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# Antidiabetic activity of leaves of *Anthocephalus indicus* A. Rich. in alloxan induced diabetic rats

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## Abstract

The present study aims to examine the antidiabetic potential of leaves of *Anthocephalus indicus* A. Rich. The aqueous extract of leaves was screened for serum glucose lowering activity. Diabetes was induced in Sprague Dawley adult male rats by intra peritoneal injection of alloxan monohydrate at 80mg/kg bw. to the rats. Aqueous leaves extract of *Anthocephalus indicus* A. Rich. at 400mg/kg bw was given orally to control and diabetic rats for 21 days. Blood samples taken from retro orbital plexus of rats were analysed for serum glucose level, total cholesterol, high density lipoprotein (HDL- cholesterol) and low density lipoprotein (LDL- cholesterol) as per standard kit method. The rats feed with aqueous leaves extract showed significant reduction in blood glucose, total cholesterol, triglycerides, HDL and LDL as compared to diabetic rats. Aqueous leaf extract results for antidiabetic activity were compared with standard drug glibenclamide at 10mg/kg bw. and the antidiabetic acitivity was found to be significant. Histopathological study of liver and pancreas of rats showed that alloxan caused damage in the liver cells and degeneration of pancreatic islet cells. Administration of aqueous leaves extract caused an improvement in damaged liver cells and degenerated pancreatic islet cells. Thus, *Anthocephalus indicus* can be considered as a good natural antidiabetic drug.

**Keywords:** Anthocephalus indicus, antidiabetic, leaf extract, histopathology

## Introduction

Diabetes mellitus is a major public health problem in developed as well as developing countries. [1] It is ranked seventh among the leading causes of death and third when its fatal complications are taken in to account. [2] Diabetes is a syndrome, initially characterized by a loss of glucose homeostasis. The disease is progressive and is associated with oxidative stress with high risk of diabetic dyslipidaemia which is responsible for micro and macro vascular complications of diabetes mellitus [3]. Disease leads to long term damage to β- cells of pancreas which helps in insulin secretion and failure of various organs like eyes, kidneys, nerves, heart and blood vessels [4, 5]. Currently available therapy for diabetes and diabetic dyslipoproteinemia include insulin and various oral antidiabetic agents such as sulfonylurea, metformin, -glucosidase inhibitors, troglitazone [6] and anti dyslipoproteinemic agents as gemfibrozil and flavastatin [7], but these are known to have number of serious adverse effects in patients [8 ,9].

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian medicinal systems mention the use of plants in treatment of various human ailments [10]. Herbal drugs are easily available at low cost, are comparatively safe and people have faith in such remedies [11]. There are many plants and plant products (active, natural principles and crude extracts) which have been mentioned or used in the Indian traditional system of medicine and which have shown experimental or clinical anti-diabetic activity [12]. Among the major phytochemical constituents of plants credited with hypoglycemic action are glycosides, alkaloids, glycans, triterpenes, mucilages, polysaccharides, oils, vitamins, saponins, glycoproteins, peptides, amino acids and proteins [10].

*Anthocephalus indicus* A. Rich. belongs to the family Rubiaceae commonly known as Kadamba. It is used as herbal remedy that has been mentioned in ancient Indian medical literatures for the treatment of fever, anaemia, diabetes, uterine complaints, menorrhagia, blood and skin diseases, diarrhoea, colitis, stomatitis, dysentery and in improvement of semen quality [13, 14]. Phytoconstituents in the plant consist of indole alkaloids, terpenoids, sapogenins, saponins, terpenes, steroids, fats and reducing sugars [15]. The leaves of Kadamba contains glycosidic indole alkaloids; cadambine, 3 dihydrocadambine and isodihydrocadambine [16], two non- glycosidic alkaloids; cadamine and isocadamine [17], two monoterpenoid alkaloid; aminocadambine A and aminocadambine B [18], saponins and phenolic compounds [15, 19]. In traditional system of remedies, warm aqueous extract of kadamba leaves have been used to

alleviate pain, swelling and for cleansing and better healing of wounds as well as for the treatment of menorrhagia. *Anthocephalus indicus* leaves have been reported to possess antimicrobial, antioxidant, analgesic, antipyretic, anti-inflammatory and hepatoprotective activity [19-22]. In literature, the antidiabetic study of alcoholic extract of stem bark and alcoholic and aqueous extract of roots of Kadamba is mentioned but no report is found on antidiabetic study of leaves [15]. Therefore the aim of the research work was to study the antidiabetic potential of aqueous leaves extract of *Anthocephalus indicus*.

## Material and Methods

### Chemicals

Alloxan monohydrate was purchased from Sd-fine Chemical Ltd. Mumbai. Blood Glucose, total cholesterol, HDL cholesterol and triglyceride were determined by commercially available kits from Span diagnostics. All other chemical were of analytical grade. Glibenclamide (standard drug) was purchased from local market medical store.

### Collection of Plant Material

The leaves of *Anthocephalus indicus* A. Rich. were collected from the field grown plants found in Kalyan, Mumbai region. The plant was identified with the help of "The Flora of Presidency of Bombay" and the voucher specimen was authenticated from Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai [23]. The leaves were washed properly under running tap water, shade dried, powdered and stored in an airtight bottle.

### Preparation of extract

The aqueous extract of leaves was prepared by boiling the 100 g of leaf powder in 300ml of distilled water as a solvent for 1 hour. After boiling the solution was allowed to cool at room temperature and then filtered through Whatman filter paper. The filtrate was then concentrated at 100 C to evaporate the solvent. After evaporation of solvent the gummy residue 30 g is stored at 4 C till further use. The gummy residue was redissolved in distilled water at 100 mg/ml of concentration for antidiabetic study [29].

### Preliminary phytochemical analysis of leaf extract

The aqueous leaf extract of *Anthocephalus indicus* was subjected to qualitative tests for the analysis of various active constituents viz. alkaloid, flavonoid, tannins, glycosides, steroids, phenols coumarins and quinones using test procedures [24, 25].

### Antidiabetic study in alloxan induced diabetic rats

Animal study was performed at Animal House of Bombay Veterinary College, Mumbai. Sprague Dawley adult male rats of 150-180 g were used for the study [23]. Total 60 rats were kept in controlled conditions, temperature 25-26 C, relative humidity 60-80% and 12/12 hour light/dark cycle and provided with standard pellet diet (Lipton India, Ltd) and water *ad libitum* [23]. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Bombay Veterinary College, Mumbai, India (REG No. MVC-IAEC-05112 /2012/CPCSEA) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All the animals were acclimatized for seven days before conducting the study [26].

### Experimental Design

Rats were divided with into 4 groups with six rats in each group. These groups were as follows; Group I: Control rats on standard pellet diet and water *ad libitum*, Group II: Alloxan treated diabetic rats on standard pellet diet and water *ad libitum*, Group III: Alloxan treated diabetic rats on standard pellet diet, water *ad libitum* and aqueous leaf extract (400 mg/kg bw.), Group IV: Alloxan treated diabetic rats on standard pellet diet, water *ad libitum* and standard drug glibenclamide (10 mg/kg bw.). Diabetes was induced by intraperitoneal injection of ice cold saline solution of alloxan monohydrate 80 mg/Kg b.w in rats of group II, III and IV respectively [27]. After three days of injection, diabetes was confirmed by blood glucose estimation using kit by GOD-POD method. Rats with serum glucose level 250 mg/dL were selected for the study [28]. After 21 days of feeding rats were fasted overnight and blood was withdrawn from retroorbital plexus [23]. The blood was centrifuged within 1 hour of collection and serum was separated. Serum was used for the estimation of glucose using kit by GOD-POD method, total cholesterol, triglycerides, high density lipoprotein (HDL- cholesterol), low density lipoprotein (LDL-cholesterol) using Span Diagnostic kits [10, 29] Thereafter, rats from all the groups were anaesthetized using chloroform inhalation. The peritoneum was stripped open, the pancreas and livers were quickly harvested and preserved in 4% formalin solution. The tissues were processed histologically using haematoxylin and eosin staining technique [30].

### Statistical Analysis

Data was analysed statistically using one way analysis of variance (ANOVA) using SPSS 20.0 software and post hoc Dunnett's test at p 0.05 to determine significant differences among treatment means. The values are expressed as mean ± standard deviation (SD) [26].

## Results

### Preliminary phytochemical analysis of leaf extract

Preliminary phytochemical analysis of aqueous leaves extract of the plant revealed the presence of alkaloids, flavonoids, tannins, glycosides, phenols, coumarins and quinones (Table 1)

**Table 1** Preliminary phytochemical analysis of aqueous leaf extract of *Anthocephalus indicus*

| Phytoconstituents | Observation |
|---|---|
| Alkaloids | + |
| Phenols | + |
| Tannins | + |
| Steroids | - |
| Glycosides | + |
| Coumarins | + |
| Flavonoids | + |
| Quinones | + |

"+" represents presence and "-" represents

## Effect on serum biochemical parameters

Alloxan at 80 mg/kg bw was found to significantly increase (P< 0.01) the serum glucose (375.71±23.12),total cholesterol (162.5±10.45), LDL (108.09±26.86), triglycerides (82.45±13.99) but significantly decreased (P< 0.05) the serum HDL level (37.91±11.10) in the rats of group II, III and IV as compared to levels at zero day and in normal control rats. Treatment with aqueous extract of leaves of *Anthocephalus indicus* at 400 mg/kg bw showed significant reduction (P< 0.05) in serum glucose (74.76±7.54), total cholesterol (95.83±35.05), LDL (36.66±24.87), triglycerides (54.38±12.30) and significant increase in (P< 0.05) the serum HDL levels (55.51±13.01) as compared to diabetic group rats. The result of treatment with the aqueous extract of leaves of *Anthocephalus indicus* is nearby same for all the measured serum biochemical parametes when compared with the results of standard drug (glibenclamide) treated group (Table 2-6).

**Table 2** Effect of *Anthocephalus indicus* aqueous leaf extract on serum blood glucose level of alloxan induced diabetic rats

| Groups | 0 Day | After 21 Days |
|---|---|---|
| **Control** | 63.17±2.11$^{b}$ | 72.37±19.68$^{b}$ |
| **Alloxan treated** | 64.92±0.96$^{b}$ | 375.71±23.12$^{a}$ |
| **Alloxan + Extract (400 mg/kg bw.)** | 64.86±1.73$^{b}$ | 74.76±7.54$^{b}$ |
| **Alloxan + Glibenclamide (10 mg/kg bw.)** | 65.80±1.89$^{b}$ | 76.66±14.50$^{b}$ |

The values represent the mean ± SD of six rats. Results are significant at p 0.01 as per one way ANOVA statistical analysis. Same superscript indicates no significant difference between the values whereas variation in the superscript order of letter indicates proportionate difference at P 0.01.

**Table 3** Effect of *Anthocephalus indicus* aqueous leaf extract on serum total cholesterol level of alloxan induced diabetic rats

| Groups | 0 Day | After 21 Days |
|---|---|---|
| **Control** | 68.75±10.45$^{d}$ | 47.36±14.51$^{b}$ |
| **Alloxan treated** | 68.75±25$^{d}$ | 162.5±10.45$^{a}$ |
| **Alloxan + Extract (400 mg/kg bw.)** | 68.75±6.84$^{d}$ | 95.83±35.05$^{bc}$ |
| **Alloxan + Glibenclamide (10 mg/kg bw.)** | 72.91±5.10$^{cd}$ | 97.91±31.04$^{b}$ |

The values represent the mean ± SD of six rats. Results are significant at p 0.01 as per one way ANOVA statistical analysis. Same superscript indicates no significant difference between the values whereas variation in the superscript order of letter indicates proportionate difference at P 0.01.

**Table 4** Effect of *Anthocephalus indicus* aqueous leaf extract on serum triglycerides level of alloxan induced diabetic rats

| Groups | 0 Day | After 21 Days |
|---|---|---|
| **Control** | 40.34±10.35$^{b}$ | 47.36±14.51$^{b}$ |
| **Alloxan treated** | 40.34±16.86$^{b}$ | 82.45±13.99$^{a}$ |
| **Alloxan + Extract (400 mg/kg bw.)** | 42.09±17.61$^{b}$ | 54.38±12.30$^{b}$ |
| **Alloxan + Glibenclamide (10 mg/kg bw.)** | 42.10±9.41$^{b}$ | 49.03±5.37$^{b}$ |

The values represent the mean ± SD of six rats. Results are significant at p 0.01 as per one way ANOVA statistical analysis. Same superscript indicates no significant difference between the values whereas variation in the superscript order of letter indicates proportionate difference at P 0.01.

**Table 5** Effect of *Anthocephalus indicus* aqueous leaf extract on serum HDL level of alloxan induced diabetic rats

| Groups | 0 Day | After 21 Days |
|---|---|---|
| Control | 27.08±4.19$^{c}$ | 58.22±10.80$^{a}$ |
| Alloxan treated | 29.79±4.19$^{bc}$ | 37.91±11.10$^{b}$ |
| Alloxan + Extract (400 mg/kg bw.) | 28.43±6.79$^{bc}$ | 55.51±13.01$^{a}$ |
| Alloxan + Glibenclamide (10 mg/kg bw.) | 29.78±6.63$^{bc}$ | 52.80±9.95$^{a}$ |

The values represent the mean ± SD of six rats. Results are significant at p 0.01 as per one way ANOVA statistical analysis. Same superscript indicates no significant difference between the values whereas variation in the superscript order of letter indicates proportionate difference at P 0.01.

**Table 6** Effect of *Anthocephalus indicus* aqueous leaf extract on serum LDL level of alloxan induced diabetic rats

| Groups | 0 Day | After 21 Days |
|---|---|---|
| Control | 10.7±1.03$^{c}$ | 26.95±14.31$^{bc}$ |
| Alloxan treated | 11.2±1.7$^{c}$ | 108.09±26.86$^{a}$ |
| Alloxan + Extract (400 mg/kg bw.) | 10.88±0.95$^{c}$ | 36.66±24.87$^{b}$ |
| Alloxan + Glibenclamide (10 mg/kg bw.) | 10.88±0.95$^{c}$ | 35.30±23.96 |

The values represent the mean ± SD of six rats. Results are significant at p 0.01 as per one way ANOVA statistical analysis. Same superscript indicates no significant difference between the values whereas variation in the superscript order of letter indicates proportionate difference at P 0.01.

## Histopathological results of pancreas

In the pancreas of control group rats, round and oval shaped islets were evenly distributed throughout the cytoplasm. In diabetic group rats, the islets were damaged, shrunken in size (Fig 6). In rats treated with aqueous leaf extract of *Anthocephalus indicus* (400 mg/kg bw) and standard drug glibenclamide (10 mg/kg bw) islets were normal in arrangement similar to those in control group rats [1, 31].

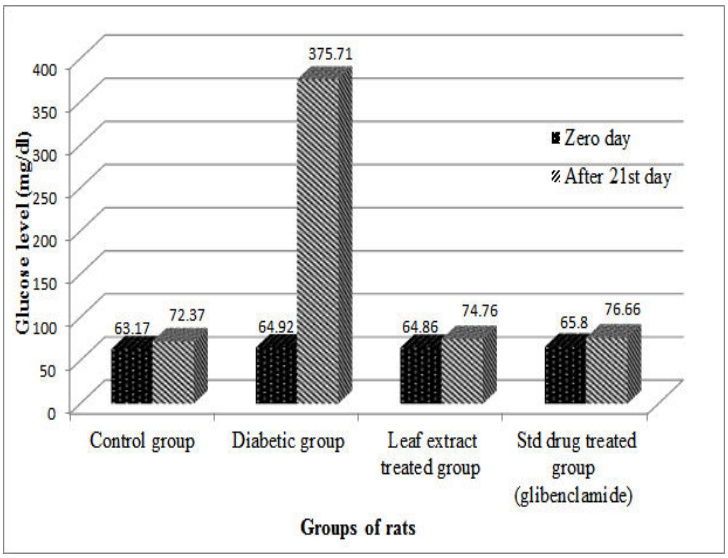


**Fig 1** Effect of aqueous leaf extract of *Anthocephalus indicus* and Std drug (glibenclamide) on glucose level of rats in comparison with diabetic rats

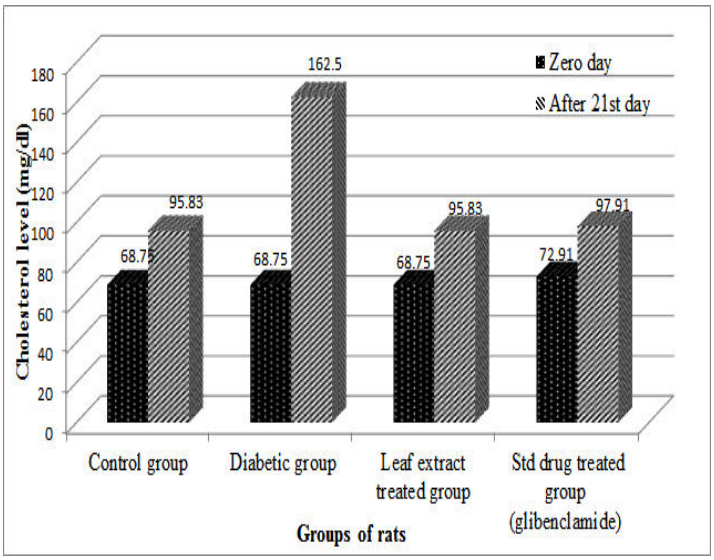


**Fig 2** Effect of aqueous leaf extract of *Anthocephalus indicus* and Std drug (glibenclamide) on serum total cholesterol level of rats in comparison with diabetic rats

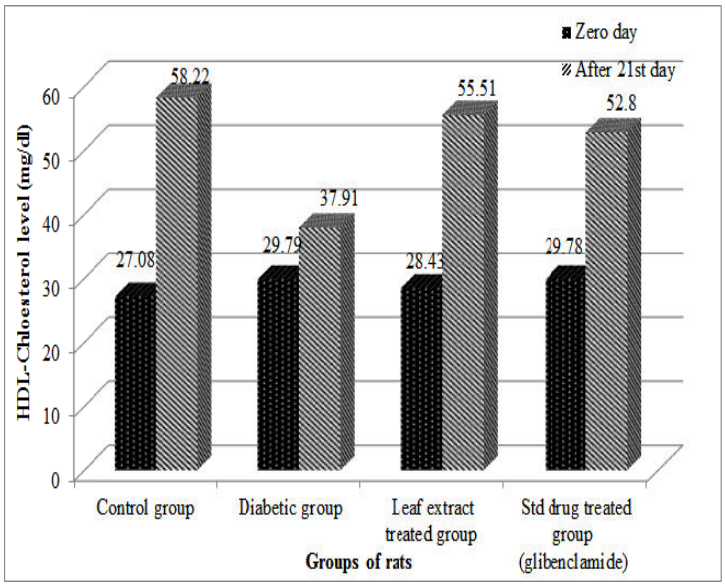


**Fig 3** Effect of aqueous leaf extract of *Anthocephalus indicus* and Std drug (glibenclamide) on serum triglycerides level of rats in comparison with diabetic rats

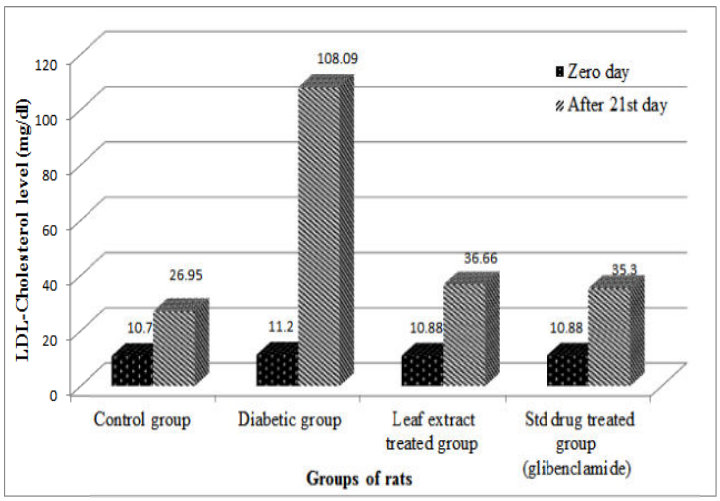


**Fig 4** Effect of aqueous leaf extract of *Anthocephalus indicus* and Std drug (glibenclamide) on serum HDL level of rats in comparison with diabetic rats

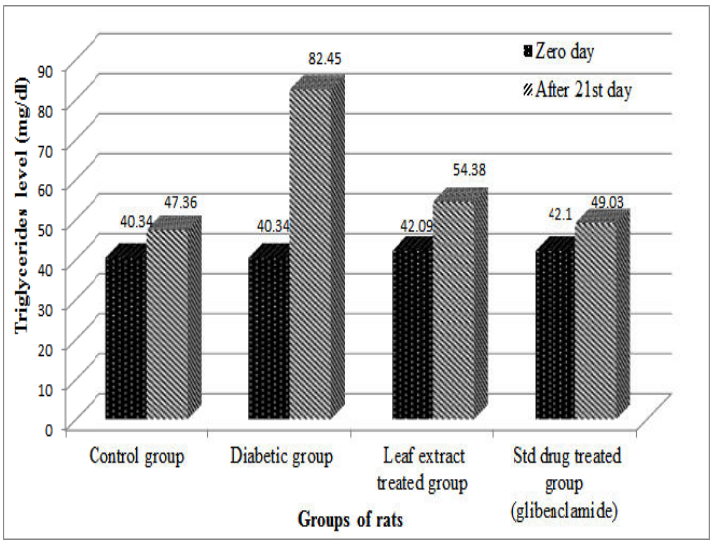


**Fig 5** Effect of aqueous leaf extract of *Anthocephalus indicus* and Std drug (glibenclamide) on serum LDL level of rats in comparison with diabetic rats

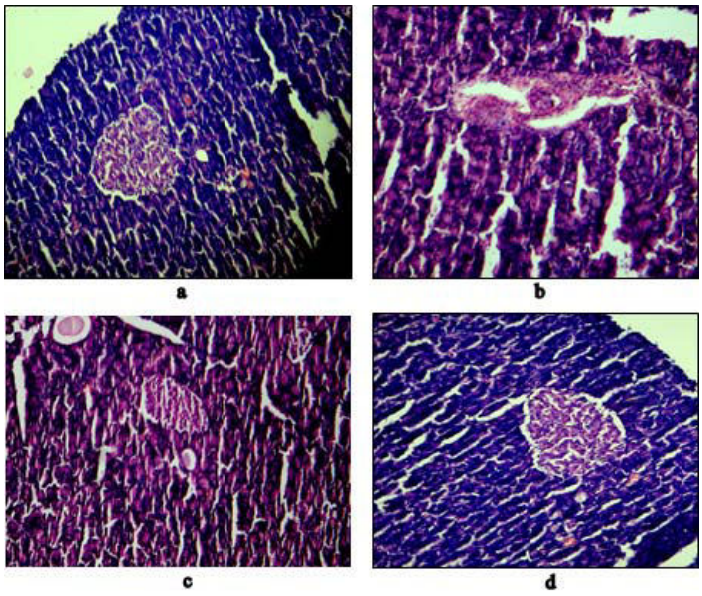


**Fig 6** T.S. of pancreas; a- control group rat with round and oval shaped islets evenly distributed throughout the cytoplasm, b- diabetic group rat with shrunken and degenerated islet cells, c and d- rats treated with leaf extract and Std drug glibenclamide showed the presence of normal isletcells similar to those in normal group rats

## Histopathological results of liver

In the normal liver tissue sections showed sinusoidal cords of hepatocytes with central vein and portal tracts. The portal tracts

showed portal triad with portal vein, hepatic artery and bile duct, whereas the diabetic rat liver tissue sections showed distortion in the arrangement of cells around the central vein, with lymphoplasmocytic infiltration in the portal tracts with destruction of some bile ducts (Fig 7). In rats treated with aqueous leaf extract of *Anthocephalus indicus* (400 mg/kg bw) and standard drug glibenclamide (10 mg/kg bw) liver cells showed normal cellular arrangement around the central vein and reduced necrosis with no lymphoplasmocytic infiltration in the portal tracts [1, 32].

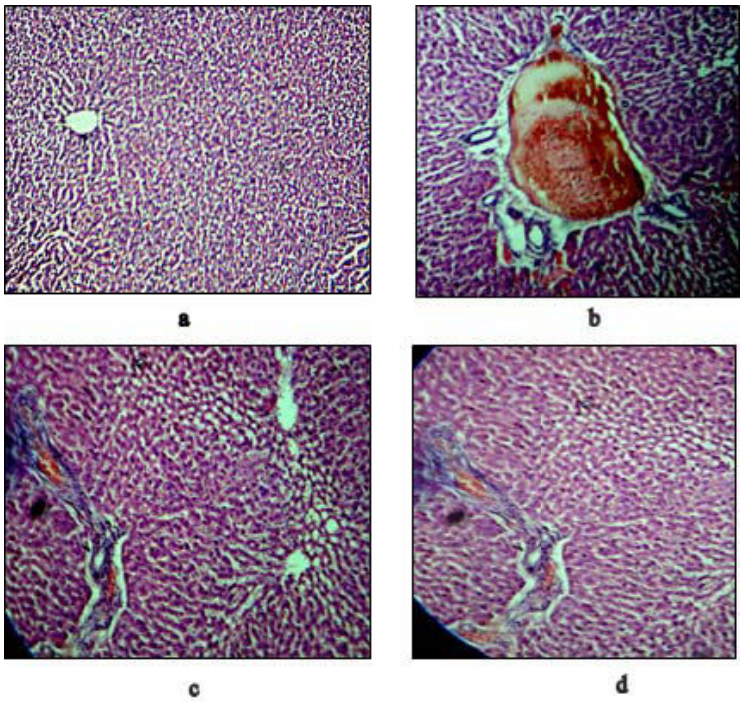


**Fig 7** T.S. of liver; a- control group rat with normal sinusoidal hepatocytes around central vein, b- diabetic group rat with lymphoplasmocytic infiltration in the portal tracts, c and d- rats treated with leaf extract and Std drug glibenclamide showed normal cellular arrangement around the central vein and reduced necrosis with no lymphoplasmocytic infiltration in the portal tracts

## Discussion

In the present study it was found that *Anthocephalus indicus* leaf extract and glibenclamide both caused a significant decrease in the serum glucose and lipid levels in alloxan induced hyperglycaemic rats. The diabetogenic agent alloxan has distinct pathological effects interfering with the physiological changes of pancreatic and liver cells. It inhibits the glucose induced secretion of insulin through its ability to specifically inhibit the glucokinase, the glucose sensor of beta cells and it causes the state of insulin dependent diabetes mellitus through its ability to induce a selective necrosis of the beta cells [33]. Due to the similarity of alloxan to glucose molecules it enters the liver cells through GLUT2 glucose transporters and cause further destructive changes in the liver cells [34].

Alloxan induced diabetes involves the degeneration of islet β- cells by accumulation of cytotoxic free radicals [2]. In the study higher levels of serum lipids in alloxan treated diabetic rats. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for cardiovascular disease [35]. The abnormally high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral deposits, since insulin inhibits the hormone sensitive lipase enzyme. The hyperlipidaemia is considered as a result of unregulated actions of lipolytic hormones on the fat deposits [35].

The majority of islet cells are formed by B cells which are responsible for producing insulin. Depletion of B cells will therefore result in insulin deficiency which leads to a disorder in carbohydrate, protein and fat metabolism with resultant hyperglycaemia [36]. In this study changes in the islet cells of diabetic rats were caused because of the destruction of B cells [31, 37]. In the rats treated with leaf extract and glibenclamide regeneration of islet cells was seen.

The histopathological examination of liver tissue of diabetic rats showed hepatic cells and portal tract degeneration with lymphoplasmocytic infiltration in portal cells as compared to that of normal rats. These results are in agreement with Buko *et al* (1996) who reported that in diabetic rats liver tissue was characterized by hydropic dystrophy and lymphocytic infiltrations. These damages may be due to oxygen free radicals (OFR) exerting their cytotoxic effect by peroxidation of membrane phospholipids leading to a change in cell permeability and loss of membrane integrity. Decreased endothelium- dependent relaxation in diabetes is linked to release of OFRs [39].

Normal range of serum glucose, cholesterol, HDL, LDL and trigycerides and the regenerative effect of the islet cells of pancreas and liver cells in the rats treated with leaf extract and glibencamide enlighten the positive effects of these agents on the production of insulin by pancreatic cells and further metabolism of glucose in liver. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, glycosides, phenols, coumarins and quinones. Flavonoids, alkaloids and phenolic compounds are known to be bioactive antidiabetic principles [40]. Flavonoids are known to regenerate the damaged beta cells and stimulate the insulin secretion in the alloxan diabetic rats [40, 41]. Phenolic compounds are found to be effective antihyperglycemic agents [40]. The antidiabetic effect of aqueous extract of leaves of the plant may be due to the presence of more than one antihyperglycemic bioactive principle and their synergistic properties.

Thus an attempt has been made to describe *Anthocephalus indicus* leaf extract as a potent sugar and lipid lowering agent and these beneficial activities may contribute to antidiabetic activities of the natural products

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