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Effect of *Averrhoa bilimbi* fruit powder on Histopathology and the Functional Indices of the Liver and Kidney of Rats fed with high fat diet

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Abstract
The effects of *Averrhoa bilimbi* fruit powder at 125, 250 and 500 mg/kg body weight in hyperlipidemic rats on the histopathology and functional indices of liver and kidney were evaluated. The serum ALT and creatinine were found to be elevated after hyperlipidemic induction in all groups except normal control. However, the ALT and BUN levels were found to be increased with *A. bilimbi* given at the dose of 500 mg/kg of body weight as well as with rosuastatin group at the end of the experiment. The fruit powder did not significantly ($P < .05$) alter the creatinine level in the serum of rats. Histological examination revealed mild tubular damage in kidney and marked fatty infiltration along with mononuclear infiltration in the liver and kidney of hyperlipidemic rats. *A. bilimbi* fruit powder administration exhibited marked reduction in the accumulation of lipid droplets as well as monocytic infiltration in liver and kidney of rats.

Keywords: *Averrhoa bilimbi*, mononuclear infiltration, creatinine

Introduction
Hyperlipidemia is an important etiological factor in inducing cardiovascular diseases (CVD) including atherosclerosis that accounts for the chief cause of death in developing countries like India. Lowering the elevated plasma lipids has been proved to decrease the danger of CVD considerably (Grundy, 1997) [1]. The present hypolipidemic medications like statins, fibric acids and bile acid binding resins are associated with various adverse effects. Therefore, attempts have been made to identify the lipid lowering effects of various medicinal plants.
Alternative medicines especially medicinal plants are available for the treatment of hyperlipidemia. Common advantages of herbal medicines are efficacy, safety, cost effective and abundance. *Averrhoa bilimbi* bilimbi belonging to Oxalidaceae family have been known for its medicinal properties for the effective management of several human ailments. The fruits of *A.bilimbi* was used in folk medicine to control obesity in certain parts of India. So, the research on plants with hypolipidemic properties has great value in modern therapeutics. However, studies must be undertaken to assess the adverse effects of the consumption of the plant as a hypolipidemic agent. The present study is aimed at evaluating the effect of *Averrhoa bilimbi* fruit powder on histopathology as well as functional indices of liver and kidney in high fat diet fed rats.

2. Material and Methods
2.1 Experimental animals
A total of forty eight male Wistar rats weighing 150-200 g were used for the study.
Animals were maintained under standard environmental conditions. The study were conducted in accordance with the Animal ethics committee of the university.

2.2 Plant materials / chemicals
The fresh fruits of *A. bilimbi* were collected locally from Thrissur district of Kerala and were authenticated by taxonomist of St. Thomas College, Thrissur. Cholesterol and cholic acid were procured from Sisco Research Laboratories private Limited, Mumbai. The reference drug used for the study was rosuvastatin, Diagnostic kits were procured from Agappe Diagnostics, Ltd.

2.3 Experimental design

Group I (G_I)	Normal control fed with standard diet.
Group II (G_{II})	Hyperlipidemic control fed with high fat diet.
Group 111(G_{I11})	Hyperlipidemic rats treated with *A. bilimbi* fruit powder at a dose rate of 125 mg/kg body weight orally from 16th day along with high fat diet.
Group 1V (G_{IV})	Hyperlipidemic rats treated with *A. bilimbi* fruit powder at a dose rate of 250 mg/kg body weight orally from 16th day along with high fat diet.
Group V (G_V)	Hyperlipidemic rats treated with *A. bilimbi* fruit powder at a dose rate of 500 mg/kg body weight orally from 16th day along with high fat diet.
Group VI (G_{VI})	Hyperlipidemic rats treated with reference drug rosuvastatin at a dose rate of 10 mg/kg body weight orally from 16th day along with high fat diet.

Blood will be collected from all the animals on days 0, 15, 30 and 45. Serum will be separated for doing serum biochemistry. The animals were sacrificed on day 45 and the liver and kidney tissues were dissected out for the assessment of antioxidant status.

2.4 Estimation of liver and kidney function indices
ALT was estimated by UV Kinetic test (Alan, 1988) [2]. BUN was estimated based on UV Kinetic test (Alan, 1988) [2]. Serum creatinine was estimated based on Jaffe Kinetic method.

2.5 Gross and histopathological examination
On day 45, all the rats were sacrificed and detailed necropsy was performed. Gross pathological findings were recorded. After sacrificing, the rat's liver and kidney tissues were dissected out and washed thoroughly in normal saline and fixed in 10 per cent neutral buffered formalin. The fixed tissue was embedded in paraffin. A thin film of 4 µm thickness was sectioned and stained with hematoxylin and eosin (Bancroft and Cook, 1994) [3]. The processed film was examined under the light microscope and photomicrographs were taken.

2.6 Statistical analysis
Data were subjected to statistical analysis using One way analysis of variance followed by Duncan Multiple range test (DMRT) and the results were expressed as Mean ± Standard error (SE) of eight rats in each group. Statistical analysis was done by using the statistical software SPSS Version 21.0.

3. Results and discussion
3.1 Effect of A. Bilimbi on ALT, BUN and creatinine
ALT is present mainly in the cytosol of the liver and in low concentrations elsewhere, is one of the most reliable and sensitive markers of hepatocellular injury or necrosis (Kim *et al*., 2008) [4]. In the present study, mean serum ALT level was found to be higher in G_{II}. These findings are in line with the results of Matos *et al*. (2005) [5] and Lee *et al*. (2008) [6] who obtained augmented serum ALT activity in hyperlipidemic rats. This increase may be due to the hepatocellular injury associated with hyperlipidemia (Xia *et al*., 2013) [7]. In this study, among the *A.bilimbi* fruit powder treated groups, G_V showed significantly higher value for ALT. Rosuvastatin group also showed significantly (P <0.001) higher ALT values than normal G_I on day 30 and 45. The ALT values of G_{III} and G_{IV} were within the normal range on day 30 and 45. These results are similar to the findings of Nagmoti *et al*. (2010) [8] who reported that administration of methanolic extract of *A. bilimbi* attenuated the increased levels of serum ALT in carbon tetrachloride (CCl_4) intoxicated rats.

After the induction of hyperlipidemia, mean BUN values of G_{II} to G_{VI} were within the normal range on day 15. There was no significant changes in the levels of BUN in animals treated with 125mg/kg body weight of *A.bilimbi* fruit powder. Hence the *A.bilimbi* fruit can be considered non nephrotoxic at that dose. Animals that were treated with *A. bilimbi* at the dose of 250 and 500 mg/kg body weight showed significantly (P <0.001) higher serum BUN levels as compared to G_I on day 30 and 45. (Table 1)

Serum creatinine levels were significantly (P <0.001) higher after the induction of hyperlipidemia. This correlates with the results of Eddy *et al*. (1996) [9] who observed an increase in serum creatinine of rats fed high fat diet indicative of renal damage associated with hyperlipidemia. The mean serum creatinine levels of G_{II} to G_{VI} returned back to normal on day 30 and 45 of the experiment.

Table 1: Effect of *A. bilimbi* fruit on ALT, BUN and serum creatinine levels in rats fed on a high fat diet. (Mean ± SE, n=8)

Parameter	Groups	0 day	15th day	30th day	45th day
ALT	G_I	53.31 ± 0.61^{A}	53.92 ± 0.55^{dB}	54.62 ± 0.32^{cB}	55.81 ± 0.40^{dC}
	G_{II}	53.31 ± 0.61^{A}	76.70 ± 1.27^{aB}	85.83 ± 0.84^{aC}	101.03 ± 0.76^{aD}
	G_{I11}	54.21 ± 0.24^{A}	73.42 ± 1.23^{bB}	55.41 ± 0.29^{cC}	56.31 ± 0.34^{cdD}
	G_{IV}	54.05 ± 0.43^{A}	69.32 ± 1.05^{cC}	56.11 ± 0.71^{cB}	56.50 ± 0.29^{cdB}
	G_V	54.17 ± 0.46^{A}	70.71 ± 1.23^{bcC}	58.3 ± 0.84^{bB}	57.34 ± 0.53^{cB}
	G_{VI}	54.54 ± 0.46^{A}	72.65 ± 0.29^{bBC}	59.10 ± 1.01^{bB}	59.94 ± 0.22^{bB}
BUN	G_I	23.50 ± 0.83^{A}	24.04 ± 0.78^{abcA}	24.74 ± 0.61^{cAB}	25.16 ± 0.72^{cC}
	G_{II}	24.05 ± 0.47^{A}	24.90 ± 0.53^{abA}	25.27 ± 0.34^{cA}	26.34 ± 0.30^{cAB}
	G_{I11}	23.47 ± 0.52^{A}	22.87 ± 0.76^{cA}	24.01 ± 0.42^{cA}	25.11 ± 0.24^{cAB}
	G_{IV}	24.21 ± 0.55^{A}	25.49 ± 0.38^{aB}	26.59 ± 0.58^{bC}	27.09 ± 0.55^{bD}
	G_V	23.75 ± 0.42^{A}	25.45 ± 0.39^{aB}	27.34 ± 0.39^{aC}	28.45 ± 0.62^{aD}
	G_{VI}	22.74 ± 0.35^{A}	$23.19 \pm 0.64b^{cA}$	24.21 ± 0.39^{cAB}	26.95 ± 0.52^{cC}
Creatinine	G_I	0.53 ± 0.01	0.53 ± 0.01^{c}	0.55 ± 0.01^{cd}	0.55 ± 0.01^{b}
	G_{II}	0.53 ± 0.01^{A}	0.71 ± 0.01^{aB}	0.81 ± 0.01^{aC}	1.09 ± 0.05^{aD}
	G_{I11}	0.54 ± 0.01^{AC}	0.71 ± 0.01^{aB}	0.54 ± 0.01^{dC}	0.53 ± 0.01^{bC}
	G_{IV}	0.54 ± 0.01^{AC}	0.70 ± 0.01^{aB}	0.57 ± 0.01^{bC}	0.55 ± 0.01^{bC}
	G_V	0.54 ± 0.01^{A}	0.70 ± 0.01^{aB}	0.56 ± 0.01^{bcA}	0.54 ± 0.01^{bA}
	G_{VI}	0.54 ± 0.01^{A}	0.67 ± 0.01^{bAB}	0.54 ± 0.01^{cdA}	0.52 ± 0.01^{bA}

Means bearing different small letter superscripts in columns differ significantly (P <0.001).
Means bearing different capital letter superscripts in rows differ significantly (P <0.001).

3.2 Gross pathology of liver and kidney

On gross pathological examination, normal control (G_I) rats showed liver that were bright red, smooth and with sharp edges (Fig1-A).The liver of hyperlipidemic rats were diffusely pale in appearance (Fig 1-B). This finding is in accordance with Wu *et al*. (2012) [10] who stated that the gross appearances of the livers from rats receiving cholesterol rich diet were enlarged with a yellowish colour. Son *et al*. (2003) [11] also reported hypertrophy of liver and had yellowish colour in cholesterol fed rats. Treatment groups showed reduction in the severity of high fat diet induced fatty liver as indicated by macroscopic appearance. (Fig 1-C, D).There were no gross changes in the kidney of animals of all the six groups.

3.3 Histopathology of Liver and Kidney

Section from normal control (G_I) animals showed normal lobular architecture showing cords of hepatocytes extended radially from central vein and hepatic artery branches around the periphery of lobule (Fig 2-A). The liver of hyperlipidemic group (G_{II}) showed extensive accumulation of lipid droplets in liver and mononuclear infiltration along with congestion in portal vein. This is in line with the reports of Al-Awad *et al.* (2013) [12] who observed infiltration of lipids, lymphocyte infiltration and congestion in high fat fed rats. Ji *et al*. (2011) [13] also observed lipid droplets in the cytoplasm of hepatocytes in the high fat fed rats.

In the present study, administration of 125 and 250 mg/kg of *A. bilimbi* fruit powder (G_{III} and G_{IV}) exhibited reduction in the accumulation of lipid droplets and monocytic infiltration. Rats supplemented with 500 mg/kg of *A. bilimbi* fruit powder (G_V) exhibited apparently normal architecture of liver with absence of lipid droplets (Fig 2-C). This can be due to the activity of *A. bilimbi* on lowering serum cholesterol and reducing lipid deposition in the liver. In rats treated with rosuvastatin, marked reduction in lipid droplets were observed. Treatment with rosuvastatin showed marked restoration of alterations towards the normal histology of liver. The treatment groups G_{III} and G_{IV} showed reduction in fat droplets in liver.

Kidneys of normal control group showed normal histology with presence of normal renal tubules and glomerulus. (Fig 2-D). In contrast, hyperlipidemic diet (G_{II}) produced fatty changes, mononuclear cell infiltration and mild tubular damage in kidney (Fig 2-E). This is in line with the report of Swapnil *et al*. (2013) [14] who observed fatty changes in kidney in hyperlipidemic rats. Co-administration of 125, 250 mg/kg body weight of *A. bilimbi* fruit powder showed moderate accumulation of lipid droplets. Group treated with 500 mg/kg body weight showed marked attenuation of pathological changes in kidney caused due to hyperlipidemic diet (Fig 2-F).These results could be attributed to effects of *A. bilimbi* fruit powder which improved the lipid profile as well as reduced the lipid peroxidation(Ambili *et al*, 2009) [15].

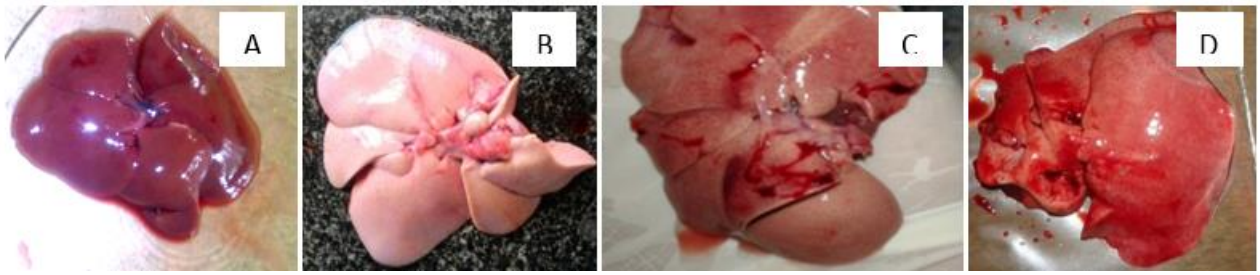

Fig 1: Gross pathology of liver (A) Normal control (B) Hyperlipidemic control - Diffusely pale liver (C) Treatment group (G_V) - Reduction in severity of hepatic lipidosis (D) Group VI - Marked reduction in hepatic lipidosis

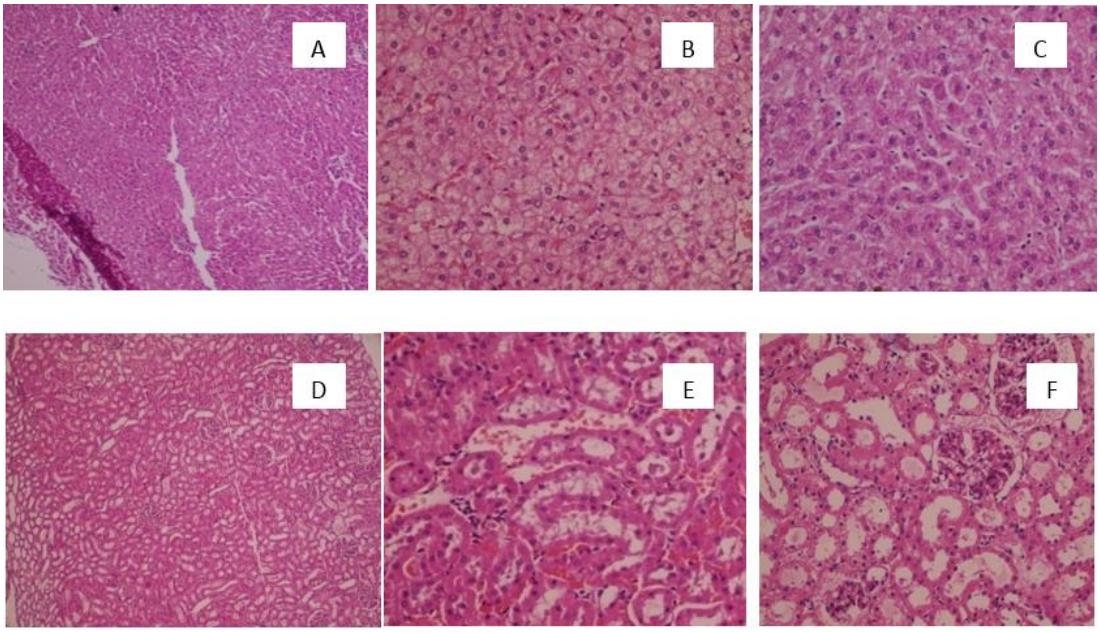

Fig 2: Histological appearance of liver (A) Normal control (H&E X100) (B) Hyperlipidemic control (H&E X400) - Hepatocytes showing degenerative changes (C) Group V (H&E X400) - Hepatocytes showing marked reduction in damages. Photomicrographs of H&E stained kidney sections (D) Normal control (H&E X100) (E) Hyperlipidemic control (H&E X400) - Degenerative changes (F) Group V (H&E X400) - Renal tubules showing marked reduction in damages.

4. Conclusions

Therefore, the present study demonstrated that *A. bilimbi* fruit powder produced significant amelioration on histopathological analysis in high fat diet induced hyperlipidemic rats. Administration of *A. bilimbi* fruit powder resulted in significant lowering of serum biochemical parameters namely ALT, BUN and creatinine. Histopathological examination showed marked reduction in lipid droplets both in liver and kidney. The histopathological study suggests that treatment of *A. bilimbi* fruit powder will reduce hepatocellular damage, reflecting its protective role in liver damage caused by high fat diet.

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