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Impact of Aqueous Extract of *Costus afer* Stem on the Functional Integrity of the Liver

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Abstract: The presence of certain enzymes in the blood and at a high concentration indicates damage to the liver, also reduced concentration of bilirubin is associated with cardiovascular risk and other diseases. This study was aimed at observing the ability of *costus afer* stem extract to maintain the integrity of the liver. *Costus afer* stem was extracted and given at concentrations of 0.25 ml/100g, 0.5 ml/100g, 1 ml/100g and 2 ml/100g once daily to induced diabetic rats, comparing their effect with that of metformin treated diabetic rats. At the end of each week three rats were sacrificed from each group and subjected to experimental and statistical analysis. Induction of diabetes using alloxan led to an increase in AST, ALT and ALP concentration in serum, as concentration of *costus afer* stem extract increased AST, ALT and ALP of the treated diabetic rats reduced. Administration of *costus afer* plant resulted in reduction of AST, ALT and ALP concentration in diabetic rats, the reduction was significant for just AST and ALT. There was no effect on serum bilirubin and albumin, serum protein concentration increased as the concentration of plant extract increased with a significant difference from the untreated diabetic rats. *Costus afer* stem extract possess the ability to restore damage caused to the liver by diabetes.
Keywords: Liver, liver enzymes, bilirubin, albulmin, total protein.

INTRODUCTION

Metabolism is principally done by the liver, the liver serves to produce energy from carbohydrate and fatty sources, absorption of these substances and ultimately regulation of these substances to ensure proper functioning of the body system.

Bilirubin is the end product of tetra pyrrole heme, the catabolism of tetra pyrrole heme is catalyzed by the enzyme heme oxygenase. Ferrous iron, carbon monoxide and biliverdin are also generated in course of this catabolism; biliverdin reductase subsequently reduces biliverdin to bilirubin [1]. Bilirubin has antioxidant property, protecting lipids from oxidation [2], anti-inflammatory properties [3]. A study by Novotry and Vitek [4] found that 6.5 % decrease in cardiovascular disease was observed when bilirubin was increased; this report was also confirmed by Huang *et al*., [5] whose result indicated high occurrence of ischemic stroke and myocardial infarction at low serum bilirubin concentration. Increased oxidative stress, elevated glucose and formation of glycated end products are associated with diabetes mellitus [1]. A survey by the US National Health and Nutrition Examination (NHANES) has proven that high serum bilirubin concentration protects against diabetes mellitus. The presence of certain enzymes in the blood and at a high concentration indicates damage to the liver, such enzymes includes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Proteins are synthesized in the liver; elevated serum albumin is likely an indication of insulin resistance as suggested currently [6].

MATERIALS AND METHODS

Chemicals and reagents

The reagent were obtained from the respective company as stated subsequently, chloroform was obtained from BDH chemicals Ltd., metformin from Merck Serono Ltd. U.K, 10 % formalin and alloxan (Qualkems Lab. Reagents), biochemical reagent kits from MINDRAY and finisher feed from Top Feed Ltd.

Experimental animals

Ninety-nine wistar rats were used for the study, three of which served for the pilot study. The animals were administered 120 mg/kg of alloxan solution made from 2.0 g of alloxan dissolved in 40ml of distilled water. The animals were thus found to be diabetic, their glucose concentration after about 2-3 days increased by two fold. *Costus afer* stem extract was prepared following the method of Emeh *et al*., [7]. The animals were grouped as follows

- Non-diabetic rats administered with just distilled water and feed, this group was tagged as negative control 1 (group 1).
- Untreated diabetic rats, this group were tagged as positive control (group 2).
- Non-diabetic rats administered with 1 ml/100 g of *costus afer* stem extract, this group was tagged as negative control 2 (group 3).
- Diabetic rats treated with 7.1 mg/kg metformin (group 4).
- Diabetic rats treated with 0.25 ml/100 g of *costus afer* stem extract (group 5).
- Diabetic rats treated with 0.5 ml/100 g of *costus afer* stem extract (group 6).
- Diabetic rats treated with 1.0 ml/100 g of *costus afer* stem extract (group 7).
- Diabetic rats treated with 2.0 ml/100 g of *costus afer* stem extract (group 8).

Method of blood and organ collection

Blood was collected from the tail of the experimental animals following the method of Graham and Ki [8] while the liver was prepared and examined following the method described by Stefan [9].

Assay of liver enzyme activities and other biochemical parameters

ALT and AST activity were determined using Reitman and Frankel method while ALP activity was determined using the method of Deutsche as described by Chuku *et al*., [10], biuret reagent method was used to estimate protein concentration, serum albumin and total bilirubin were also determined [11, 12].

Statistical analysis

Values were reported as Mean ± standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p < 0.05$).

RESULTS

Induction of diabetes using alloxan led to an increase in AST, ALT and ALP concentration in serum, as concentration of *costus* afer stem extract increased AST, ALT and ALP of the treated diabetic rats reduced. Administration of plant stem extract resulted in a significant reduction in AST concentration when compared against untreated diabetic animals, the AST concentration of all experimental animals which were given the plant extract had a higher AST concentration in blood as against the non-diabetic rats which were given just feed and water at week 1 and week 2. The reduction in ALT concentration of treated animals was also significant when compared against the diabetic untreated animal while reduction in ALP serum concentration was not significant. There was no effect on serum bilirubin and albumin, serum protein concentration increased as the concentration of plant extract increased with a significant difference from the untreated diabetic rats.

Table-1: Mean (±SD) of liver enzymes, renal and other biochemical parameters of the experimental animals

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
AST (IU/L)	81.85±56.85^{bcde}	213.83±1.55^{a}	185.40±19.64^{a}	175.13±22.14^{a}	193.70±22.00^{a}
ALT (IU/L)	247.70±63.50^{bcde}	135.80±7.20^{a}	116.60±5.63^{a}	105.33±14.69^{a}	116.47±14.27^{a}
ALP (IU/L)	93.15±99.95^{bcde}	169.77±38.13^{a}	261.63±77.02^{a}	265.77±51.60^{a}	283.03±77.81^{a}
T.Bilirubin (µmol/L**)**	4.67±0.58	5.67±0.58	6.33±1.15	6.00±2.00	5.67±0.58
T.Protein (g/L)	72.60±0.20^{d}	71.70±1.04^{d}	66.80±1.31	63.17±2.93^{ab}	67.13±5.77
Albumin (µmol/L**)**	31.15±1.35	32.07±1.72	30.17±2.15	28.90±0.26	29.07±0.21

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Superscript “b” shows significant difference, ($p<0.05$) when Group 2 is compared with other groups.
Superscript “c” shows significant difference, ($p<0.05$) when Group 3 is compared with other groups.
Superscript “d” shows significant difference, ($p<0.05$) when Group 4 is compared with other groups.
Superscript “e” shows significant difference, ($p<0.05$) when Group 5 is compared with other groups.

KEY: GRP 1= 2ml Extract; **GRP 2=** 1ml Extract; **GRP 3**= 0.50ml Extract; **GRP 4**= 0.25ml Extract; **GRP 5=** 0.10ml Extract.

Table-2: Effect of *Costus afer* stem extract on AST activity (IU/L) of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	110.33±35.64^{bdefgh}	346.67±19.60^{acef}	185.40±19.64^{b}	274.43±65.84^{a}	222.47±31.57^{ab}	234.70±46.40^{ab}	278.57±15.67^{a}	262.40±19.69^{a}
Week 2	100.67±34.43^{bdefg}	366.67±20.10^{acfgh}	158.00±47.37^{bde}	278.60±53.70^{ac}	279.43±30.25^{ac}	230.40±43.10^{ab}	209.93±3.58^{ab}	187.15±9.45^{b}
Week 3	109.67± 27.54	371.67±28.00	101.67±14.57	135.20±14.02	200.97±29.06	221.77±31.19	193.60±25.75	180.13±26.55
Week 4	98.03±15.09	210.30±15.96	53.33±37.31	71.33±26.50	219.80±45.76	190.47±20.38	152.03±95.04	138.00±34.43

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Superscript “e” shows significant difference, (p<0.0 5) when Group 5 is compared with other groups.
Superscript “f” shows significant difference, (p<0.05) when Group 6 is compared with other groups.
Superscript “g” shows significant difference, (p<0.05) when Group 7 is compared with other groups.
Superscript “h” shows significant difference, (p<0.05) when Group 8 is compared with other groups.

KEY: GRP 1= Normal control; **GRP 2=** Diabetic control; **GRP 3**= Normal (non-diabetic) rats + 1ml Extract; **GRP 4**= Diabetic rats treated with Metformin; **GRP 5=** Diabetic rats treated with 0.25ml Extract; **GRP 6=** Diabetic rats treated with 0.50ml Extract; **GRP 7=** Diabetic rats treated with 1.0ml Extract; **GRP 8=** Diabetic rats treated with 2.0ml Extract.

Table-3: Effect of *Costus afer* stem extract on ALT (IU/L) activity of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	107.20±12.40	137.73±12.43^{deh}	116.60±5.63	94.93±24.57^{b}	80.13±13.95^{b}	101.65±7.55	99.57±2.11	89.30±21.90^{b}
Week 2	99.53±3.56^{b}	144.00±10.58^{acdefgh}	108.50±17.57^{bh}	92.70±10.10^{b}	99.50±14.70^{b}	82.53±8.21^{b}	80.67±8.50^{b}	77.30±5.40^{bc}
Week 3	95.87±8.36	153.00±23.90^{fgh}	89.80±23.21	118.15±6.85	102.93±18.12	79.23±47.27^{b}	85.00±15.39^{b}	75.57±15.30^{b}
Week 4	98.70±19.34	159.57±37.37	96.23±29.65	76.10±2.40	100.63±14.53	67.27±5.30	69.40±8.67	89.27± 2.61

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Superscript “c” shows significant difference, (p<0.05) when Group 3 is compared with other groups.
Superscript “d” shows significant difference, (p<0.05) when Group 4 is compared with other groups.
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Superscript “f” shows significant difference, (p<0.05) when Group 6 is compared with other groups.
Superscript “g” shows significant difference, (p<0.05) when Group 7 is compared with other groups.
Superscript “h” shows significant difference, (p<0.05) when Group 8 is compared with other groups.

KEY: GRP 1= Normal control; **GRP 2=** Diabetic control; **GRP 3**= Normal (non-diabetic) rats + 1ml Extract; **GRP 4**= Diabetic rats treated with Metformin; **GRP 5=** Diabetic rats treated with 0.25ml Extract; **GRP 6=** Diabetic rats treated with 0.50ml Extract; **GRP 7=** Diabetic rats treated with 1.0ml Extract; **GRP 8=** Diabetic rats treated with 2.0ml Extract.

Table-4: Effect of *Costus afer* stem extract on ALP activity (IU/L) of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	103.00±17.09^{bdefh}	313.00±42.87^{a}	261.63±37.02	338.00±15.59^{a}	320.00±46.77^{a}	339.33±7.94	207.00±7.94	288.67±101.04^{a}
Week 2	122.33±24.13^{b}	348.00±37.51^{a}	230.00±28.82	233.00±57.00	274.00±31.24	266.33±47.25	252.67±32.93	241.00±57.00
Week 3	131.33±27.15	264.33±37.77	74.67±17.93	132.67±35.44	254.00±48.97	234.00±49.86	230.67±31.56	191.50±11.50
Week 4	110.33±20.07^{b}	311.67±53.67^{acdh}	85.00±31.24^{b}	85.33±36.51^{b}	225.33±63.85	231.00±50.58	215.00±58.50	153.00±43.96^{b}

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Superscript "f" shows significant difference, ($p<0.05$) when Group 6 is compared with other groups.
Superscript "g" shows significant difference, ($p<0.05$) when Group 7 is compared with other groups.
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KEY: GRP 1= Normal control; **GRP 2=** Diabetic control; **GRP 3**= Normal (non-diabetic) rats + 1ml Extract; **GRP 4**= Diabetic rats treated with Metformin; **GRP 5=** Diabetic rats treated with 0.25ml Extract; **GRP 6=** Diabetic rats treated with 0.50ml Extract; **GRP 7=** Diabetic rats treated with 1.0ml Extract; **GRP 8=** Diabetic rats treated with 2.0ml Extract.

Table-5: Effect of *Costus afer* stem extract on total bilirubin concentration (µmol/L) of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	6.00±1.00	5.67±0.58	6.33± 1.15	6.33±1.53	5.67±1.15	6.00± 0.00	6.00± 0.00	6.33±0.58
Week 2	5.00±1.00	5.67±0.58	5.00±1.73	6.50±1.50	6.67±2.08	6.67±0.58	5.67±0.58	4.00±1.00
Week 3	5.67±0.58	5.00± 1.00	4.33±0.58	3.50±0.50	3.50±0.50	5.33±1.53	5.33± 0.58	5.00±1.00
Week 4	5.33±1.08	6.00±0.00	6.00±1.00	4.00±1.00	6.00± 1.00	6.50±0.50	5.83±1.61	6.33±1.53

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Superscript "g" shows significant difference, ($p<0.05$) when Group 7 is compared with other groups.
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Table-6: Effect of *Costus afer* stem extract on total protein concentration (g/L) of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	63.07±2.69	49.23±8.99^{c}	66.80±1.31^{b}	57.67±12.23	58.07±3.50	55.17±0.06	64.37±0.64^{b}	60.57±2.35
Week 2	74.00±13.11^{b}	38.73±9.36^{acdegh}	63.03±7.82^{b}	76.90±2.20^{b}	76.80±4.90^{b}	59.73±10.18	69.20±3.40^{b}	75.47±0.21^{b}
Week 3	79.33±5.77^{bf}	35.07±9.00^{acdefgh}	59.90±7.04^{b}	73.00±10.54^{b}	66.33±5.86^{b}	56.53±8.50^{ab}	70.27±4.02^{b}	68.33±5.13^{b}
Week 4	70.33±0.15^{b}	45.50±2.40	69.03±2.57	76.10±10.27^{b}	68.07±3.41^{b}	69.50±2.43^{b}	72.80±5.35^{b}	67.00±1.70^{b}

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Table-7: Effect of *Costus afer* stem extract on albumin concentration (µmol/L) of alloxan-induced diabetic *albino wistar* rats

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6	GRP 7	GRP 8
Week 1	30.13±2.45	26.83±1.61	30.17±2.15	22.00±7.33	23.83±5.40	22.13±3.05	26.27±1.42	23.90±1.47
Week 2	33.70±8.12	25.00±3.00	28.97±0.45	30.20±0.00	24.47±4.08	25.73±2.03	27.20±0.17	31.47±0.70
Week 3	35.70±6.62	24.33±2.08	27.80±2.43	30.95±2.95	29.15±2.85	32.97±9.81	26.70±0.79	28.15±0.35
Week 4	33.23±3.35	29.15±0.15	28.27±1.27	29.43±2.15	30.20±2.38	29.90±2.42	30.70±3.03	27.60±0.60

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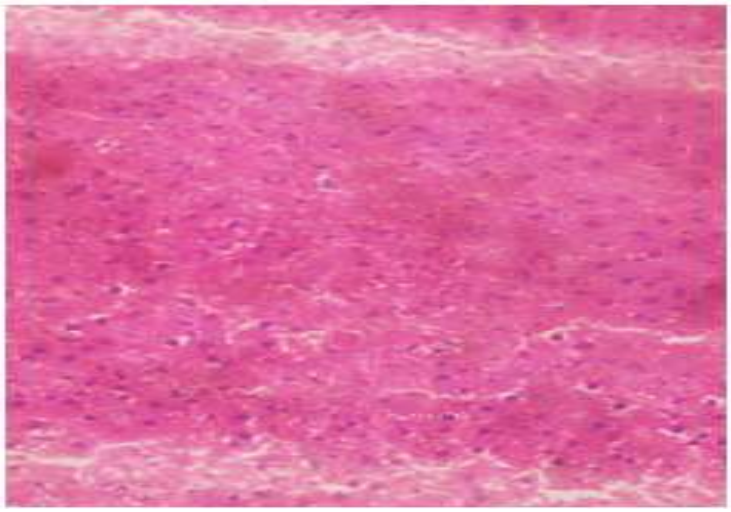


Plate-1: Liver photomicrograph of the normal control animal showing normal hepatocytes; magnification x400

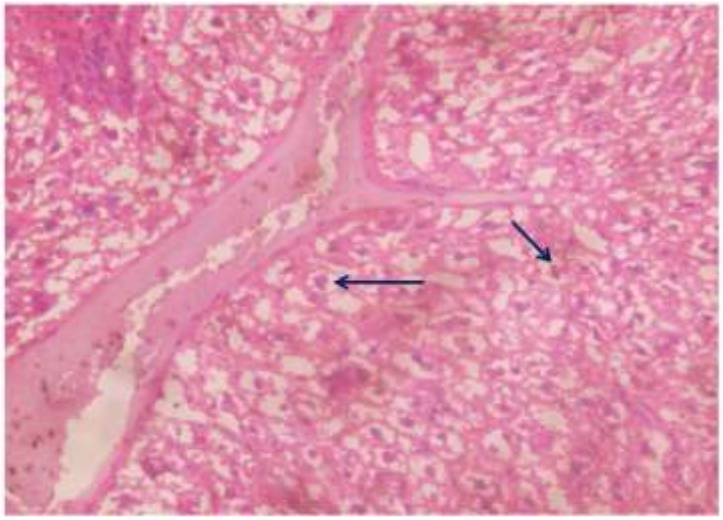


Plate-2: Liver photomicrograph of the diabetic control animal showing severe hepatocellular distortion; magnification x400

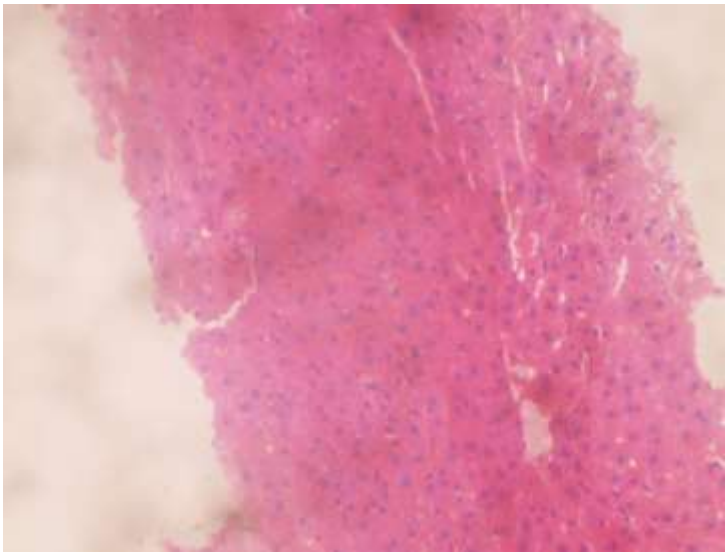


Plate-3: Liver photomicrograph of normal animals that received 1ml *Costus afer* extract; magnification x400

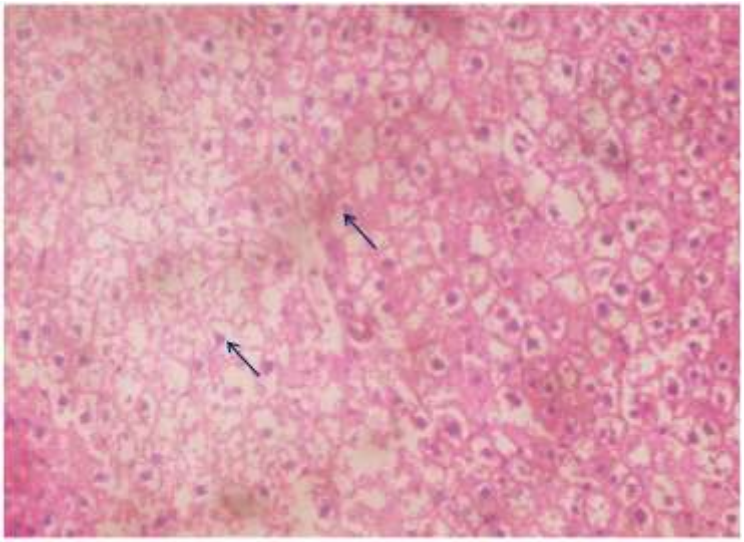


Plate-4: Liver photomicrograph of animals treated with metformin showing severe hepatocellular distortion after week 1, magnification x400

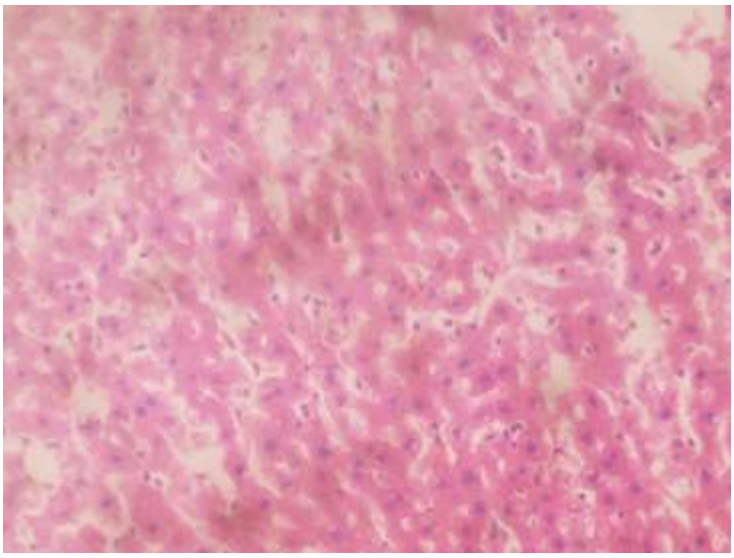


Plate-5: Liver photomicrograph of animals treated with metformin showing increased inflammatory cells within the sinusoids after week 2, magnification x400

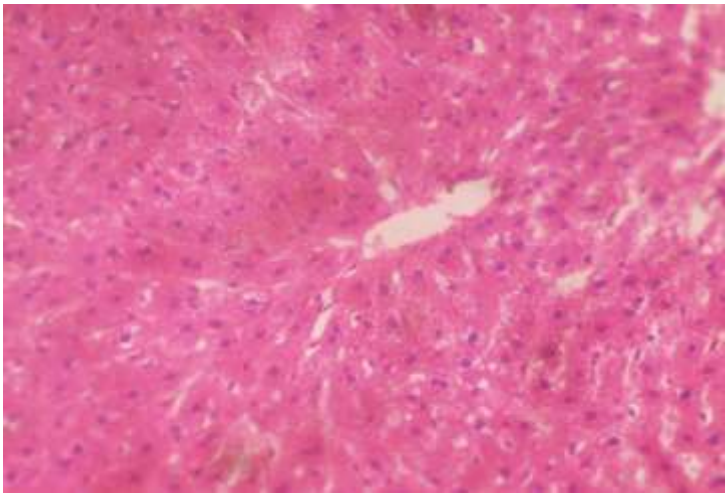
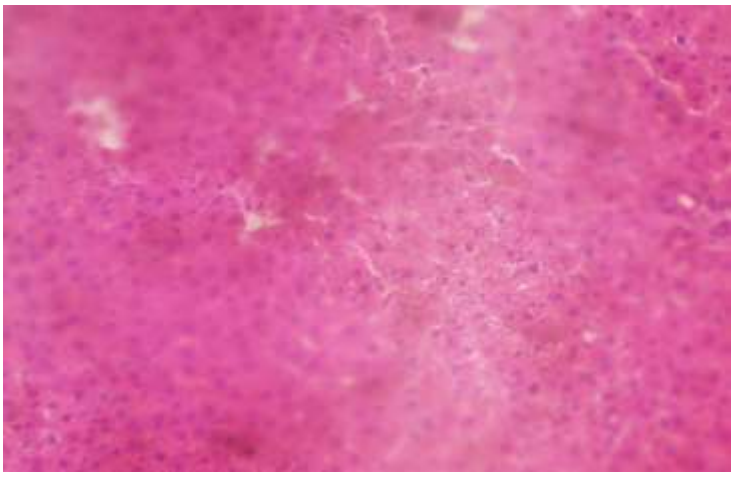


Plate-6: Liver photomicrograph of animals treated with metformin showing normal histology with hypertrophy of kuffer cells after week 3, magnification x400



Plates-7: Liver photomicrograph of the animals treated with metformin showing normal histology after week 4; magnification x400

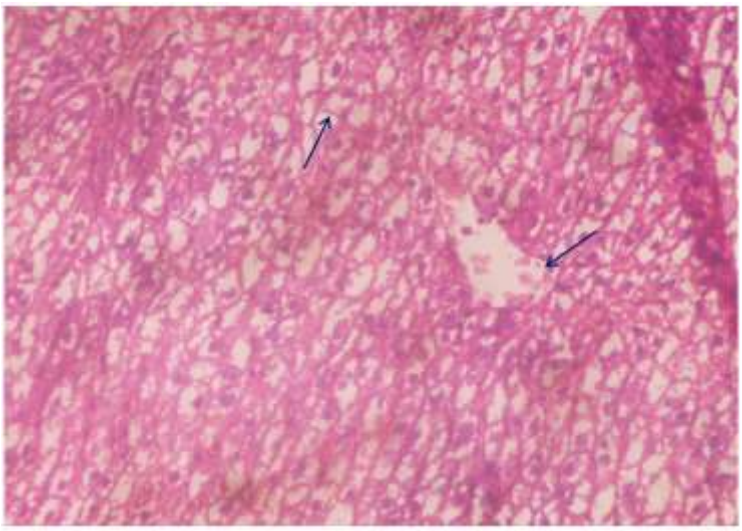


Plate-8: Liver photomicrograph of animals treated with 0.25ml of *Costus afer* extract showing severe hepatocellular distortion after week 1; magnification x400

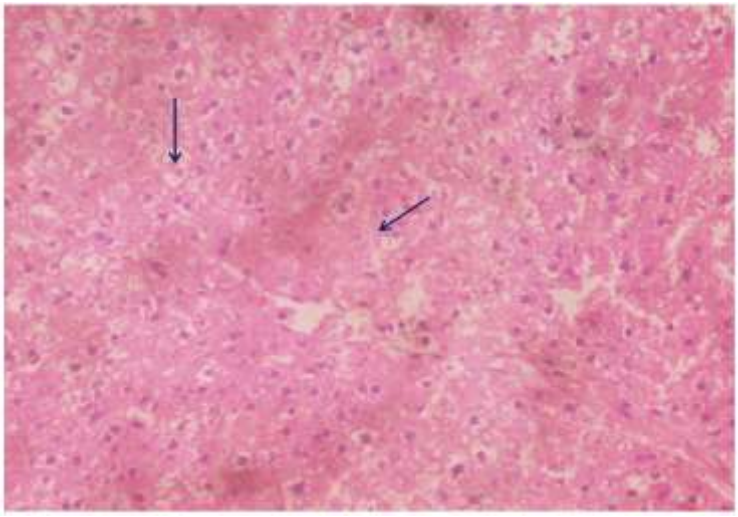


Plate-9: Liver photomicrograph of animals treated with 0.25ml of *Costus afer* extract showing reduced hepatocellular distortion after week 2; magnification x400

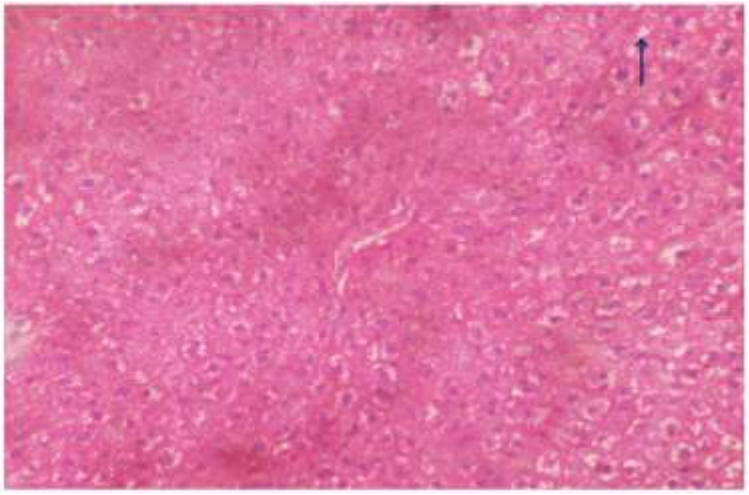


Plate-10: Liver photomicrograph of animals treated with 0.25ml of *Costus afer* extract showing more reduced hepatocellular distortion after week 3; magnification x400

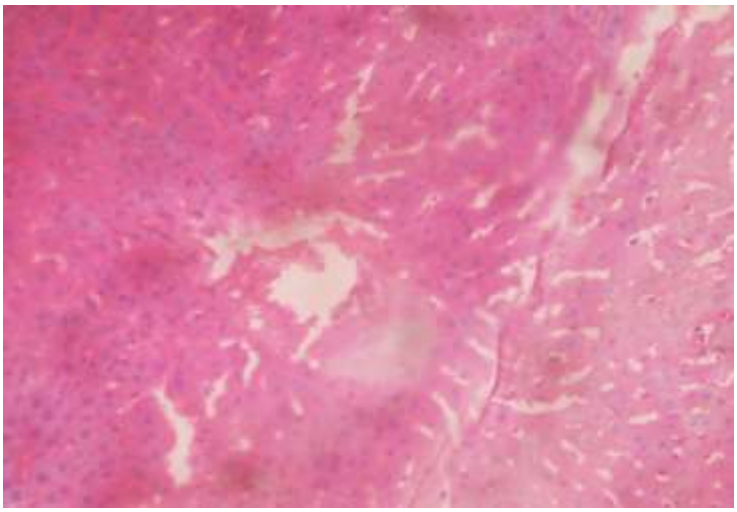


Plate-11: Liver photomicrograph of animals treated with 0.25 ml of *Costus afer* extract showing normal histology after week 4; magnification x400

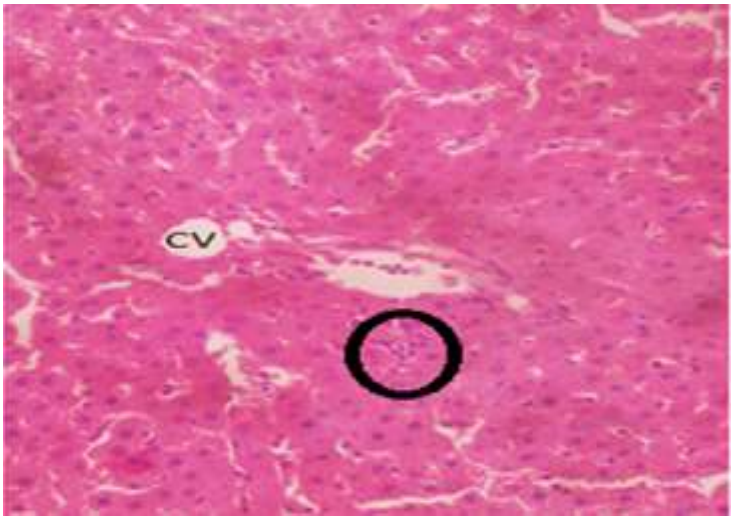


Plate-12: Liver photomicrograph of animals treated with 0.50 ml of *Costus afer* extract showing increased inflammatory cells in the sinusoids with normal hepatocytes after week 1; magnification x400

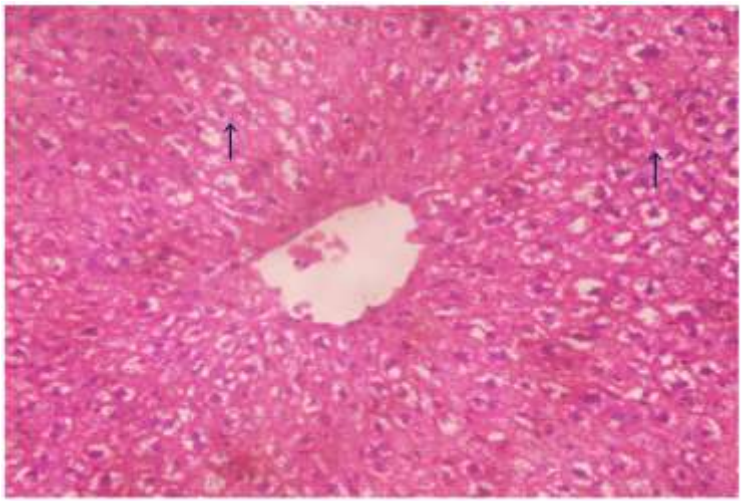


Plate-13: Liver photomicrograph of animals treated with 0.50 ml of *Costus afer* extract showing hepatocellular distortion after week 2; magnification x400

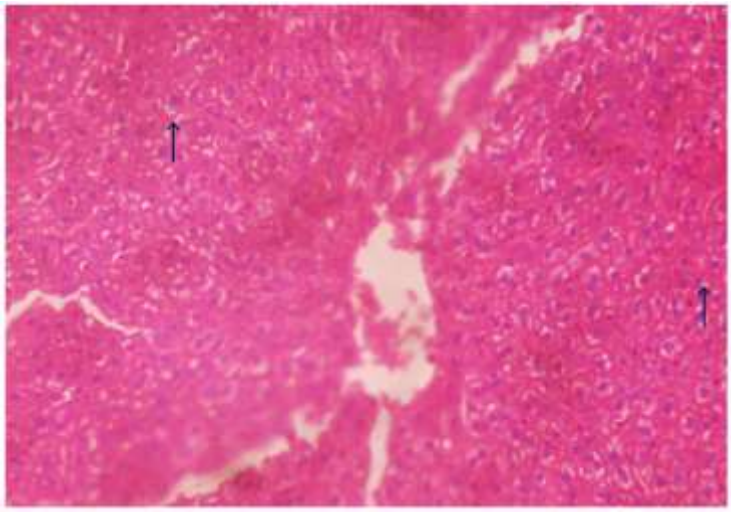


Plate-14: Liver photomicrograph of animals treated with 0.50 ml of *Costus afer* extract showing mild hepatocellular distortion after week 3; magnification x400

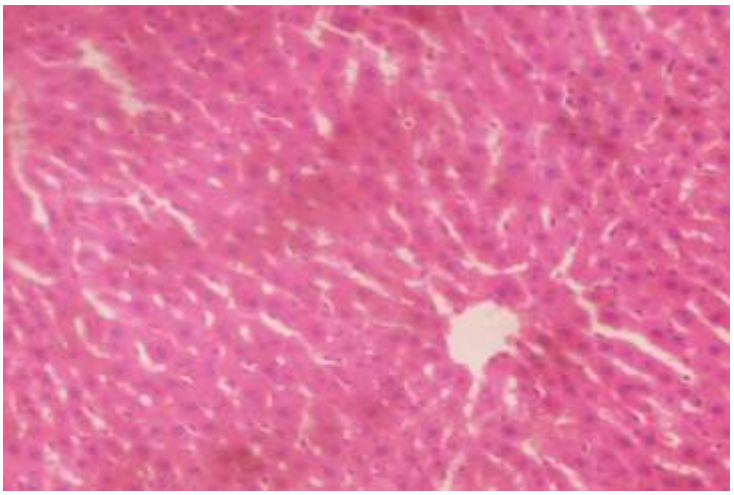
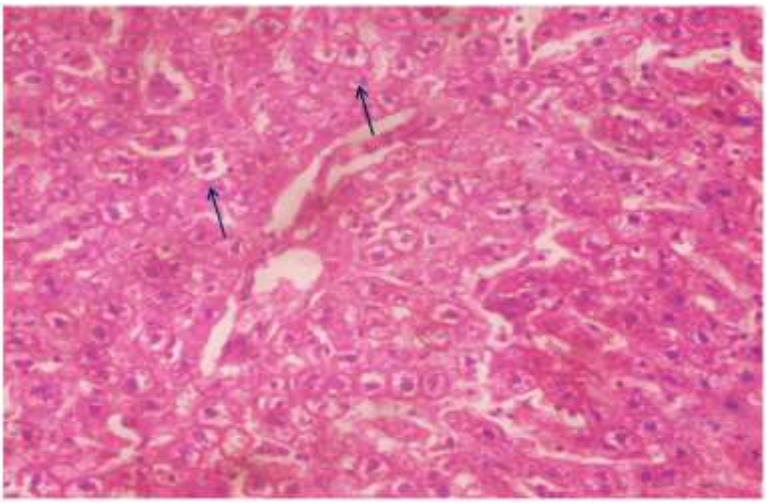
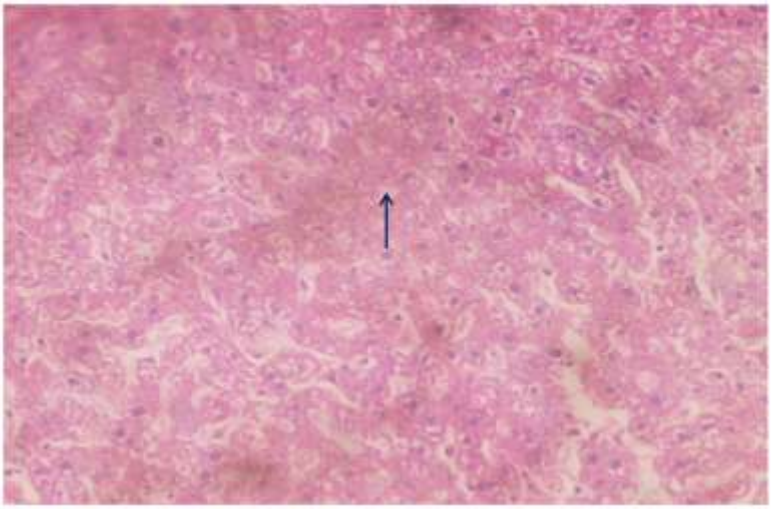


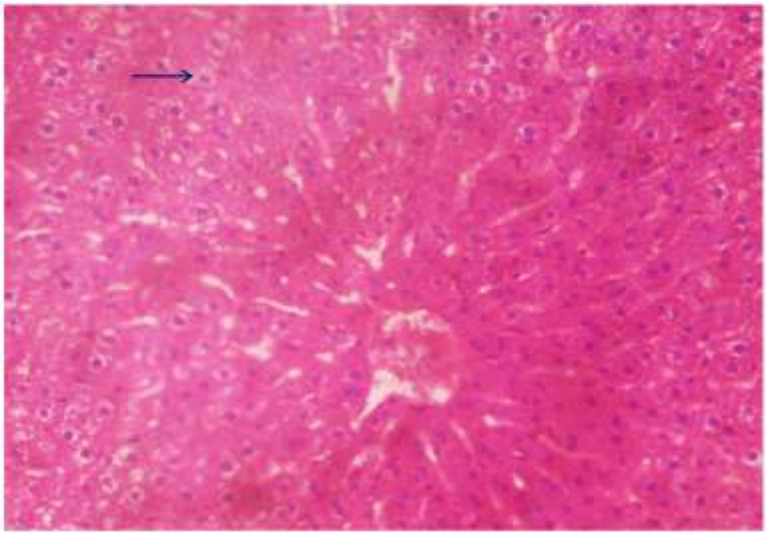
Plate-15: Liver photomicrograph of animals treated with 0.50 ml of *Costus afer* extract showing normal histology after week 4; magnification x400



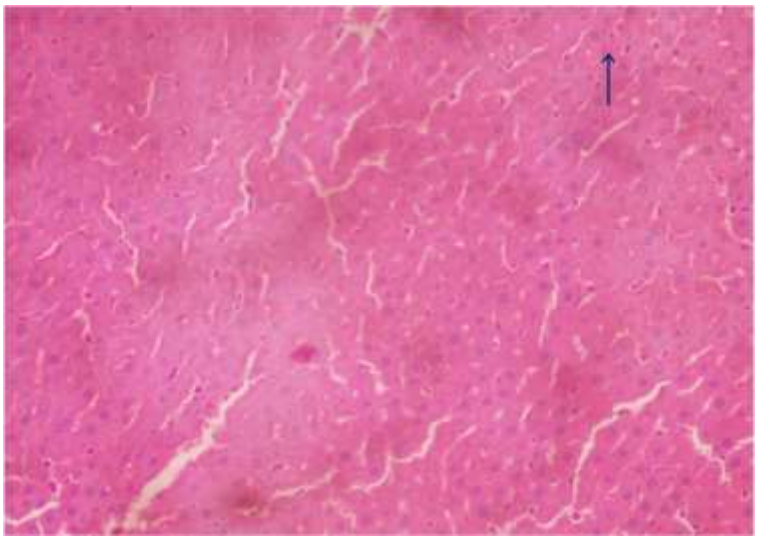
Plates-16: Liver photomicrograph of animals treated with 1.0 ml of *costus afer* extract showing severe hepatocellular distortion with increased inflammatory cells after week 1; magnification x400



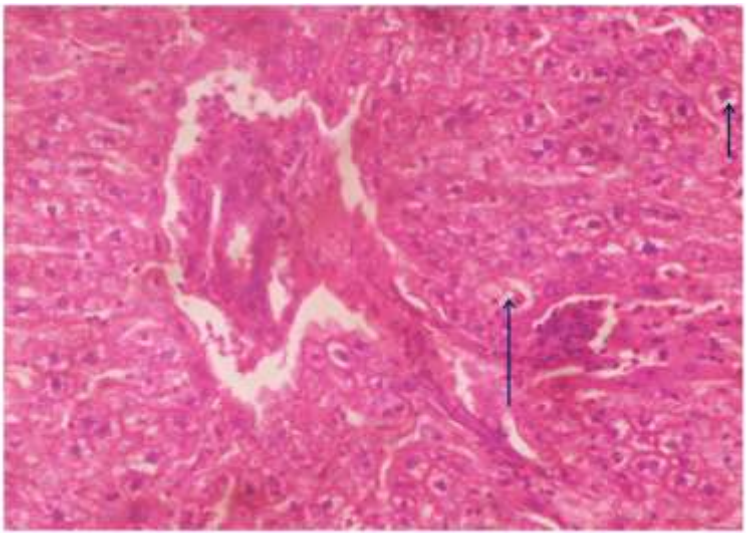
Plates-17: Liver photomicrograph of animals treated with 1.0 ml of *costus afer* extract showing reduced hepatocellular distortion after week 2; magnification x400



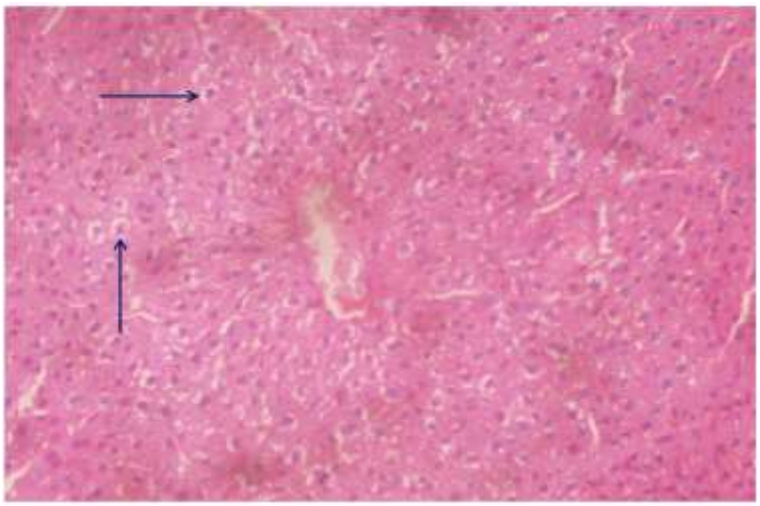
Plates-18: Liver photomicrograph of animals treated with 1.0 ml of *costus afer* extract showing mild hepatocellular distortion after week 3; magnification x400



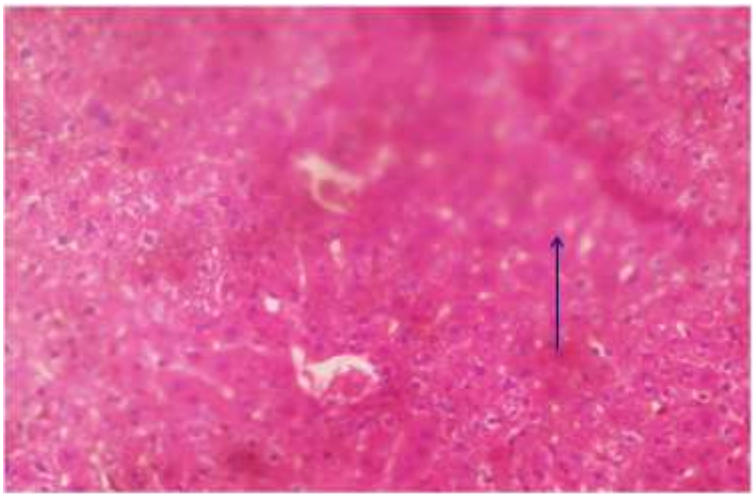
Plates-19: Liver photomicrograph of animals treated with 1.0 ml of *costus afer* extract showing normal histology after week 4; magnification x400



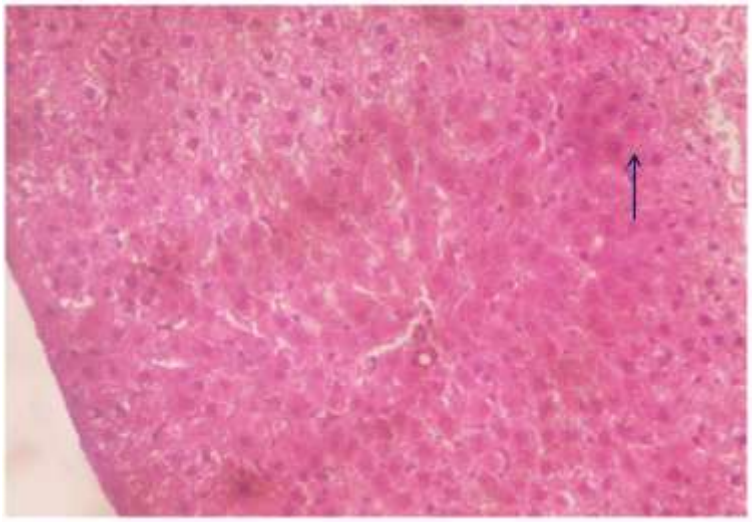
Plates-20: Liver photomicrograph of animals treated with 2.0 ml of *costus afer* extract showing increased inflammatory cells in sinusoids after week 1; magnification x400



Plates-21: Liver photomicrograph of animals treated with 2.0 ml of *costus afer* extract showing hepatocellular distortion after week 2; magnification x400



Plates-22: Liver photomicrograph of animals treated with 2.0 ml of *costus afer* extract showing mild hepatocellular distortion after week 3; magnification x400



Plates-23: Liver photomicrograph of animals treated with 2.0 ml of *costus afer* extract showing increased inflammatory cells in sinusoids after week 4; magnification x400

DISCUSSION

The rise in serum level of liver enzymes, total bilirubin and total protein have been attributed to the damaged structural integrity of the liver [13, 14] which are considered as a sensitive marker of liver injury [15]. These manifestations are a consequence of a metabolic alteration with an increase of glyconeogenesis, ketogenesis that occur in diabetic animals. Increase in serum level of AST as observed in this study may reflect damage of liver cells. Serum ALT level is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis [16], also a mild or higher activity of AST indicates liver injury or myocardial infarction [17, 18]. Increase in serum ALP as shown by the untreated animals may be considered as a sensitive indicator of cholestasis in early stages or mild circumstances preceding other indicators such as hyperbilirubinemia [16]. ALT or AST is a liver specific enzyme and its elevated concentrations are usually linked to lot of health problems. Such suspected health issues include viral hepatitis, diabetes, congestive heart failure, liver damage, problems associated with the bile ducts like biliary tract obstruction [19]. Costus afer stem extract from observed histological examination in this study had the potential to restore the integrity of the liver, at the end of fourth week the initial observed damages to the liver such as inflammation and severe hepatocellular distortion caused by the induced diabetes was observed no more. This positive effect observed on the liver alongside earlier reported positive effect on glucose concentration proves the claim of this plant to be used in the management and treatment of diabetes [20].

Insulin resistant is linked with elevated serum albumin concentration; the effect of serum albumin on development of diabetes is not independent [20]. Based on this study there was no effect on serum bilirubin and serum albumin, while the elevated protein concentration might be due to the plant nutrient composition and its antioxidant potential to prevent oxidation of lipid membrane.

CONCLUSION

Aqueous extract of *costus afer* stem has the ability to restore reversible damage of the liver and also reduce liver enzymes in the blood both of which were caused by diabetes. The plant extract has no deleterious effect on serum bilirubin and serum albumin.

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