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Assessment of the effects of *Saccharum barberi* ethanolic extract on serum enzymes and liver histopathology of albino Wister rats and guinea pigs

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Abstract
The biochemical and histopathological effects of ethanol extract of powdered saccharum berberi stem on albino wistar rats and Guinea pigs were carried out, Forty albino wistar rats and forty guinea pigs of mixed sexes were randomly assigned into four study groups of ten (10) each. Group I is the control group while group II, III and IV received a gradual dose of 100mg/Kg, 200mg/Kg and 300mg/kg body weight respectively for a period of twenty one days.
The serum enzymes: - Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), α-glutamyl transferase (GGT) and liver histopathology were assessed.
The plant extract produced a significant effect on the serum enzymes for both animals at $P< 0.05$. There was little damage to the liver progressively. The extract appeared to be safe at the dose level of 300mg/Kg body weight in albino wistar rats and Guinea pigs, however due to the progression with the dose level, a more serious damage could arise at higher concentrations.

Keywords: *Saccharum barberi*, histopathological, biochemical, damage

Introduction
Life, health, disease and decay are inseparable from man and hence man has sought to fight and control disease and pain, with the use of plants and animals in their environment. Recent scientific record by WHO (world health organization) showed that over one billion people uses herbal plants and over twenty-one thousand medicinal plants had been discovered, including the species saccharum barberi (Das and Oudhia, 2000) [1].
The plant saccharum barberi, belong to the family of poaceae and genus saccharum is about (3–5)m tall and (2–3)cm width. The plant have spiral alternate leaves and it is a monocotyledon. They are mostly found in rain forest area of the world.
This plant had been found useful in the treatment of the following ailments: bedsores, ulcer, skin burn, cancer, malaria, cold, cough, diarrhea, spleen, tumor etc. (Begun 2002) [2]
Serum enzymes activity were determined by the feeding of the powdered stem extract of saccharum plants to examine their effect on the guinea pigs and albino wistar rats. An assessment of the liver histopathology were equally assessed.

Method
a) Experimental design
The albino wistar rats and Guinea pigs were divided into four groups each (A, B, C and D). Each group is made of Ten Albino wistar rats and Guinea pigs. Group A is the control while Group B, C and D were fed with 100mg/Kg, 200mg/Kg and 300mg/Kg body weight for a period of twenty-one days, there were sacrificed. The serum and liver taken for analysis.

b) Enzyme assay
1. Determination of alkaline phosphates
2. The enzyme reacted with the substrate nitro phenyl phosphate leading to the formation of para – nitro phenol +phosphate. The absorbance was taken at 405nm and converted to activity of this enzyme.
3. U/L = 3300 × DA 405 (nm/min)
4. Determination of Aspartate amino transferase (Rietman and Frankel 1957) [3]
5. This was done by monitoring the oxaloacetate hydrozone formed on the reaction of this enzyme with 2,4 – dinitrophenyl hydrazine. The intensity of the colour complex is proportional to the enzyme activity.
6. Determination of Alanine amino transferase (Rietman and Frankel 1957) [3]
7. The enzyme activity is monitored by the pyruvate hydrozone formed on reaction with 2,4 – dinitro phenyl hydrazine and the absorbance taken at 546nm of the colour complex.
8. Determination of the α – Glutanyl transferase (GGT) (Szasz 1969) [4]
9. This enzyme reacts with para – nitroanilide producing a substance, whose absorbance is read at 405nm.

c) Histological studies of the liver
The liver of the albino wistar rats and Guinea pigs were prepared for viewing under the microscope using the following methods.

1. Dehydration
The liver sample water were removed because of the embedding media. They were immersed in multiple water

baths and progressive higher concentration of ethanol. Copper sulphate were used to check the presence of water.

2. Clearing

The liver tissues are immersed in Xylene to remove the alcohol and make them transparent.

3. Embedding

The liver tissues were clean with high grade paraffin which fill the spaces and cavity in the tissues. The tissues were laid in glass slide and stained with Heamatoxylin and cosin (I & II). The micrograph were captured using phenix MC 1000 digital microscope and PHMIAS.

Results

Table 1: effect of saccharum barberi extract administration on ALT, AST, ALP and GGT in Albino wistar rats.

Group	Mean value ALT (g/dl)	Mean value AST (g/dl)	Mean value Alp (g/dl)	Mean value GGT (g/dl)
A (control group)	91.63±4.35	0.166±0.07	77.23±3.66	63.21±1.04
B (100mg/Kg)	81.69±3.50	0.166±0.08	64.29±6.05	62.52±1.16
C (200mg/Kg)	232.94±4.36	0.168±0.00	66.29±2.11	69.46±0.81
D (300mg/Kg)	323.51±2.8	0.158±0.00	73.84±5.07	72.58±1.04

Values are mean ±SD (n = 10) {P < 0.05}

Table 2: Effect of saccharum bar Beri extract administration on ALT, AST, ALP and GGT in Guinea pigs.

Group	Mean value ALT (g/dl)	Mean value AST (g/dl)	Mean value Alp (g/dl)	Mean value GGT (g/dl)
A (control group)	207.55±5.05	0.164±0.06	73.15±1.21	69.20±1.08
B (100mg/Kg)	110.95±0.69	0.164±0.06	67.25±1.40	74.98±0.87
C (200mg/Kg)	89.43±2.62	0.156±0.00	68.84±1.47	76.59±0.55
D (300mg/Kg)	243.85±3.58	0.152±0.00	76.96±0.82	80.61±0.37

Values are mean ± SD (n = 10) {P < 0.05}

Graphical illustrations of data obtained.

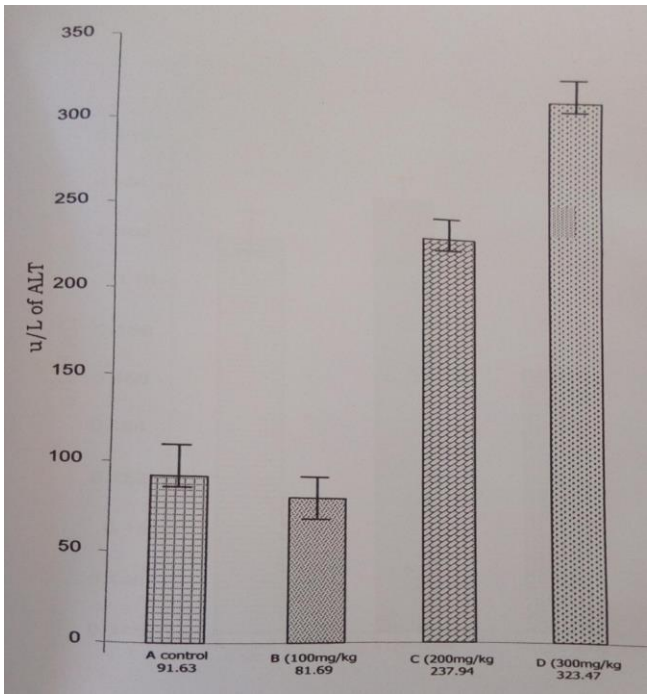


Fig I: Effect of saccharum berberi extract on ALT of Albino wistar rats.

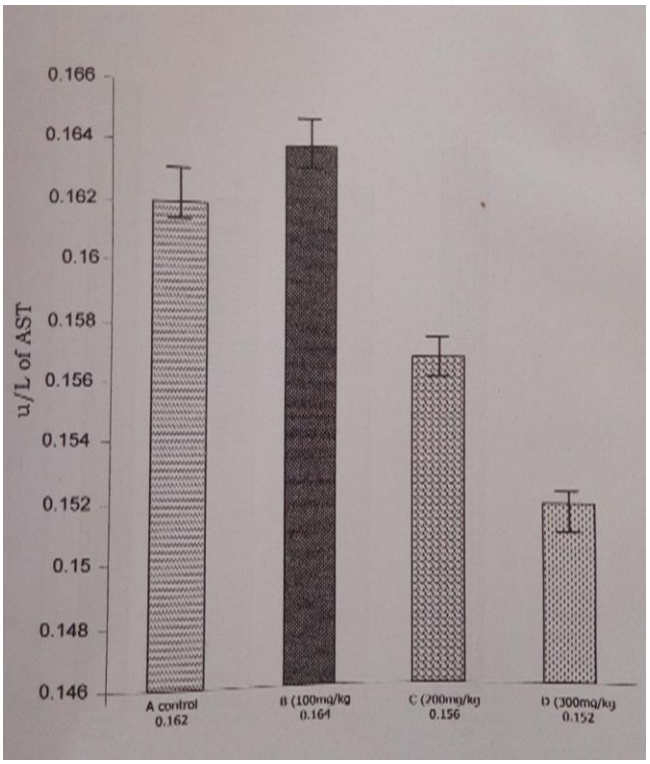


Fig 2: Effect of saccharum berberi extract on AST of Albino wistar rats.

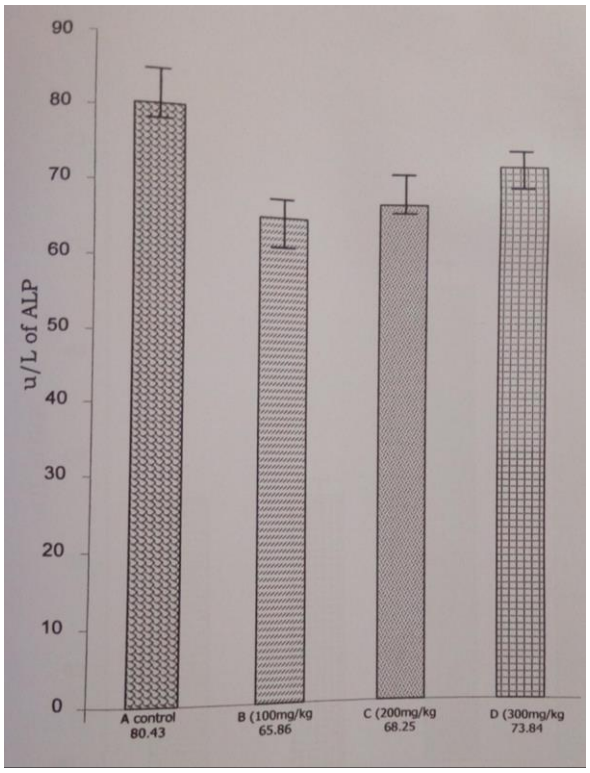


Fig 3: Effect of saccharum berberi extract on ALP of Albino wistar rats.

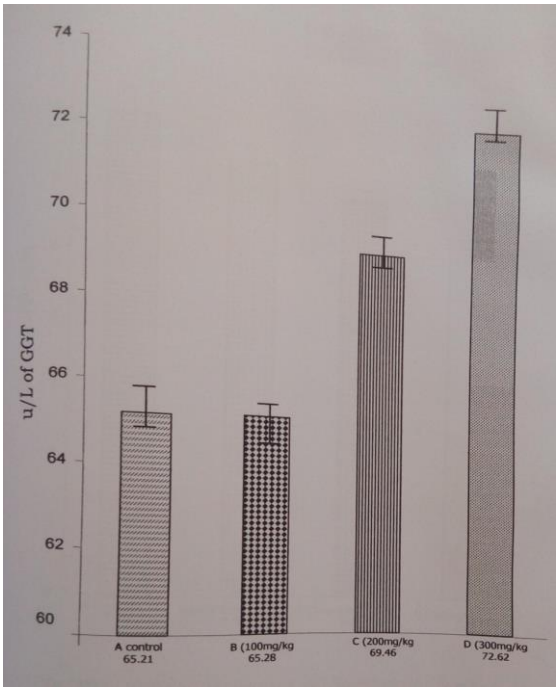


Fig 4: Effect of saccharum berberi extract on GGT of Albino wistar rats.

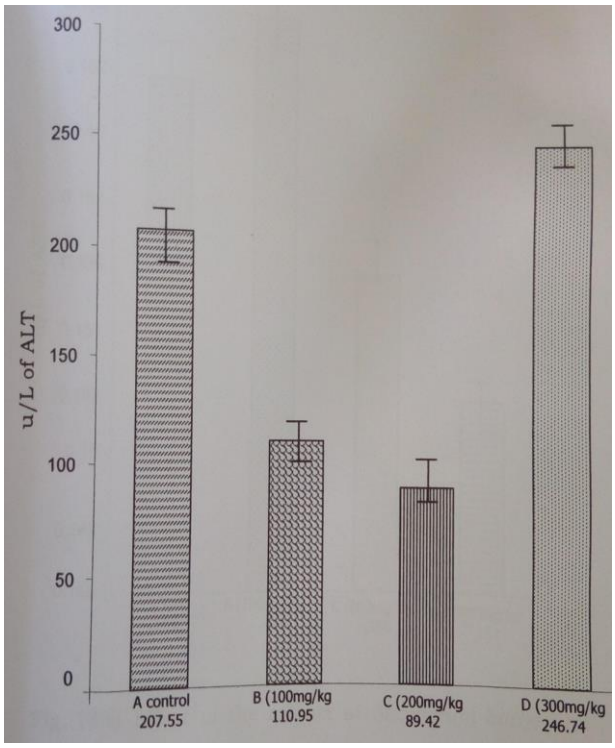


Fig 5: Effect of saccharum berberi extract on ALT of Guinea pigs.

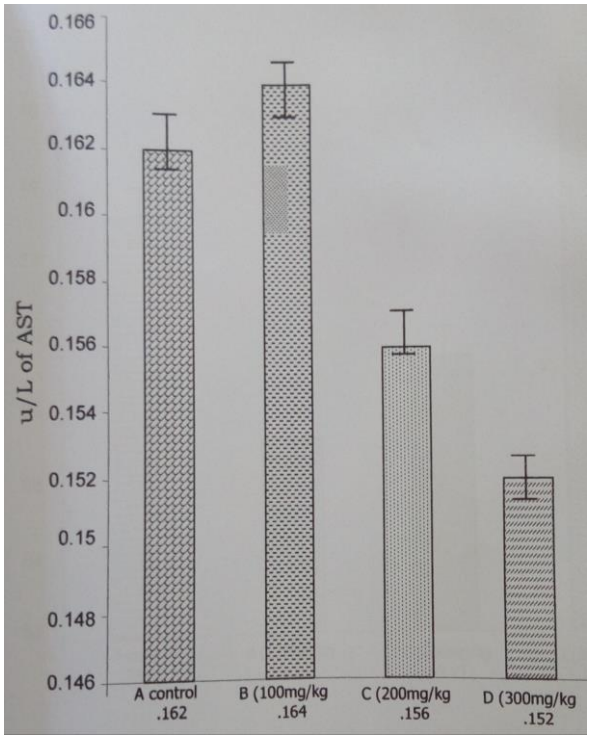


Fig 6: Effect of saccharum berberi extract on AST of Guinea pigs.

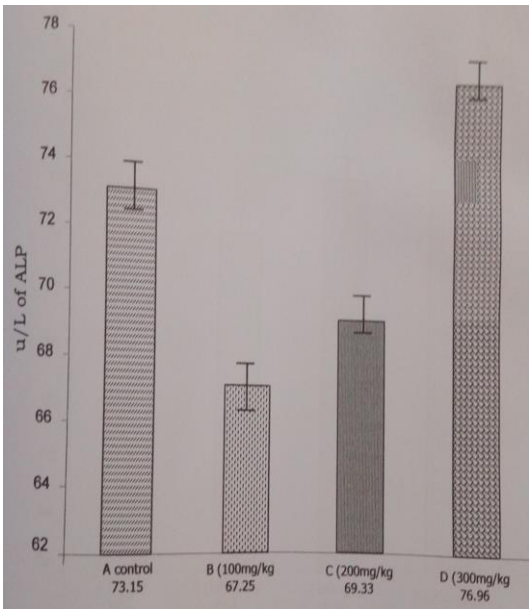


Fig 7: Effect of saccharum berberi extract on ALP of Guinea pigs.

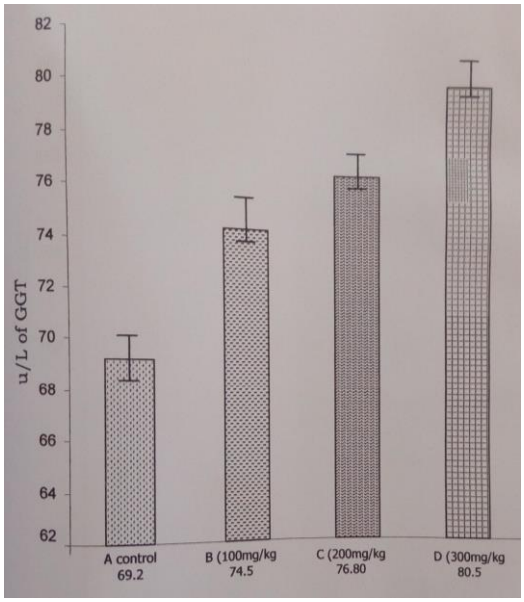
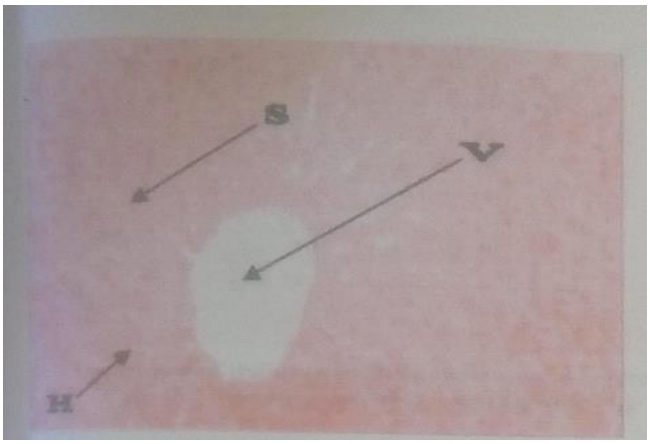


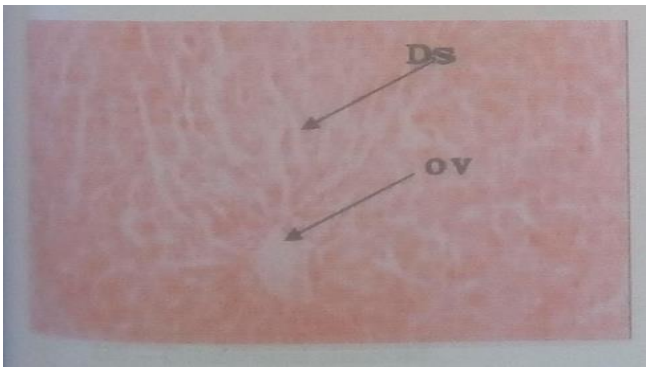
Fig 8: Effect of saccharum berberi extract on GGT of Guinea pigs.

Results of Histological analysis.



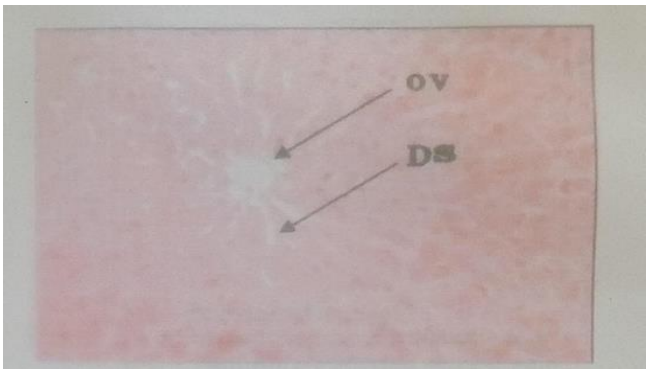
Histology of the normal liver showing the central vein (v), hepathocyte (h), and Sinusoid (s)

Plate 1: Control (Group 1): Picture micrograph of liver section of rate treated with placebo (normal saline)



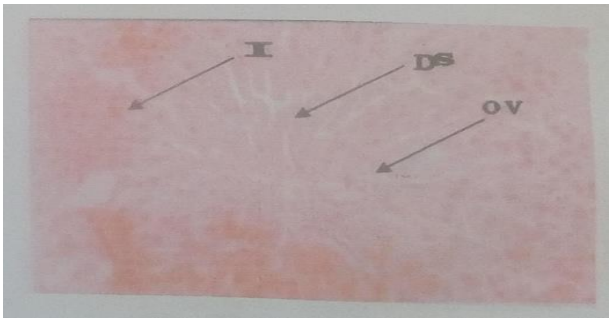
DS – Dilatated sinusoid
OV – Partially obliterated central vein.

Plate 2: (Group 2): Picture micrograph of liver section of rats treated with 100mg/kg body weight of extract (Albino wistar rat)



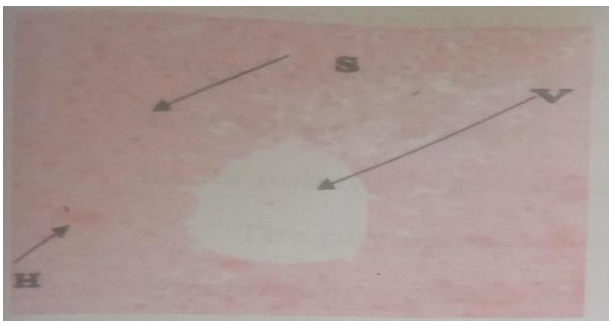
DS – Dilatated sinusoid
OV – Partially obliterated central vein.

Flate 3: (Group 3): picture micrograph of liver section of rats treated with 200mg/kg S. barberi extract body weight.



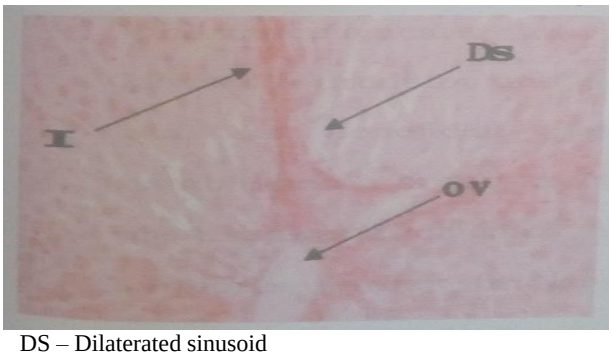
DS – Dilatated sinusoid
OV – Partially obliterated central vein.

Plate 4: (Group 4): Picture micrograph of liver section of rats treated with placebo 300mg/kg bodweight of extract (Alblino wistar rat)



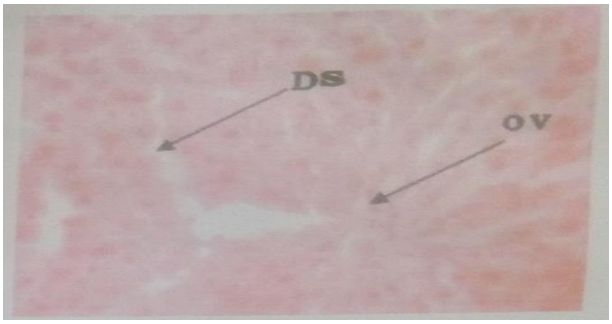
Histology of the normal liver showing the central Vein (V), Hepathocyte (H), and Sinusoid (S)

Plate 5: Control (Group 1): Picture micrograph of liver section of Guinea pig treated with placebo (normal saline mgx2)



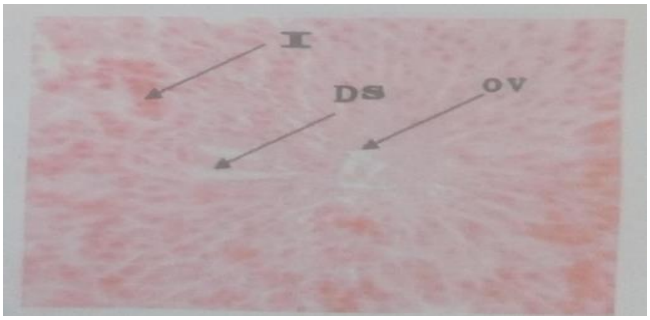
DS – Dilaterated sinusoid
I – Moderate degree of inflammation
OV – Partially obliterated central vein

Plate 6: (Group 2): Picture micrograph of liver section Guinea pig treated with 100mg/kg body weight of extract (Guinea pig)



DS – Dilaterated sinusoid
I – Moderate degree of inflammation
OV – Partially obliterated central vein.

Plate 7: (Group 3): Picture micrograph of liver section of Guinea pig treated with 200mg/kg body of extract (Guinea pig)



I – Moderate degree of inflammation
DS – Dilaterated sinusoid
OV – Partially obliterated central vein

Plate 8: (Group 4): Picture micrograph of liver section of Guinea pig treated with 300mg/kg body of extract (Guinea pig)

Discussion

The serum enzymes activity determined for ALP, AST, ALT and GGT at statistically significant $P<0.05$ as given in Table I and II shows a fluctuating value. Most values for ALP, AST and ALT shows an decreasing values with increasing trend for GGT for both the Albino wistar rats and Guinea pigs. The study actually shows an implication of the extract on the serum enzymes at the dose level given. The Test values obtained for the albino wistar rats and Guinea pigs showed that the enzymes ALP, ALT and GGT to have a significant effect, while the AST have no significant effect. ($P<0.05$).

The relevance of the effect of the extracts on the albino wistar rats and Guinea pigs Show that the extract at higher concentration more than what is given will have a more significant effect on the serum enzymes due to the liver injury (Nelson and Cox, 2008), similar study correlates to this fact as CCl4 challenge in rats result to an elevated level of ALP, ALT and AST. (Szymonik – Lesuk *et al*, 2003, Turkey *et al* 2005) [7, 6].

The result of the liver histopathology show sinusoidal dilution and congestation, this is more prone at higher dose level.

This progressive damage increase with dose level is a good indicator as it can become complicated and cause biliary cirrhosis which may be difficult to manage at this level. (young and Cletra, 1992) [8].

Conclusion

In conclusion it is of paramount important to actually go into the medicinal plants research and the methodology on which they could be used to treat chronic ailments to avoid serious setbacks.

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