, the organs were preserved in 10% formalin,

which was made by dissolving 10 milliliters in 90 milliliters of distilled water. This procedure was performed for histological investigations.

Using increasing alcohol grades (70, 80, 90, and absolute), tissue was dehydrated. After 30 minutes of clearing in Xylene, the tissue was impregnated with wax and then embedded in paraffin wax. After being sectioned at 4 μm on a rotary microtome, they were stained for 15 minutes with Mayer's haematoxylin, rinsed in an acid-and-alcohol mixture (one milliliter of hydrochloric acid added to 99 milliliters of 70% alcohol).

After five to ten minutes of washing under running tap water, the tissue preparations were counterstained for two minutes using 1% aqueous eosin. Dehydrated, cleaned, and mounted using the neutral balsam procedure for microscopic inspection after being rinsed in water to get rid of extra eosin. According to Avwioro (2014), the bright field Leitz microscope was used to report any indication of histological alterations.

Statistical Analysis

The data was statistically analyzed using Graphpad Prism version 5.0 to examine the biochemical and hematological analyses. The significance threshold will be set at $p \leq 0.05$.

Results and Discussion:-

Depicts the effects of Asa river water on PCV of rats which shows no significant difference between the treated groups compared with control, hence, no effect on PCV of the rats as shown in figure 3.1. The effect on Haemoglobin concentration of the rats was illustrated and shown in figure 3.2. Treated groups are not significantly different from control, hence, no effect on haemoglobin concentration. Figure 3.3, 3.4, 3.5 and 3.6 showed the Red blood cell (RBC), MCV, MCH and MCHC of the rats and treated groups were not significantly ($P < 0.05$) different from control. Hence, no effect on the concentrations. there is no significant difference on MCV concentration, hence no negative impact on the metabolic environment that produces red blood cells. Moreover, Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) also, indicate blood level conditions. A low level is an indication of anaemia (Aster, 2004) with no significant difference on MCH concentration. Furthermore, MCHC has no significant difference.

WBC concentration of the rats having no significant difference between 20% treated group compared with control while other treated groups (40%, 60%, 80%, 100%) were all significantly ($P < 0.05$) decreased in concentration when compared with the control as shown in figure 3.7. Increase in neutrophil concentrations compared with the control and other treated groups of 20%, 40%, 60%, 80%, 100%. As in figure 3.8, lymphocytes and monocytes (figure 3.9 and 3.10). Platelets showed a significant increase which was observed in 60%, 80% when compared with control as shown in figure 3.11. The primary roles of white blood cells and their derivatives include the production, transportation, and distribution of antibodies in the course of an immunological response in addition to the protection of the body against foreign invaders by phagocytosis. As a result, animals with high white blood cell counts can create antibodies during phagocytosis, while those with low levels are more prone to sickness and have a higher level of disease resistance as reported by Soetan *et al.*, 2013, also, they are flexible enough to adjust to often occurring illness conditions (Isaac *et al.*, 2013).

Figure 3.12 to figure 3.15 showed the effect on serum Total protein, Albumin, Globulin, ALP. In fig 3.12, 3.13, 3.14 and 3.15, a significant increase was observed in 20%, 40%, Figure 3.16 and 3.17 shows a non-concentration dependent significant increase in all the treated groups of serum AST and ALT activity when compared with the control. An insignificant increase was observed in serum bicarbonate in all the treated group when compared with control as shown in figure 3.18. A significant decrease of serum sodium and chloride concentration was observed in all the treated groups when compared with the control as shown in figure 3.18 and 3.19 while treated groups were not significantly different from one another; hence, the effect was not concentration dependent. An insignificant decrease of total bilirubin and creatinine was observed in treated groups as shown in figure 3.21 and 3.22 when compared with the control while treated groups were not significantly different from one another, hence no effect on total bilirubin. No significant difference of serum conjugated bilirubin between the treated groups and control. No significant difference of serum conjugated bilirubin between the treated groups and control (figure 3.23). No significant difference in serum unconjugated bilirubin between the treated groups when compared with control and treated groups were not significantly different from one another as shown in figure 3.24.

Decreased activities of antioxidant enzymes like Superoxide dismutase (SOD) and non-enzymatic antioxidant, reduced glutathione (GSH) concentration in liver and the kidney was observed as shown in figure 3.25, 3.28 and figure 3.29, 3.32 respectively. Significant elevation in hepatic innate SOD might be as a result of natural response of hepatocyte trying to salvage the liver from various oxidative damages. This has been occasioned by the river which has significant serum elevation of AST and ALT activities. Moreover, excessive generation of reactive oxygen species caused by this river, in the kidney might have resulted in significant decrease in SOD activity which might latter result in kidney malfunction. This is also in line with earlier report of Singh and Pandey (2021) who reported significant decrease in renal SOD and GSH in Stinging catfish treated with industrial effluents. Moreover, renal total protein in treated groups was not significantly different from control hence no effect on renal total protein.

The components of biological membranes are susceptible to damage from free radicals and active oxygen. These free radicals' actions cause lipid peroxidation, which is linked to aging, inflammation, and cancer (Ames *et al.*, 1993). Additionally, they harm cells by reacting covalently with the macromolecules that make up cells (Ajayi *et al.*, 2010). The constituent of the river did not show lipid peroxidation in hepatocyte biomembrane as well as other protein entities in the cell. Reduced glutathione (GSH) is a biological antioxidant that isn't enzymatic and is present in body tissue. Alkoxy radical, superoxide anion, and hydrogen peroxide are examples of non-radical substances that it works to eliminate from tissue. Reduced level of GSH has been observed in various disease state. In hepatic tissue, various treatment groups showed no significant different in GSH concentration compared with control. Moreover, in renal tissue, various treatment groups showed significant increase in GSH concentration when compared with control. Increase in GSH concentration in some groups might be as a result of hepatic and renal tissues trying to compensate for oxidative stress.

The constituents did not show lipid peroxidation in hepatocyte biomembrane as well as other protein entities in the cell, while the treated groups is significantly decreased in MDA concentration compared with the control, the result obtained was also corroborated earlier report of possible oxidative stress by free radical generation in renal tissue evident by significant elevation of urea and creatinine with corroborated earlier report of Singh and Pandey (2021) who reported significant elevation in renal and hepatic MDA concentration in industrial effluent treated Stinging catfish.

Histopathological changes observed in the liver and the kidney of the rats treated with different concentrations of Asa river in the form of periportal cellular infiltration and congestion of the renal cortical interstitium are in agreement with the observations of Bhushan *et al.*, 2013 in Cypermethrin and beta-cyfluthrin-induced biochemical and histopathological alterations in rat liver, George *et al.*, 2014 in renal failure caused by the increased level of creatinine and urea levels as markers and Emmanuel *et al.*, 2020 in biochemical and histomorphological alteration in liver and kidney of *Rattus norvegicus* administered with tetracycline.

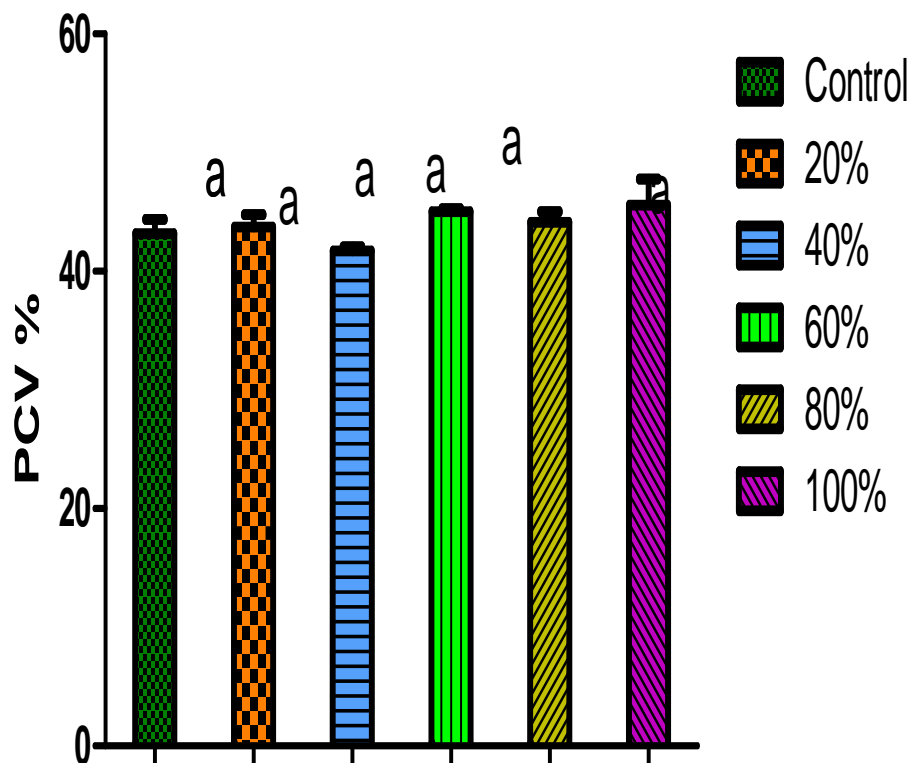
The liver exhibits an important role in detoxifying xenobiotics and metabolic byproducts, making it vulnerable to harm from direct exposure to hazardous chemicals. Liver and kidney abnormalities were brought on by presence of metals in the water on *Rattus norvegicus* which may also suggest that the hepatic and renal functions may be modulated by the metals through direct or indirect effects on these organs. These effects on the concentration and duration dependant as reported by Shahid *et al.*, 2020, Shahid *et al.*, 2021, Bianca *et al.*, 2021, Alessio *et al.*, 2022.

The liver sections from treated *Rattus norvegicus* at different concentrations of 20,40,60,80 and 100 ppm of Asa river showed changes when compared with those from the control rats as shown in Table 3.1. Section of liver of *Rattus norvegicus* treated with 20% of Asa river showed mild periportal cellular infiltration as in Table 3.1, Section of liver from 40% also showed severe central veinous congestion and mild periportal cellular infiltration, Section of liver from 60% showed mild to moderate periportal cellular infiltration, from 80% showed mild periportal cellular infiltration and from 100% showed mild congestion of the renal interstitium.

The microscopic observations of the sections of the control, the glomerular and surrounding tubules in the kidneys of rats showed typical renal architecture as demonstrated in Table 3.2, the kidneys from treated *Rattus norvegicus* at different concentrations of 20,40,60,80 and 100ppm of Asa river showed changes when compared with those from the control rats as shown in plate 3.1. Section of kidney of *Rattus norvegicus* treated with 20% of Asa river with no visible lesion. Section of kidney from 40% also showed mild congestion of the renal interstitial and few tubules appeared degenerated, Section of kidney from 60% showed severe congestion of the renal cortical interstitial, from 80% and 100 % shown significant bleeding and congestion at the renal interstitial.

The microscopic intensity degenerative changes in the concentrations of the kidney as it increases, and as the pathological changes observed in the higher concentrations is an indication of toxicity as reported by Khalid *et al.*, 2018 and Olonisakin *et al.*, 2019 and likewise the liver as reported by Abdel *et al.*, 2012, Sultan *et al.*, 2016 and Kalaiyarasi *et al.*, 2017. The toxicological evaluation of the rats treated with the concentrations of the river indicates that if usage of the water is continuous over time, It will provide toxicological concerns, as demonstrated by elevated serum, liver, and kidney levels as well as pathological alterations (Kazi *et al.*, 2018 and Sarajpreet *et al.*, 2018).

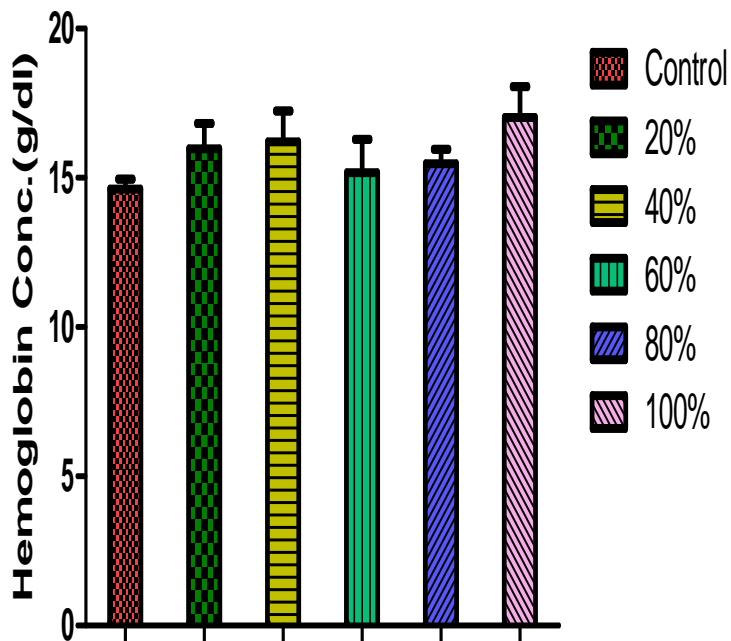
Figure 3.1:-



Effect of different percentage of Asa water on PVC of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

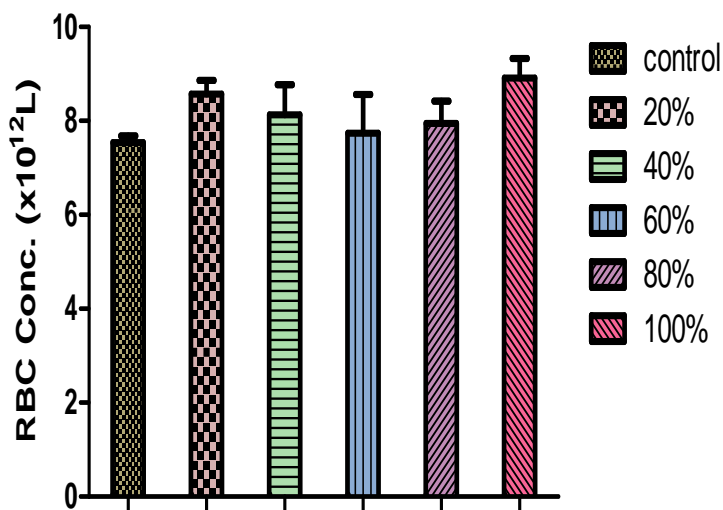
Figure 3.2:-



Effect of different percentage of Asa water on Hemoglobin of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
 Bars with alphabet 'a' are not significantly different from control.
 No significant difference between the treated groups

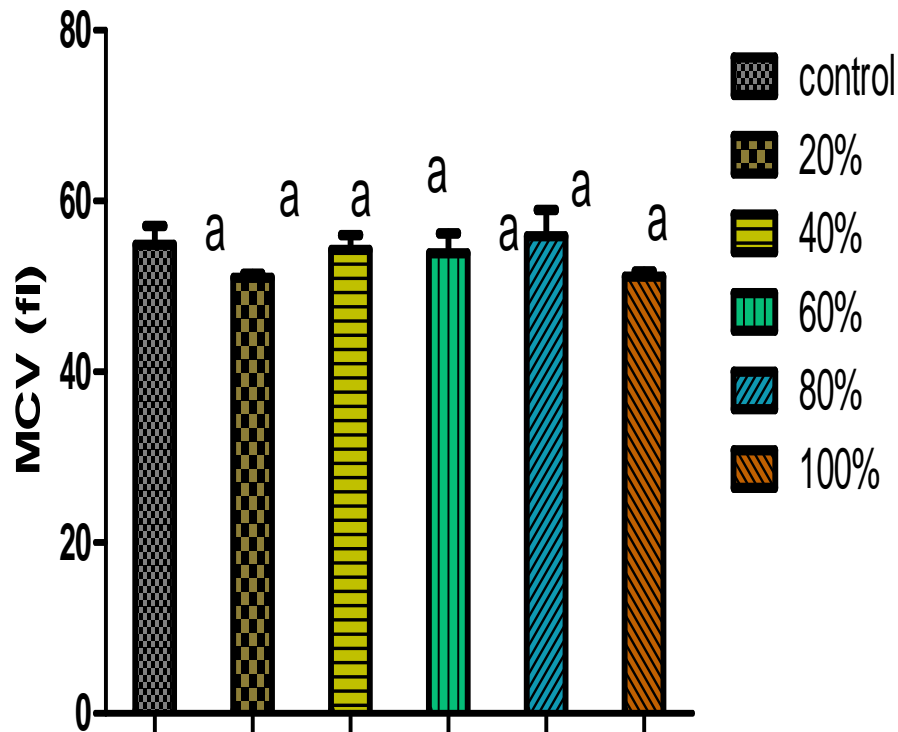
Figure 3.3:-



Effect of different percentage of Asa water on RBC conc. of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

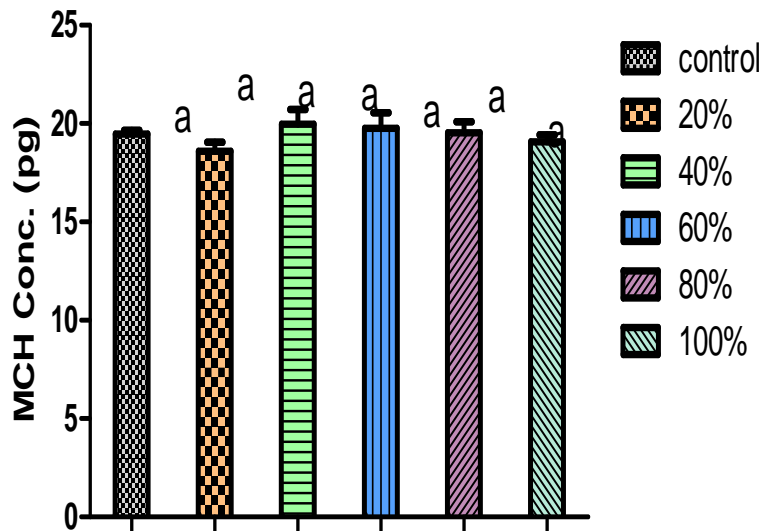
Figure 3.4:-



Effect of different percentage of Asa water on MCV conc. of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

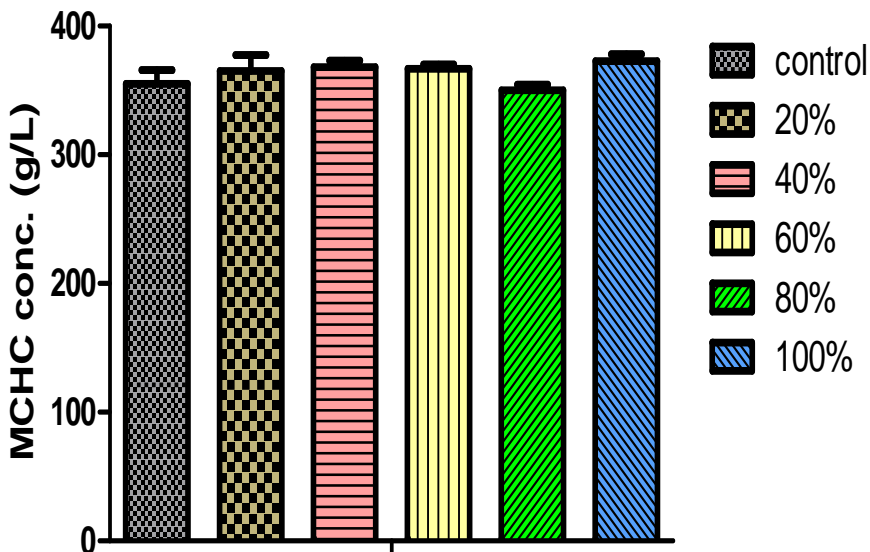
Figure 3.5:-



Effect of different percentage of Asa water on MCH conc. of rats

Level of significance was taken at (P<0.05) for five rats per group.
 Bars with alphabet 'a' are not significantly different from control.
 No significant difference between the treated groups

Figure 3.6:-

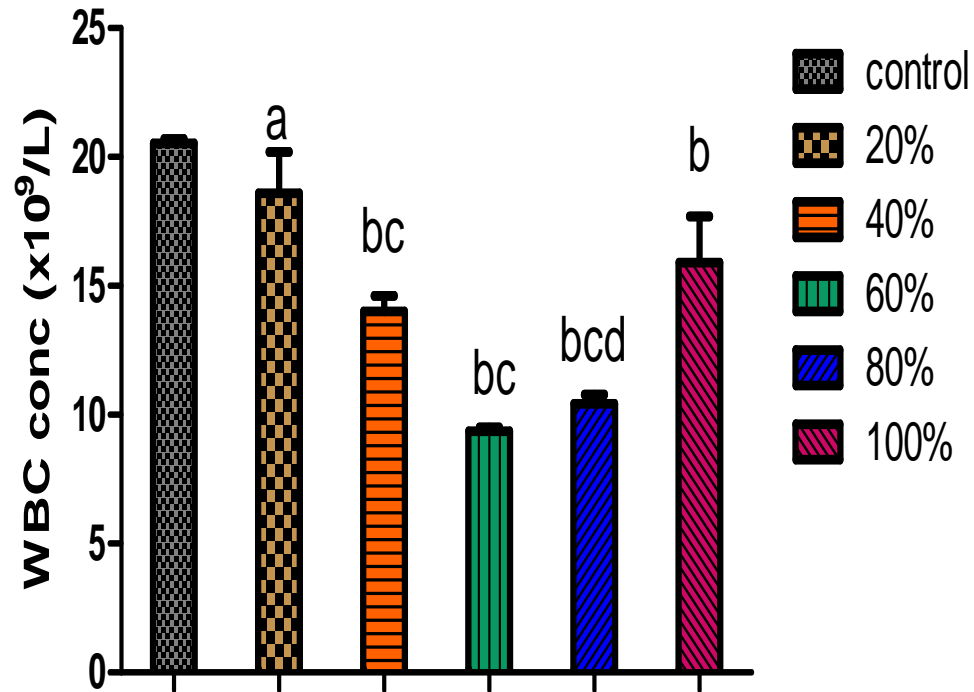


Effect of different percentage of Asa water on MCHC conc. of rats

Level of significance was taken at (P<0.05) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

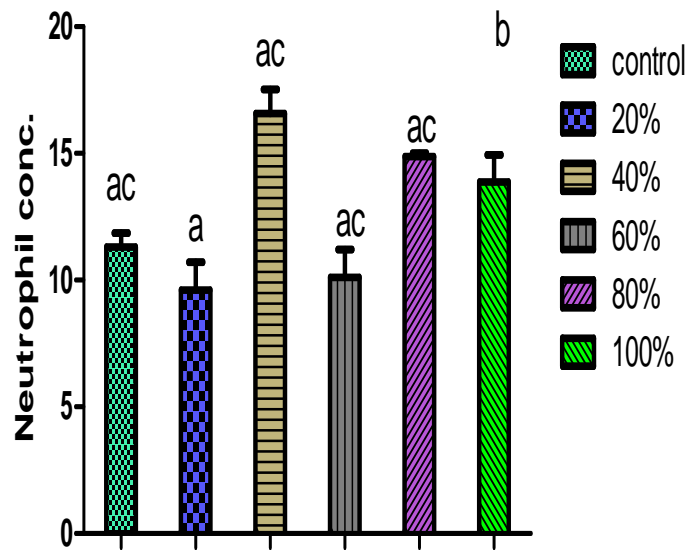
Figure 3.7:-



Effect of different percentage of Asa water on WBC conc. of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bar with alphabet 'a' are not significantly different from control.
Bars with alphabet 'b' are significantly different from control.
Bars with alphabet 'c' are significantly different from 20% Asa water
Bar with alphabet 'd' significantly different from 60% Asa water

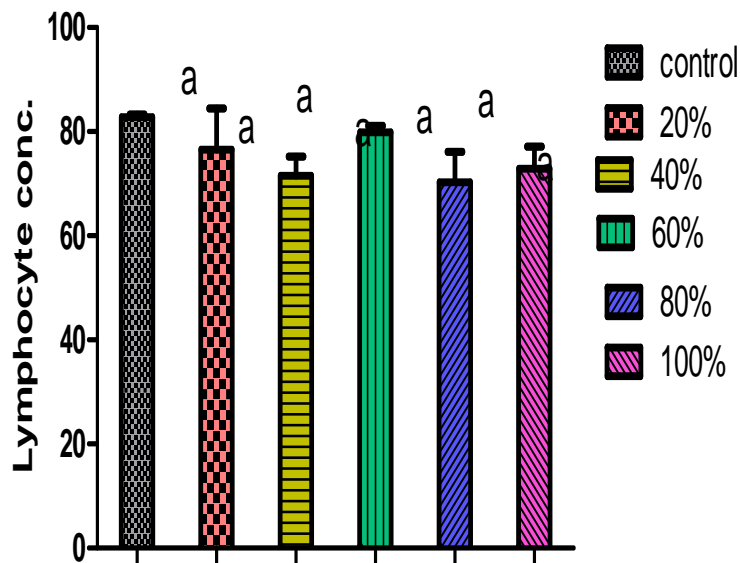
Figure 3.8:-



Effect of different percentage of Asa water on Neutrophil conc. of rats

Bar with alphabet 'a' are not significantly different from control.
 Bar with alphabet 'b' are significantly different from control.

Figure 3.9:-

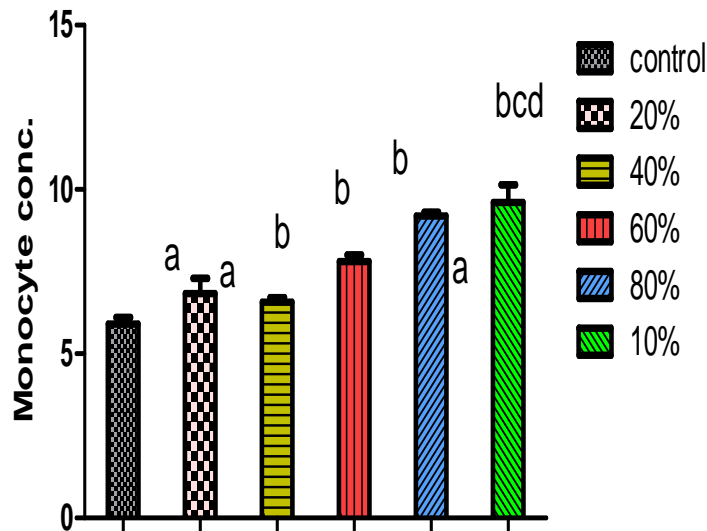


Effect of different percentage of Asa water on Lymphocyte conc. of rats

Level of significance was taken at (P<0.05) for five rats per group.
 Bars with alphabet 'a' are not significantly different from control.

No significant difference between the treated groups

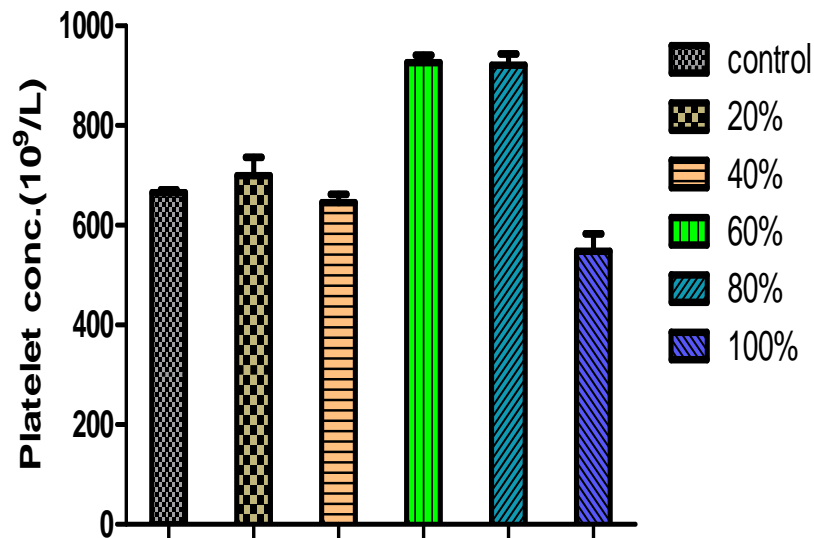
Figure 3.10:-



Effect of different percentage of Asa water on Monocyte conc. of rats

Level of significance was taken at (P<0.05) for five rats per group.
 Bar with alphabet 'a' are not significantly different from control.
 Bars with alphabet 'b' are significantly different from control.
 Bars with alphabet 'd' significantly different from 40% Asa water

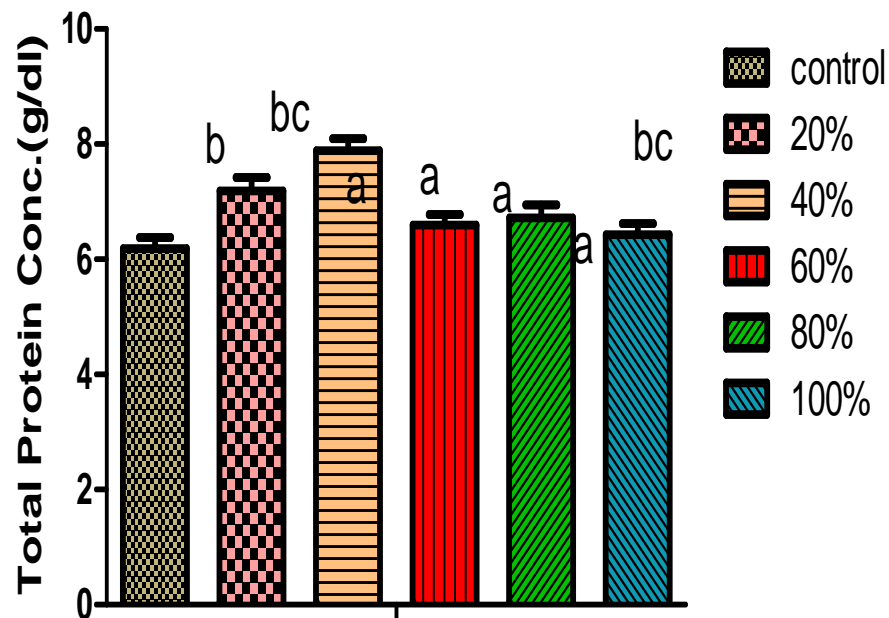
Figure 3.11:-



Effect of different percentage of Asa water on Platelet conc. of rats

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bar with alphabet 'a' are not significantly different from control.
Bars with alphabet 'b' are significantly different from control.
Bars with alphabet 'c' are significantly different from 20% Asa water
Bars with alphabet 'd' are significantly different from 40% Asa water
Bars with alphabet 'e' are significantly different from 60% Asa water

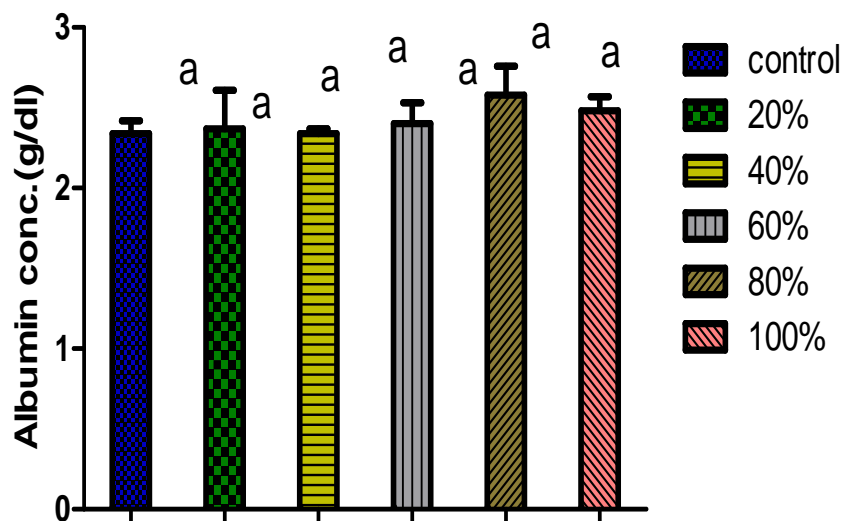
Figure 3.12:-



Effect of different percentage of Asa water on serum total protein

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
Bars with alphabet 'b' are significantly different from control

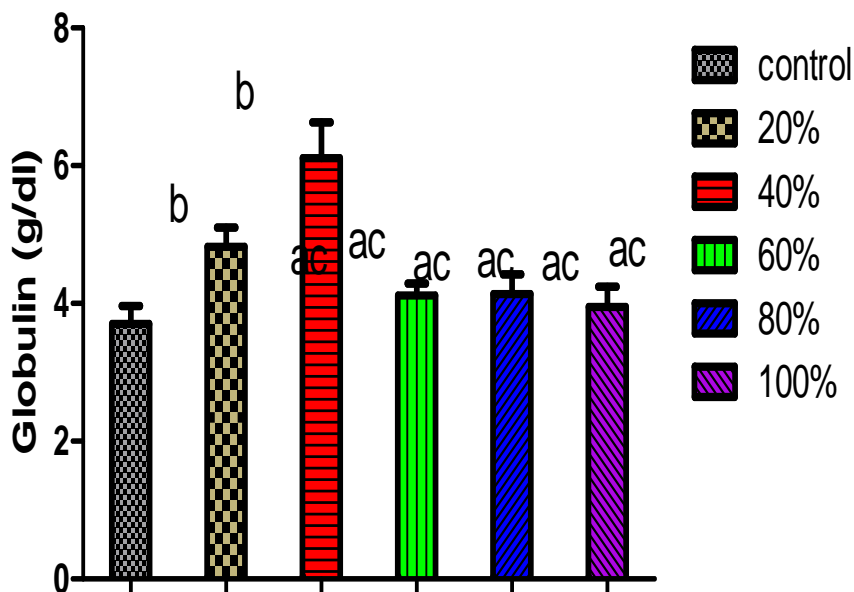
Figure 3.13:-



Effect of different percentage of Asa water on serum Albumin

Level of significance was taken at (P<0.05) for five rats per group.
 Bars with alphabet 'a' are not significantly different from control.
 No significant difference between the treated groups

Figure 3.14:-

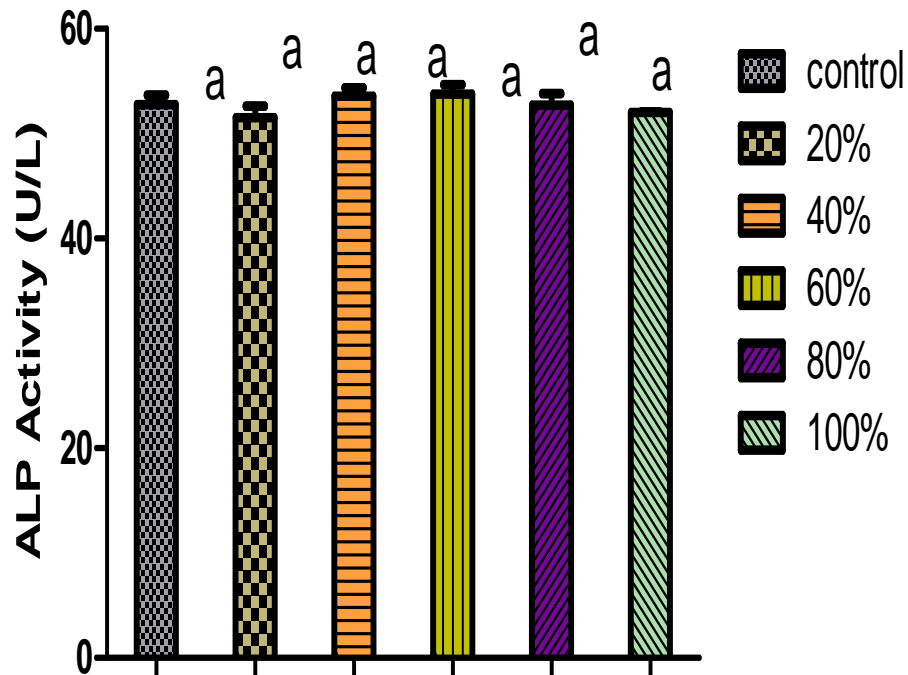


Effect of different percentage of Asa water on serum Globulin

Level of significance was taken at (P<0.05) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.
Bar with alphabet 'b' significantly different from control.
Bars with alphabet 'c' are significantly different from 40% Asa water.

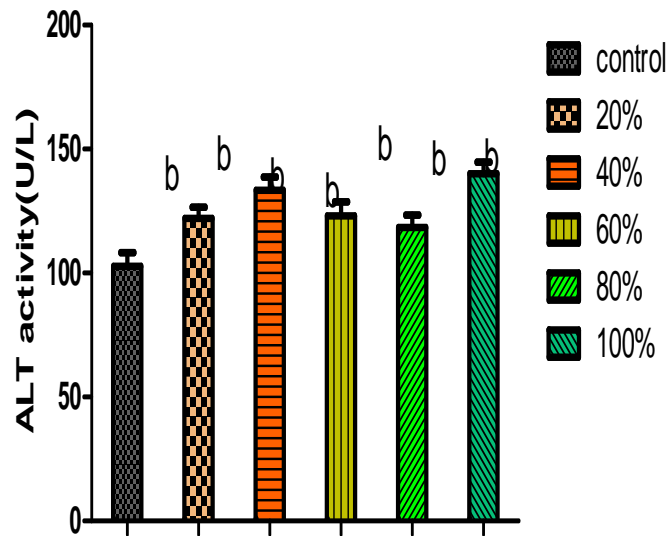
Figure 3.15:-



Effect of different percentage of Asa water on serum ALP activity

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

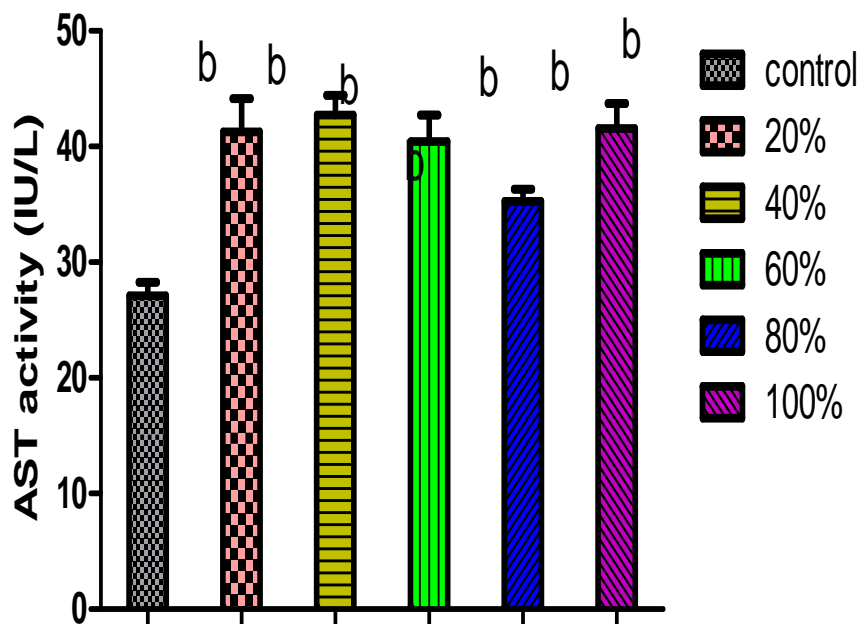
Figure 3.16:-



Effect of different percentage of Asa water on serum ALT activity

Level of significance was taken at (P<0.05) for five rats per group.
Bar with alphabet 'b' significantly different from control

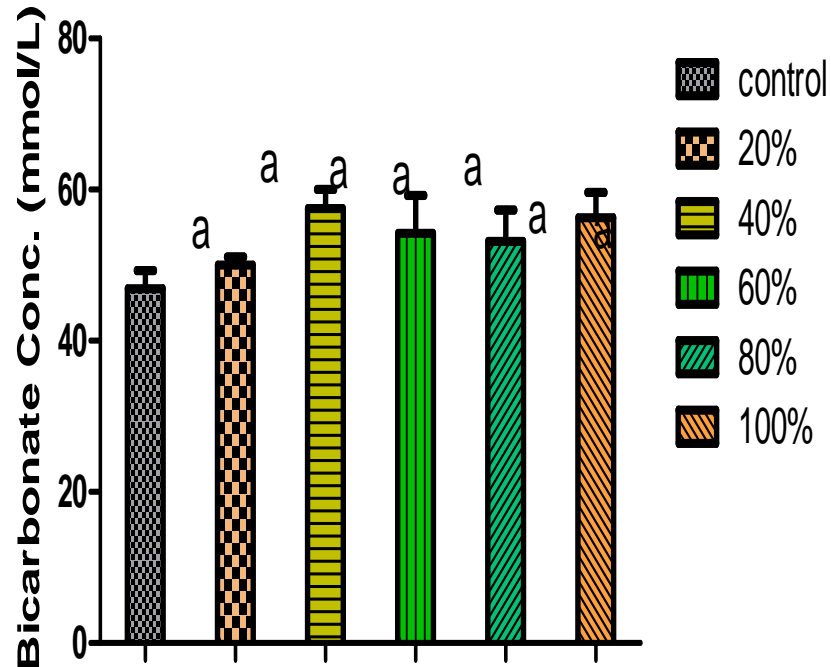
Figure 3.17:-



Effect of different percentage of Asa water on serum AST activity

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'b' are significantly different from control.

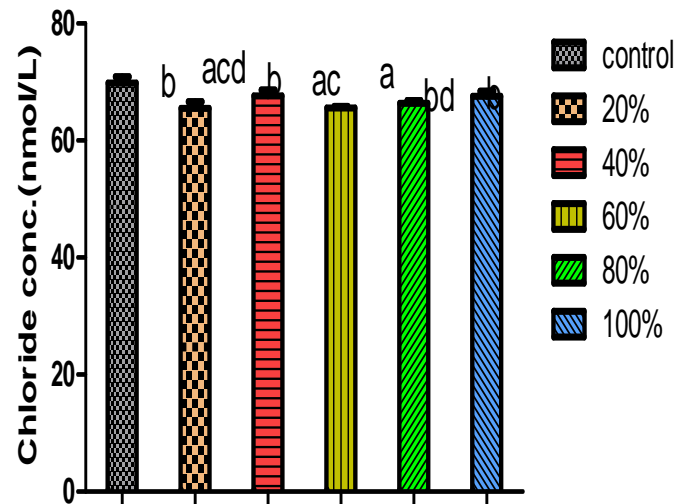
Figure 3.18:-



Effect of different percentage of Asa water on serum Bicarbonate conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant different between the treated groups

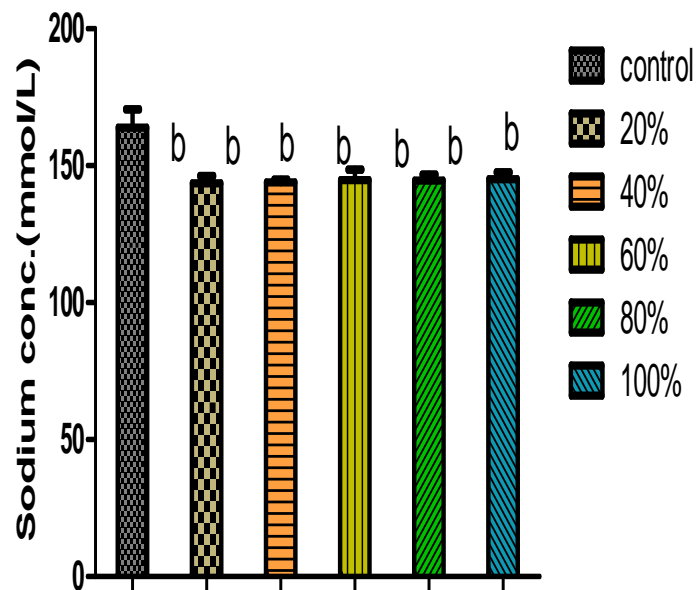
Figure 3.19:-



Effect of different percentage of Asa water on serum Chloride conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
Bar with alphabet 'b' significantly different from control.

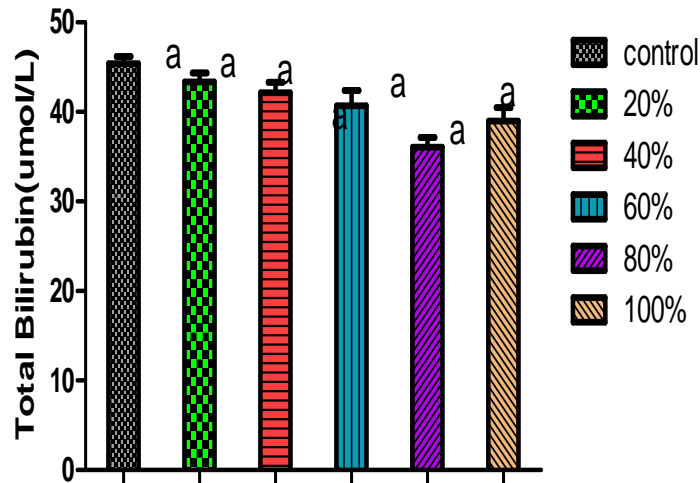
Figure 3.20:-



Effect of different percentage of Asa water on serum sodium conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'b' are significantly different from control.

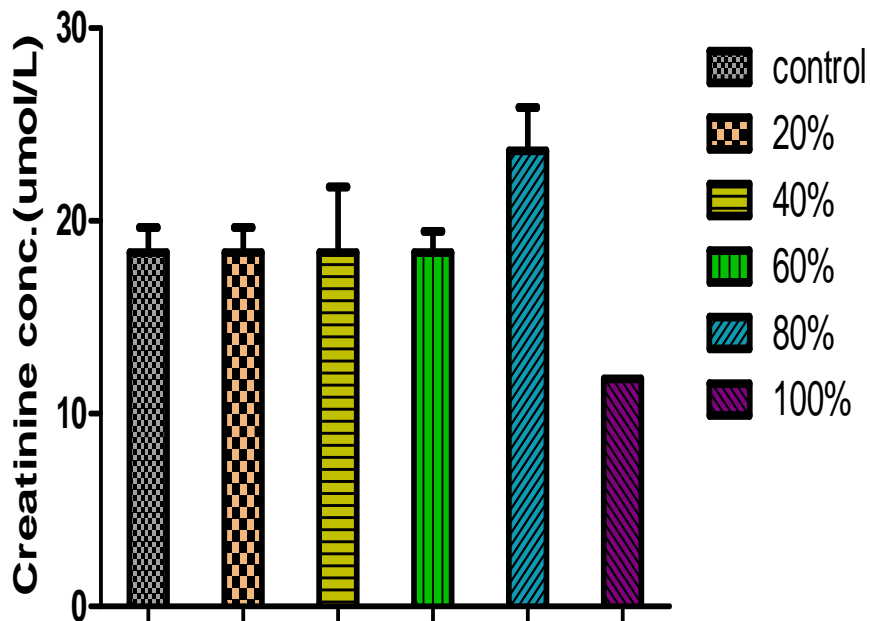
Figure 3.21:-



Effect of different percentage of Asa water on serum Total Bilirubin.

Level of significance was taken at (P<0.05) for five rats per group.
 Bars with alphabet 'a' are not significantly different from control.
 No significant different between the treated groups

Figure 3.22:-

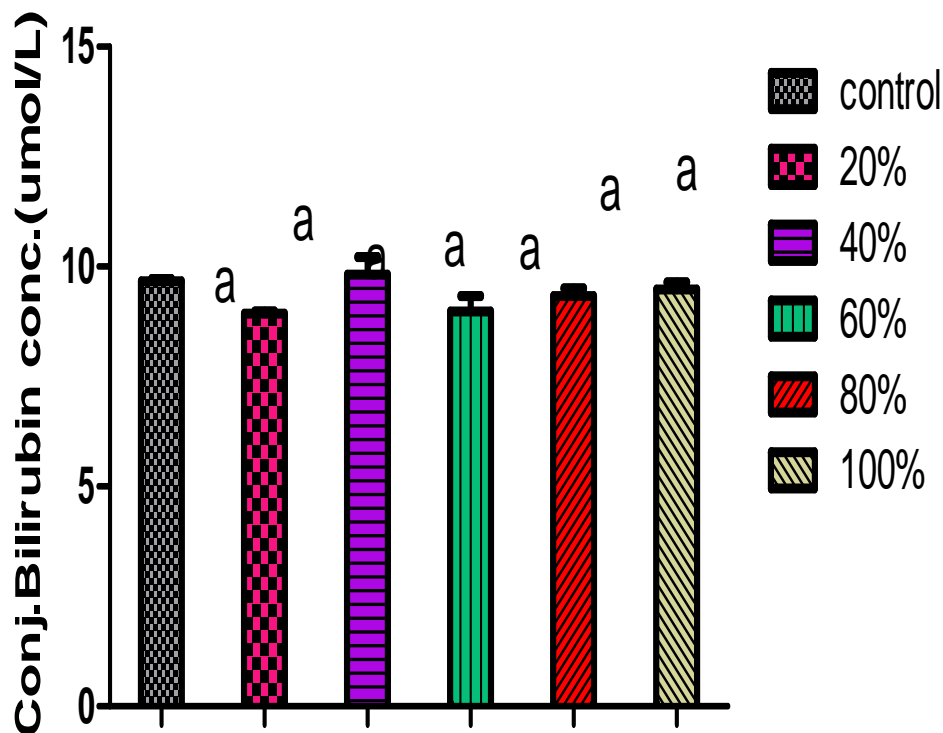


Effect of different percentage of Asa water on serum creatinine.

Level of significance was taken at (P<0.05) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.
Bar with alphabet 'b' significantly different from control.
Bars with alphabet 'c' are significantly different from CRH
Bars with alphabet 'd' are significantly different from 80% Asa water.

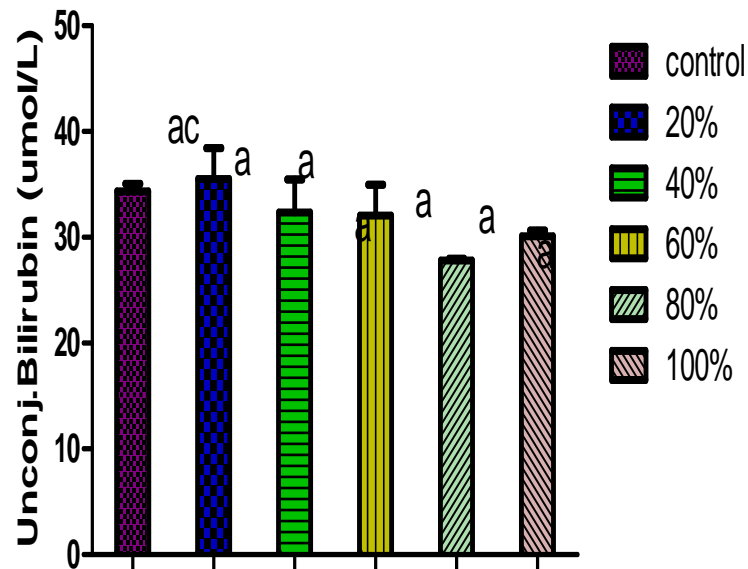
Figure 3.23:-



Effect of different percentage of Asa water on serum conj. Bilirubin.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
No significant difference between the treated groups

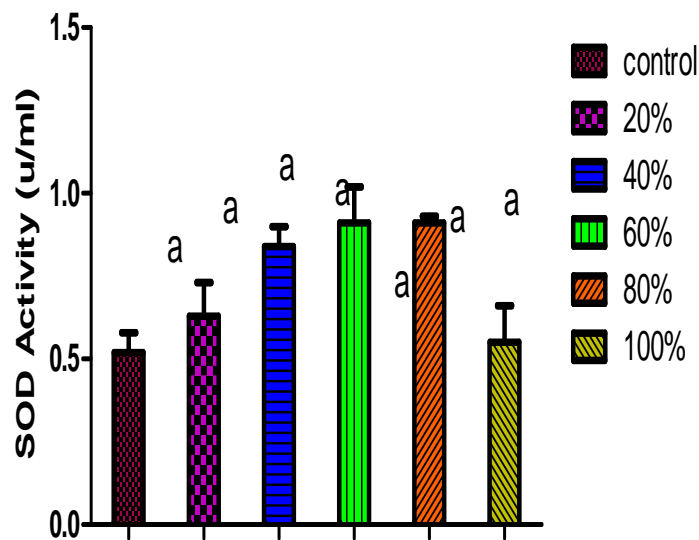
Figure 3.24:-



Effect of different percentage of Asa water on serum unconj. Bilirubin.

Level of significance was taken at (P<0.05) for five rats per group. Bars with alphabet 'a' are not significantly different from control. Bar with alphabet 'c' significantly different from CRH.

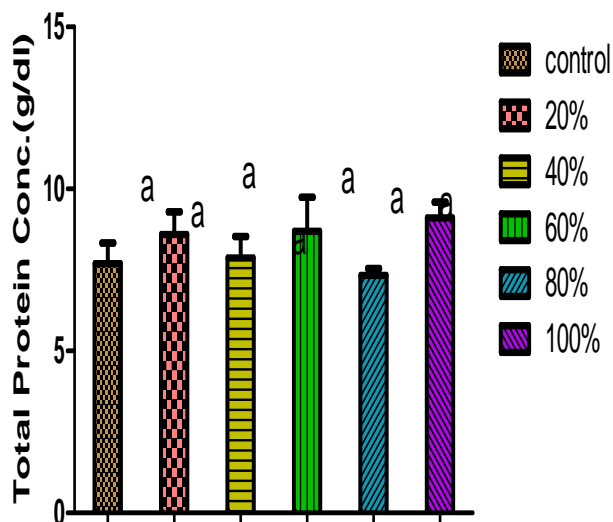
Figure 3.25:-



Effect of different percentage of Asa water on Liver SOD activity.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.

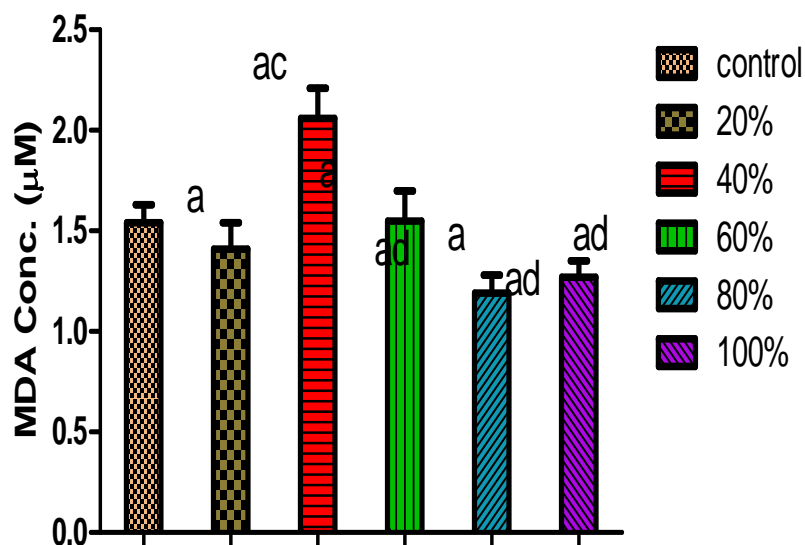
Figure 3.26:-



Effect of different percentage of Asa water on Liver Total protein conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.

Figure 3.27:-

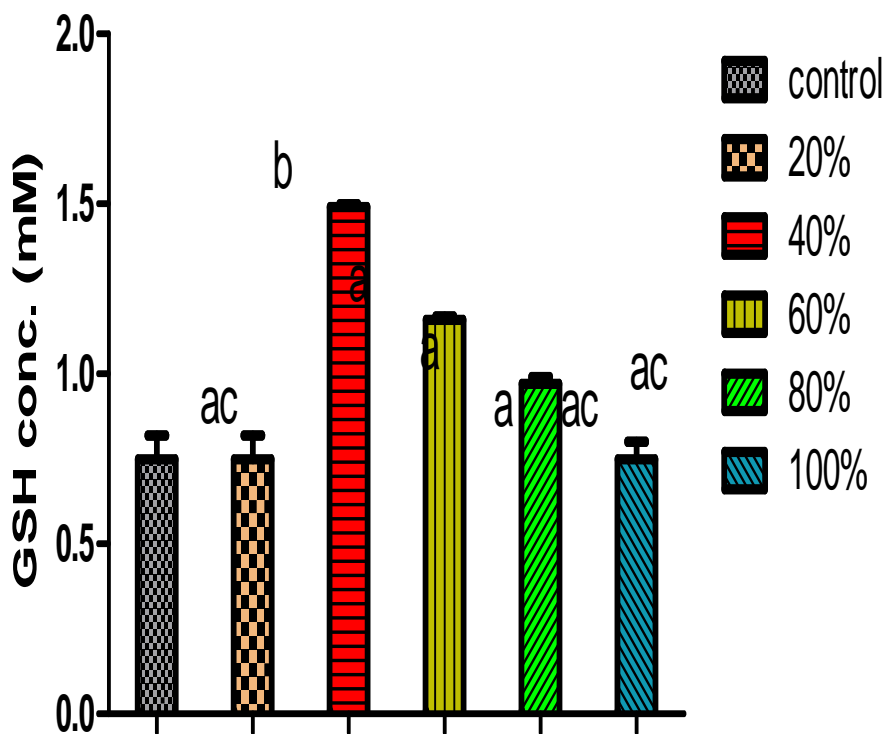


Effect of different percentage of Asa water on Liver MDA conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.
Bars with alphabet 'c' are significantly different from 20% Asa water.
Bars with alphabet 'd' are significantly different from 40% Asa water

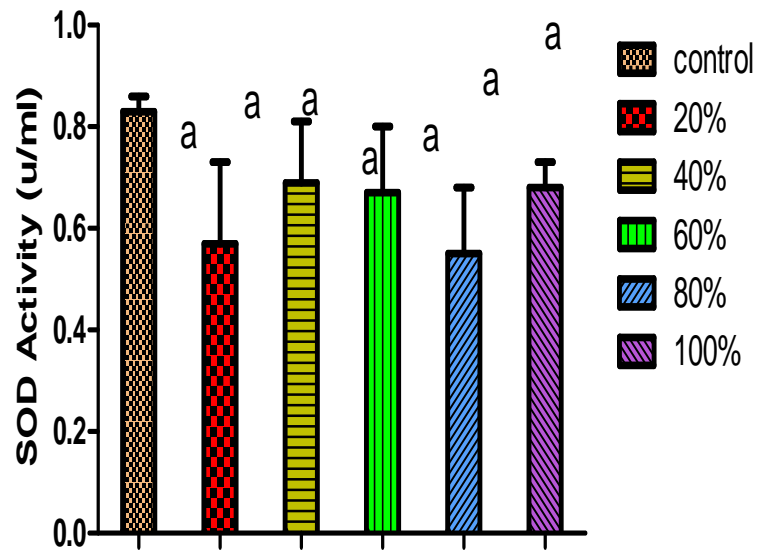
Figure 3.28:-



Effect of different percentage of Asa water on Liver GSH conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.
Bar with alphabet 'b' significantly different from control
Bars with alphabet 'c' are significantly different from 40% Asa water

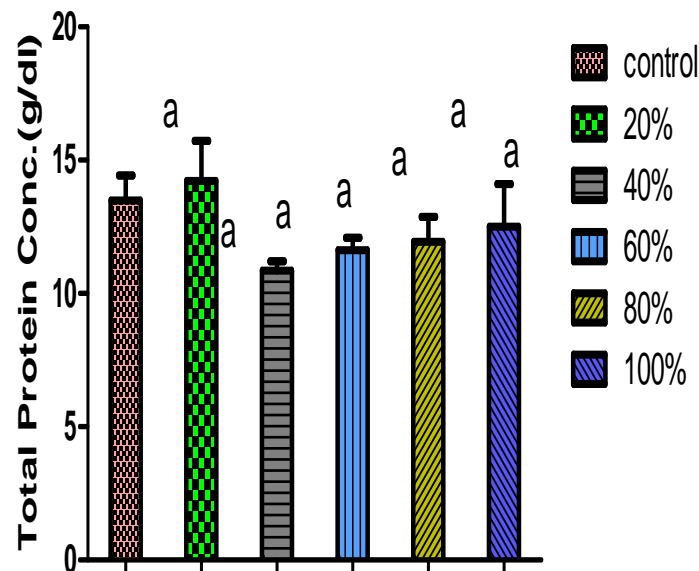
Figure 3.29:-



Effect of different percentage of Asa water on Kidney SOD activity

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bar with alphabet 'a' not significantly different from control.

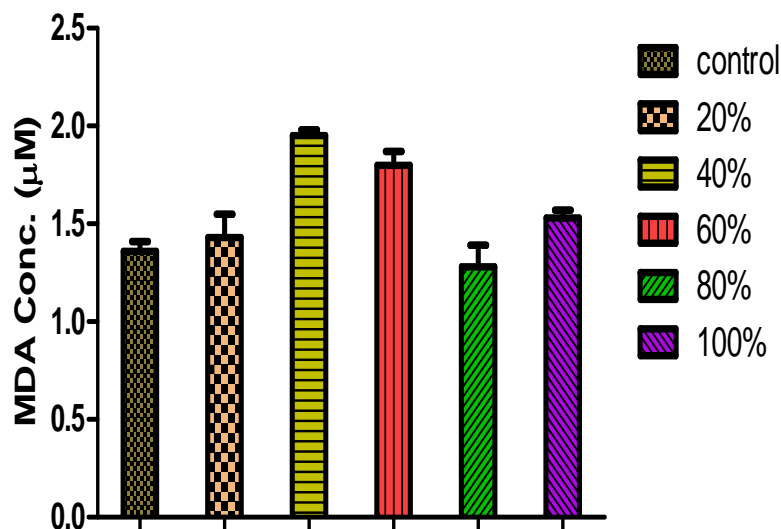
Figure 3.30:-



Effect of different percentage of Asa water on Kidney Total protein conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.
Bars with alphabet 'a' are not significantly different from control.

Figure 3.31:-



Effect of different percentage of Asa water on Kidney MDA conc.

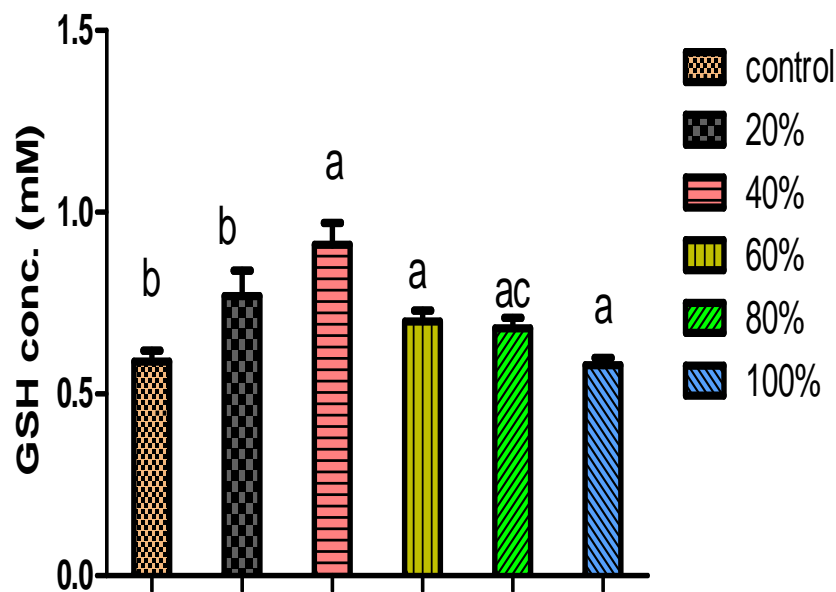
Level of significance was taken at ($P < 0.05$) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.

Bars with alphabet 'b' are significantly different from control.

Bars with alphabet 'c' are significantly different from water Hyacinth.

Figure 3.32:-



Effect of different percentage of Asa water on Kidney GSH conc.

Level of significance was taken at ($P < 0.05$) for five rats per group.

Bars with alphabet 'a' are not significantly different from control.

Bars with alphabet 'b' are significantly different from control.

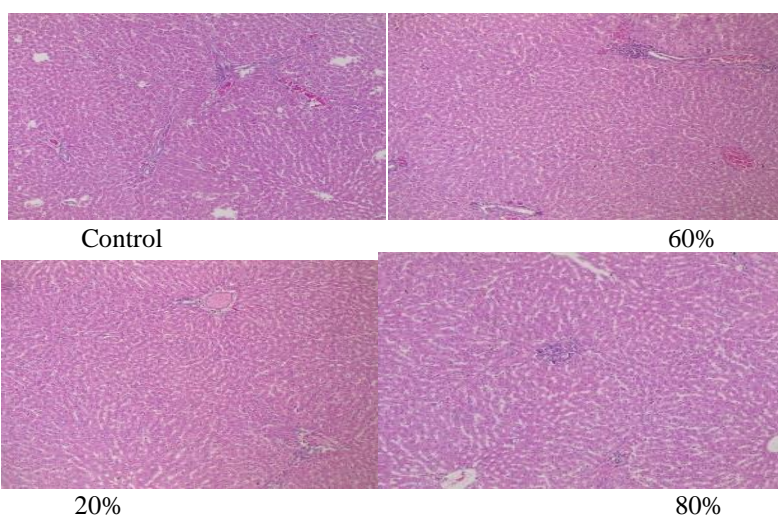
Bar with alphabet 'c' significantly different from 40% Asa water.

Table 3.1:- Aberrations observed in the Liver of Rats treated with different concentrations from Asa water.

Organ	Concentrations	Histopathological changes	Significance
Liver	20	Mild periportal cellular Infiltration.	+
	40	Severe central veinous Congestion. Mild periportal cellular Infiltration	+
	60	Mild to moderate periportal cellular infiltration	+
	80	Mild periportal cellular infiltration	+
	100	Mild congestion of the renal interstitium+	+

Table 3.2:- Aberrations observed in the Kidney of Rats treated with different concentrations from Asa water.

Organ	Concentration	Histopathological changes	Significance
Kidney	20	No visible lesion seen.	-
	40	Mild congestion of the renal interstitium. Few tubules appear degenerated.	+
	60	Severe congestion of the renal cortical interstitium.	+
	80	Severe congestion and hemorrhage at the renal interstitium.	+
	100	Severe congestion and hemorrhage at the renal interstitium	+



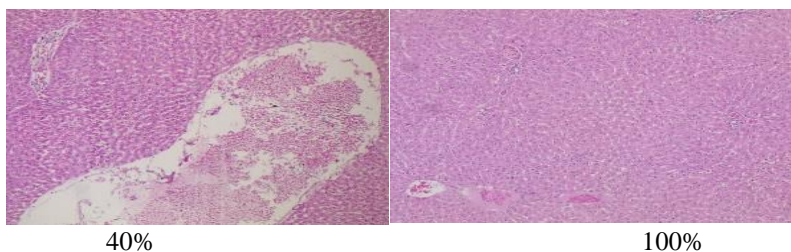


Plate 3.1:- Aberration observed in the Liver of rats with different concentrations from Asa water.

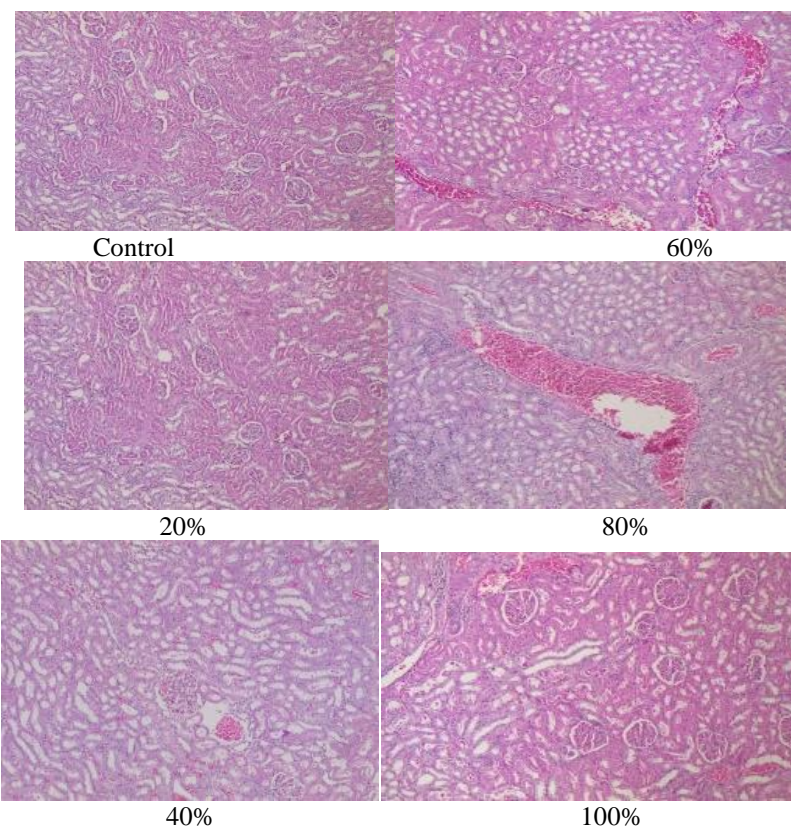


Plate 3.2:- Aberration observed in the kidney of rats with different concentrations from Asa water.

Conclusion:-

The Asa river did not significantly affect the haematological and biochemical indices of the experimental animals when compared with the control except the ALT and AST posing deleterious effect on the histopathological indices of liver and the kidney of the experimental rats at various concentrations, most affected at the higher concentrations. Innate hematocrit production mechanism was not affected by various components of the sites as negating the alteration which has been linked to various human pathological conditions.

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