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Histological and Morphometric Effects of Liv 52 on Acetaminophen Induced Liver Toxicity in Adult Albino Rats

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Abstract: *Paracetamol a commonly used analgesic has been associated with liver toxicity and resultant alteration of its histomorphology with eventual liver malfunctions. This hepatotoxicity has been shown to be as a result of prolonged use or overdoses of paracetamol. On the other hand LIV52 also known as livercare, a herbal formulation has been shown to have restorative effects on the liver induced hepatotoxicity. However there is paucity of data on its restorative histo-morphological effects on the acute induced liver hepatotoxicity or its chronic usage. In addition, Data on the restorative effects of LIV52 on the liver induced hepatotoxicity when used in varied doses is also lacking. This study aimed at determining the histo- morphological and morphometric restorative effects of varied doses of LIV52 following the liver hepatotoxicity induced with paracetamol. The research was conducted on November 2017 to July 2018.A total 25 rats of 150gms-170gms was included in the study and paracetamol drug was used to induced hepatotoxicity . A total of 30 rats was divided into 6 group, control group, paracetamol induced group, liv52 100mg group, liv52200mg group, liv52300mg group and liv52500mg group Daily weighing and feeding of rats was done, control group received DMSO alone for same schedule. Other group was induced with paracetamol for 5 days thereafter treated with liv52 of varied dose,100,200,300,500mg/kgbwt/day for 16 days while one group did not received the treatment , all groups was sacrificed after the experiment , liver was removed and weighed ,and morphometric measurement was determine by use of a ruler and caliper, while liver volumes was determine using displacement method (Archimedes principle).all liver was processed and stained with H.E stain for histological examination and the liver stromal tissue, hepatocytes cell ,kupffer cell ,central vein and portal triad was assessed using light microscope. For morphometric assessment, for paracetamol induced group, the percentage liver body ratio reduced significantly($p<0.05$) as compared to control group ($p>0.05$),while there was no significant different($p>0.05$ with the treatment with varying doses of liv52. In light microscopy Paracetamol induced group, shows dilated sinusoid capillaries, necrosis of paracentral vein and areas of parenchymal necrosis ,as compared to normal control group which had normal liver, liv52 treated group shows the varying features of healed liver parenchymal when was treated with varying doses. Hence the present study conclude that liv52 (livercare) has dose depend hepatoprotective effects in a paracetamol induced hepatotoxicity.*

Key words: *hepatotoxicity, liver volume and morphological changes*

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I. Introduction

Liver, the main organ of metabolism, excretion and detoxification in the human body has been shown to be affected by many agents causing serious health problems worldwide[123]. The rapid growth of morbidity and mortality from liver failure are attributable to the increasing number of chemical compounds and environmental pollutions[456] . Liver plays many essential roles in maintaining the normal physiology process through detoxification and metabolism process hence vulnerable to liver toxicity [7]. A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage [8].Liver damage has been shown to cause an end stage liver damage which is fatal and patient may requires liver transplant which is cumbersome and a burden to developing countries mostly[91] its estimated that 1600-2000 people died worldwide due to liver failure and 30% requires liver transplant daily[10] .The histo- cyto-architecure of the liver has been shown to be affected by many agent causing drug induced liver failure DILF[3], among them is acetaminophen commonly used analgesia[5], when either used in overdose or prolong period of time hence may leads to accumulated toxicity leading to hepatotoxicity[11-12] . The paratecamol injurious effects to the liver cells are due to its associated metabolites NPQ1 (N-acetyl-para-benzoquinone imine) that causes hepatocytes

to undergo oxidative stress hence leading to bursting of hepatocyte mitochondria cells [13] Which generate oxygen radicals and nitrogen species which leads to necrosis to these hepatocelluar cells of the liver, causing liver damage and live failure [14]-[11]. paracetamol overdose is associated with 56000 emergency and 4000 fatalities each year in causality department in USA[15]. Among these, 37% is cause by paracetamol alone [15] . it has also been documented that together with alcohol causes synergistic effects and cause severe liver failure[16] among other medication like acetylsalicylic acid, codeine, and oxycodone[3] .many remedies has come up for treatment of liver failure[17], among them is LIV52 also known as liver care[18]. Liv52 herbal formulation, al has been shown to be effectively restore the functional and the biochemical aspects of the liver following the injurious effects of acetaminophen[19]. LIV52 has six formulation namely: **Capparis spinosa 17 mg, Cichorium intybus 17 mg,Solanum nigrum 8 mg,Cassia occidentalis 4 mg ,Terminalia arjuna 8 mg ,Achillea millefolium 4 mg,Tamarix gallica 4 mg**[19]**. The** functional and biochemical restorative effects of liv52 have been documented and this research was to find out the actual histological and morphometric changes in liver due to drug induced hepatotoxicity using paracetamol as induction.

II. Material And Methods

Study designs experimental laboratory based study, used of rats as animal model, Albino males rats of 140-170gm was obtain from Jomo Kenyatta University of agriculture and technology small safari house, where they acclimatized through feeding and weighing for one week before experiment. The total of 30 rats was sampled , and randomly assigned into treatment group ,one group was not treated put on DMSO only for the rest of experiment, all group was put on paracetamol for 5 day, one group continue with DMSO alone for the rest of remaining 16 days and the other four group was treated with LIV52 from 6th day for the rest of 16 days, the experiment was running for 21 days .

Study Design: Research was experimental laboratory based study using rat as animal model
Study Location: The experiment was carried out at JKUAT safari animal house
Study Duration: November 2017 to July 2018.
Sample size: 30 albino rats.
Sample size calculation: The sample population was drawn after the resources equation which gave a total of 25 rats to be included in the experiment, 5 rats was assign to control group and 20 rats was assign to experimental group

Subjects & selection method: the rats was assign to their respective group through simple random sampling
Group A (N=5 rats)-no treatment on feeds, water and DMSO only for 21 days
Group B (N=5 rats) –Paracetamol daily for 5 day and then DMSO for 16 days;
Group C (N=20 rats) –Paracetamol daily for 5 days and then LIV52 for 16 days

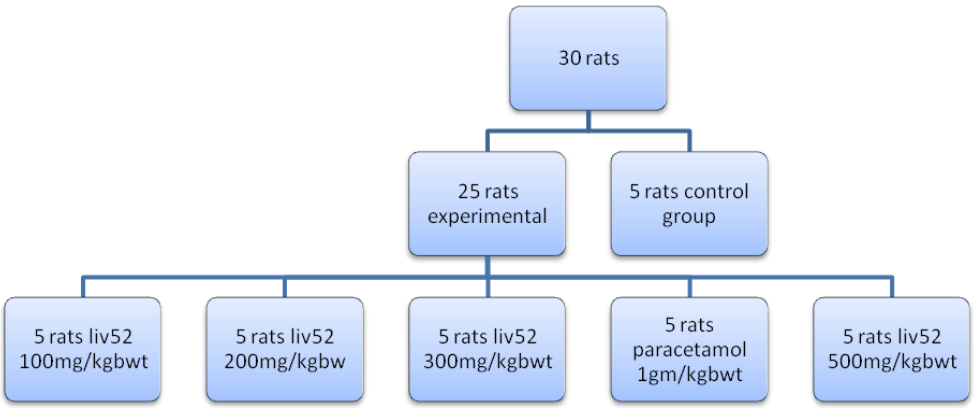


DIAGRAM 1; Flow chart showing group's samples

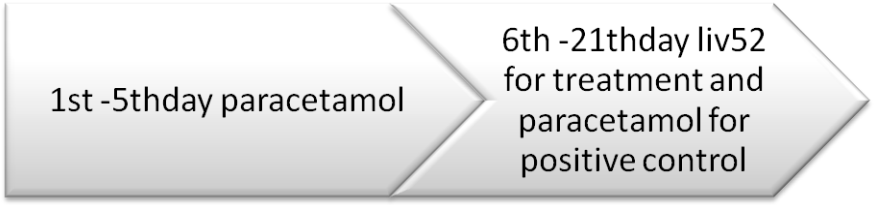


DIAGRAM 2; Flow diagram showing how paracetamol and liv52 was given

Procedure methodology

After written informed consent and obtaining letter from animal ethics committee of Jomo Kenyatta University of Agriculture and Technology .Procurement of drugs, paracetamol, liv52 (livercare) and DMSO (dimethyl sulfoxides) was done. Rats was assigned to their respective groups by simple random sampling as **(Diagram 1**), where daily weighing and administration of drugs was done between 8-10Am, paracetamol and liv52 was dissolved in 5% DMSO and converted into animal equivalent dose before administration[20-21]. Induction of hepatotoxicity was done using Paracetamol drug which was administered for five day for all group apart from control group who continued with 5%DMSO alone for 21 days, one group did not received treatment of liv52 ,while the other four groups continue with treatment of varied doses of liv52 for 16 day . All rats was sacrifice on 21st day after completion of treatment (**Diagram 2)**

ASSESSING LIVER DIMENSION

The liver was obtained, measured using a ruler and a caliper for width, length and thickness.

Estimation of percentage liver body ratio for the all groups

The rats terminal weight was determined and livers was weighed using weighing scale and both was used to determine the percentage liver body ratio by the following equation.

Percentage liver body ratio =liver weight÷ terminal weight in grams ×100.

Estimation of liver volume and histological changes

The liver volumes was determined using displacement method or water immersion method (WIM) Archimedes principle. Where the liver was immersed in graduated beaker with normal saline and displaced saline was measured. The means volumes and standard deviation (±SD) was calculated.

After volume determination the whole liver was fixed in formalin for 24 hours, later dehydrated using graduated alcohol:50%,60%,70%,80%,90%, and 100% for one hour, then cleared with xylene for 12 hours. The tissue was then infiltrated with paraffin wax for 12 hours and embedded in paraffin wax, microtome sledge was used to cut the embedded tissue into thin section of 5µm,then it was floated in water at 37c and stuck in glass the covered with thin film slip was applied . 20 slides was picked in each liver using simple uniform random sampling, it was then stored in an oven at 37c for 12 hour then viewed under light microscope, the microscopic fields were selected randomly in each liver section. The microscope stage was then moved along the X and Y directions every time, till the entire section had been studied, photos was taken using LABOMED ivu 3100 imaging camera softener with pixel pros[22]. Results was entered on excel sheet.

Statistical analysis

Data was analyzed using SPSS version 26 (SPSS Inc., Chicago, IL). ANOVA used to ascertain the significance of differences variables and confirmed by Mann-Whitney test. The level $P < 0.05$ was considered as the cut off value or significance differences.

III. Result

1. MORPHOMETRIC CHANGES

There was statistical significant different ($p<0.05$) in liver weight , volume and percentage liver body ratio for paracetamol induced group (**Table1)** as compared with liv52 treatment group with no statistical significantly different ($P>0.05$). This suggest that liver weight, liver volume and liver body ratio for paracetamol induced group reduces as compared with control and liv52 treatment groups with varied doses. However there was no significant different $p>0.05$ with control group when rats was treated with varying doses of liv52 (100mg/kgbwt,200mg/kgbwt ,300mg/kgbwt and 500mg/kgbwt .)

The liver dimensions: the thickness, length and width of the paracetamol induced group was statistically significantly $p<0.05$ as compared to control and those received the treatment of liv52 ,the length means was (2.125±.0500*,) width (2.075±.0957*) and thickness (0.925±.0500**),however there was no statistical significant different ($p>0.05$) with control for treated group with liv52, 100mg,200mg 300mg and 500mg dosage and all livers had 6 lobes in all groups (**Table 2)**

Table 1. Shows the mean liver weight of the study animals, comparative terminal liver to body ratio, and the liver volumes between the experimental groups compared with the control

The study Parametres	*ANIMALS GROUPS*					
	Control group	***Paracetamol treated group (1gm/kg/bw)***	***LIV52 treated groups (100Mg/Kg/bw)***	***LIV52 treated groups (200Mg/Kg/bw)***	***LIV52 treated groups (300Mg/Kg/bw)***	***LIV52 treated groups (500Mg/Kg/bw)***
Mean Terminal Liver	12.025±1.701	4.800±.58**	9.325±.359	10.600±1.591	11.050±1.843	10.550±1.389

weight(gms)						
The percentage ratios of the Mean Terminal body weights compared with terminal liver weight (grams)	5.31%±0.51	3.87%±0.278*	5.12%±0.13	5.5095%±0.26	5.61%±.44	5.26%±0.13
Terminal Liver volume (*by displacement method in* Mls)	11.225±1.6378	4.675±0.0957*	8.550±.2646	9.825±1.5349	10.375±1.5349	9.825±1.8446

KEY
All values are expressed as the mean ± the standard error of the mean (SEM).
*P<0.0001
**P<0.005
***P<0.05different from controls

Diagram3; Showing the measurement of thickness, width and length

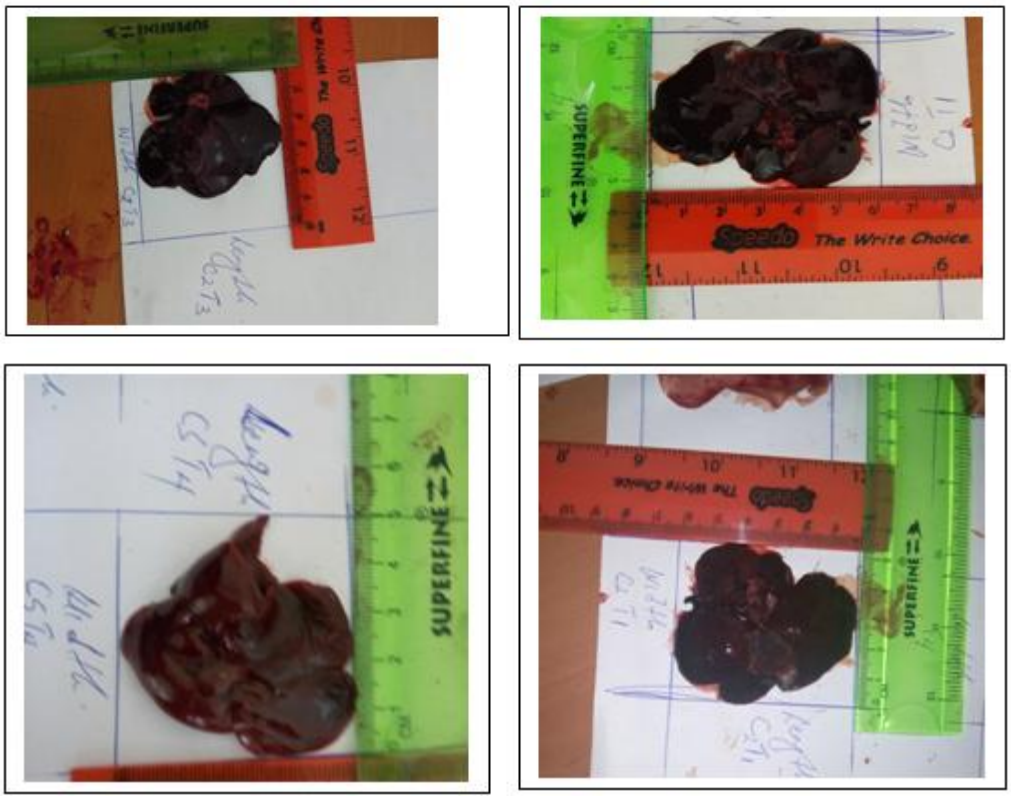


Figure 1 photographs showing the measurement of liver width, length and thickness for control group ,paracetamol induced group, liv52 treated group with 100mg/kgwt group,200mg/kgbwt,300mg/kbwt and 500mg/kgbwt

Table 2.showing liver dimension; liver lobes, liver width, length and thickness in various treatment with liv52 drugs

	ANIMALS GROUPS					
Study parameters	***Control group***	***Paracetamol treated group***	***LIV52100MG/kg bwt***	***LIV52 200MG/kgbet***	***LIV52 300MG/kgbwt***	***LIV52 500MG/kbwt***
Liver lobes	6	6	6	6	6	6
Liver width	2.30±0.081	2.07±0.095*	2.27±0.050	2.25\±0.057	2.22±0.095	2.40±0.081
Liver length	2.47±0.097	2.12±0.050*	2.22±0.050	2.27±0.050	2.35±0.057	2.60±0.081
Liver thickness	1.12±0.0957	0.925±0.05**	1.07±0.095	1.07±0.095	1.35±0.057	1.35±0.129

KEY; All values are expressed as the mean ± the standard error of the mean (SEM).
**P*<0.0001

**P<0.005
***P<0.05*different from controls*

***2.* MORPHORLOGICAL CHANGES WITH PARACETAMOL AND LIV52 VARYING DOSES**

Light microscopy the livers from the paracetamol induced groups shows prominent dilatations of sinusoidal capillaries, central veins and branches of the portal vein, there was also micro vesicular hemorrhage and cetrilobular necrosis in the livers (**Figures 3 A**). As compared with the normal liver for control group (**Figures1,) which** shows normal liver features. Many hypertrophied hepatocytes containing large cytoplasmic vacuoles were also seen with Necrotic foci accompanied by kupffer cell infiltrations in paracetamol induced group (**Figure 3, C**). Additionally, there were hemorrhagic areas around the portal triad and central vein with para- central vein necrosis. The liver cells hepatocytes shows dense cytoplasm with dark nuclei, suggestive of cellular degeneration which may leads to liver necrosis. many kupffer cells were found in the liver sections which might havelead to extensive necrosis and hemorrhages as in (**Figure 3 B).** While the control and treated group with liv52 show normal hepatocytes, sinuisoid, capillarities, central vein ,portal triad and few kupffer cells, the liver cell treated with liv52 100mg dose had slight changes as compared to 200mg, 300mg and 500mg which shows fully recovery. liver treated with 100mg of liv52(**figure 4**) shows presents of widespread areas of liver cells congestion and hemorrhage in the centrilobular and midzonal areas of the liver which means there was slight different with paracetamol induced group. While liver with 200mg (**figureE**) had moderate congestion and necrosis of single hepatocytes, and this was limited to the area immediately around the centrilobular vein, and those treated with 300mg and 500mg **(figure F and G**) which depicts minimal congestion and hemorrhagic area around the centrilobular vein and extending into the mid zone cells; most lobules of the liver was not affected. Areas of confluent necrosis limited to the liver cells surrounding the centrilobular vein. Involving all hepatocytes in the centrilobular zone; bridging of areas of *necrosis between centrilobular zones is common.*

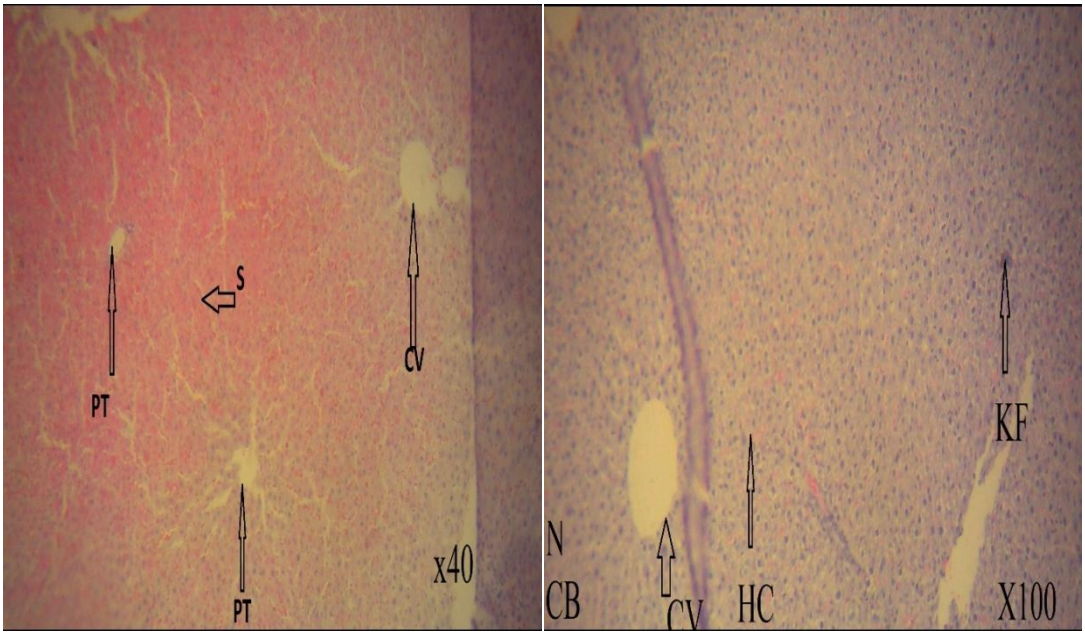
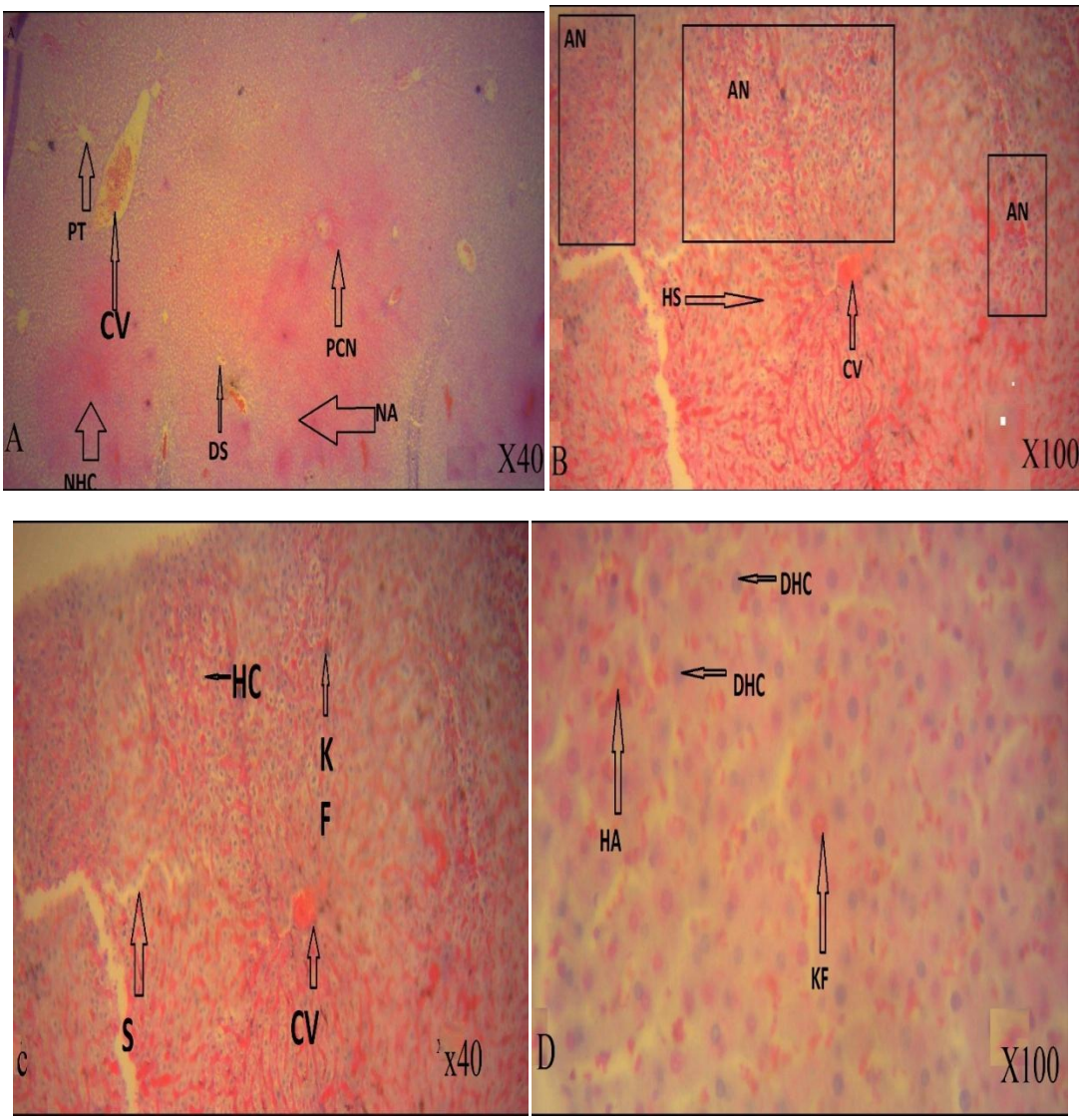
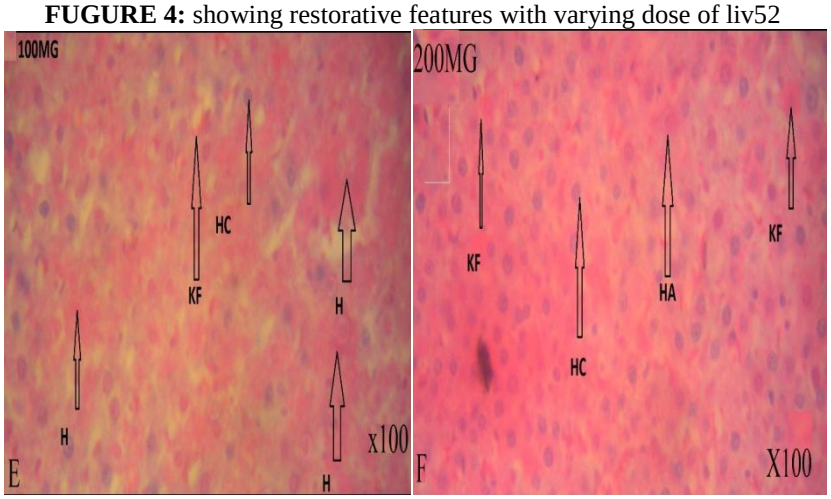


FIGURE 2*;Normal control group****:S*** *sinusoids,* ***CV*** *central vein ,****PT*** *portal triad .****magnification X40 and X100(stain with H and E)***



FIGURES 3, LABEL A and B: *Paracetamol induced group;Shows dilatation of sinusoids **DS**, area of necrosis **NA**,paracentral vein necrosis **PCN**,**CV** central vein ,**PT** portal traid* **,magnification x40,FIGURE B***; paracetamol induced group in area of necrosis **AN,** hemorrhage sinusoids **HS**, blood in **CV*****Magnification x100*** **FIGURE C and D**.*paracetamol induced liver showing several kupffer cell **KF** ,dilatated hypetrophic hepatocytes **DHC,**heamorrgic area **HA,**magnification 100x(**stain with H and E**)*

FUGURE 4: showing restorative features with varying dose of liv52



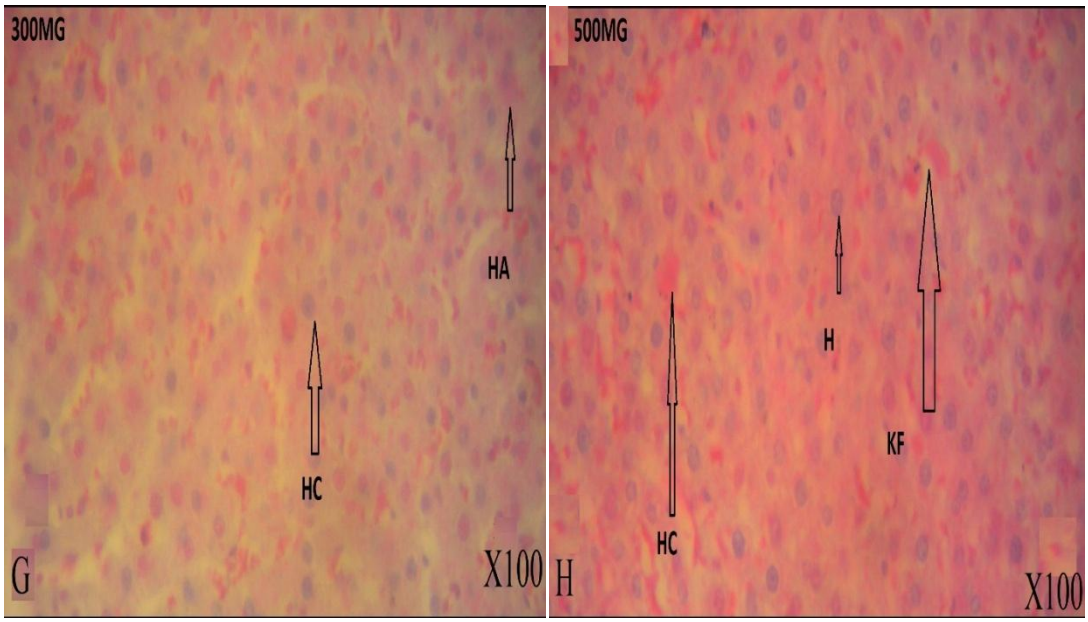


FIGURE 4 E: Diagram showing liver treated with liv52 100mg dose hemorrhage H,kupffer cell KF,dilated hepatocytes H magnification 100x stain with H and E)
FIGURE 4 F:Diagram showing liver treated with liv52 200mg (AED)kpffer cell KF,hepatocytes H,hemorrhage area HA magnification100x(stain with H and E)
FIGURE4 G:Diagram showing liver treated with liv52 300mg (AED)hepatecytes cell HC,hemorrhage area magniH.Amagnification100x (stain with H and E)
FIGURE4 H:Diagram showing liver treated with liv52 500mg(AED)minimal few kupffer cell KF and normal hepatocytes cell ,Minimal hemorrhage cells HC, no area of necrosismagnification100x,(stain with H and E)

IV. Discussion

The liver is the main organ of metabolism and detoxification and continuous expose to chemical agent intentionally or unintentionally may lead to hepatic cell damage[2324], though many interest has emerge to find a solution to this problem.liv52 (livercare) has proven to play a vital role in treatment of hepatotoxicity due to many chemicals agents, viral diseases and other live toxic substances [25] .Liv52 has shown to be hepatoprotective to hepatic liver cells and physiological restoration of liver and other organs[24].In the current study the rats induced with paracetamol drug reduced in weight significantly for the first 5 day on treatment and later picked when liv52 treatment was introduced,same observation encountered by other authors[19-25] ,and this could be explain when physical being and food intake of rats was observed during the study[25]. From the present study the morphometric and histo-cytoarchitecture data obtained suggest that hepatotoxic induced livers shown derange liver volumes ,width length and liver thickness with histological changes dilated hepatocytes cells, necrosis of liver par central vein as compared with normal group[4-5-10], but when treated with varying dose of Liv.52 herbal formulation it Exhibited a beneficial reversal effects of morphometric and histological parameters [19-25-24]. The Liv.52 herbal formulation treatment also significantly reverse liver cell injury and necrosis in hepatic tissues, this observation was also found by [24] which indication that it has hepatoprotective properties. The mechanism behind the beneficial action of Liv.52 herbal formulation in the present experimental study could be because of its potent antioxidant and other hepatospecific actions by six components formulation[19],

Conflicts of interest
I declare that there is no conflicts of interest and no Ethical conflicts among the authors or the experimental methodology.

V. Conclusion

In conclusion, oral administration of liv52 herbal formulation reverses the hepatotoxicity in paracetamol induced rats model .These findings suggest that the liv52 was effectively restores the function and cyto-architecture of the liver ,hences this study elude that liv52 has therapeutically approach to liver hepato-protective property.

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