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CHEMICAL CONSTITUENTS OF *PAULOWNIA TOMENTOSA* (THUNB) STEUD. *FAM*. SCROPHULARIACEAE AND ITS ROLE AGAINST HYPERGLYCEMIA

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ABSTRACT

Aim: The present work on the fruits of *Paulownia tomentosa* (Thunb.)Steud. (Scrophulariiaceae) has led to the isolation of three iridoidal compounds (1-3) identified as catalpol, aucubin and paulownioside and new hydrocarbon compound 4 was identified as cyclohexanone. Isolation of these constituents was performed using chromatographic techniques. Their structures were established through chemical and spectral analysis. Our study was to demonstrate the antidiabetic activity of the methanolic extracts obtained from *Paulownia tomentosa,* and investigated in normal and alloxan-induced diabetic rats, glibenclamide was used as a reference drug. **Methods:** Fifty six male albino rats will be used in the present study. Animals will be divided into 7 groups each group will be of eight rats, to evaluate the efficacy of the fruits of *Paulownia tomentosa.* **Results:** Triglycerides, total lipids also a significant decrease in liver function enzymes (AST, ALT and ALP) while elevation of Liver glycogen content, total protein and albumin in diabetic rats given methanolic extracts. *Paulownia tomentosa* extracts showed recovery in hyperglycemia and successfully recovery the alterations of all biochemical parameters measured. Histopathological study of liver was also carried out to confirm the protection offered by the methanolic extract. **Conclusions:** Data showed that *Paulownia tomentosa* possessed strong antioxidant activity, remarkable antidiabetogenic effect comparable to glibenclamide, a well-known liver protecting herbal formula.

KEYWORDS: Alloxan; *Paulownia tomentosa*, Iridoids, Aucubin, Enzymes, Hypoglycemic.

INTRODUCTION

Paulownia tomentosa (Thunb.)Steud., belongs to the family Scrophulariaceae, it is commonly known as princess tree.[1] *Paulownia tomentosa* is a genus that is composed of a number of species. They are all native to China except *P. fortunei* which extends into Vietnam and Laos. *Paulownia tomentosa* (Thunb.)Steud. Also name a princess or empress tree.[2] *P. tomentosa* is a rich source of geranylated flavanones, furanoquinones, iridoides and flavonoids were also present.[3]

Diabetes mellitus (DM) is a global epidemic affecting more than150 million people, a number that is expected to double by 2025.[4] It's a chronic disease caused by inherited and/or deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Currently available synthetic oral antihyperglycemic agents may be associated with an increased risk of unwanted effects on prolonged use Edwinet al.[5] so there is clear need to investigate new herbal medicine having fewer side effects, easy available and economic.[6] Alloxan is economical to induce Diabetes mellitus to screen antidiabetic herbals in experimental animals. It has been shown to produce diabetes by damaging islet cells of pancreas by liberating oxygen radicals. These free radicals are also involved in the other tissue damage (liver and kidney etc.) which occurs in the progression of DM.[7]

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage and also detoxification and synthesizes useful principles. Hepatotoxic agents cause structural abnormality in the liver which alters normal physiological functions of this organ.

Herbal remedies have greater advantages because of their effectiveness, minimal side effects in clinical experience and relatively low costs hence there is a growing interest in this field. Such a traditional medicinal plant is *Paulownia tomentosa* (Thunb.) Steud., well known for its folkloric use. Accordingly the hypoglycemic activity of the methanolic extracts of leaves and fruits of *P. tomentosa* (Thunb.) Steud., were carried out for the first time as mentioned and the results will be discussed.

MATERIALS AND METHODS

Plant material

Fresh leaves and fruits of *Paulownia tomentosa* (Thunb.)Steud., were collected from Agricultural Museum, Dokki, Giza, Egypt in July and September 2007 respectively. The plant materials were identified by Mrs. Terase Labib, head of the taxonomy at El-Orman Botanical Garden. A voucher specimen (No.06-06-03-16) was kept at the Herbarium of El-Orman Botanical Garden.

Extraction & Isolation

Mature fresh fruits (320g) of *P. tomentosa* (Thunb.) Steud.Were subjected to complete extraction by cold maceration in absolute methanol. The extract was collected, filtered and evaporated under vacuum to yield 38.48 g. The residue was mixed with 90ml distilled water and successively extracted on cold with chloroform, ethyl acetate, methanol respectively. The different extracts were evaporated separately under vacuum till dryness. The methanol extract were subjected to silica gel column chromatography (CC) by (Chloroform – Methanol – Water) to give catalpol (1), Aucubin (2) and paulownioside (3) and new hydrocarbon.

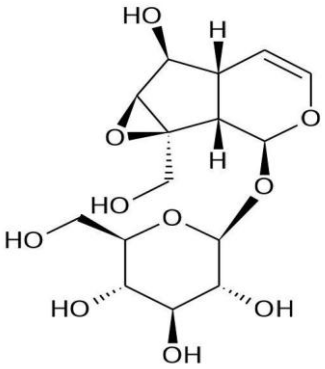
Compound 1

It was isolated as white crystalline powder by preparative TLC from fractions (1-15) CHCl3-MeOH-H2O (80:20:2) then purified and crystallized from methanol; its m.p.= 203-205, It is highly soluble in water and methanol also soluble in ethanol, acetone, but almost insoluble in lipophilic organic solvents such as chloroform, benzene, and petroleum ether & Rf value is (0.21) in solvent system *n*-butanol-H2O (9:1).

Spectral analyses of compound 1

1H-NMR and 1H-1H COSY spectra revealed the presence a contiguous sequence of cross peaks, which can be explained as follow; two cross peaks represent the correlation of H-3 at δ 6.58 (1H, br*s*, H-3) with H-4 appeared at δ 5.05 (1H, br*d*, *J* = 5.6 Hz), a sharp doublet signal appeared at δ 5.16 (1H, *d*, *J* = 4 Hz) corresponding to H-1, which is in turn correlated with H-9 at δ 2.23 (1H, *m*). Furthermore, H-5 at δ 2.17 (1H, *m*) appeared to correlate with H-6 at δ 3.87 (1H, br*d*, *J* = 8.4 Hz) and H-9. H-7 appeared at δ 3.62 (1H, brs) as indicated by COSY spectrum. Moreover, the methylene hydroxy appeared at δ3.72 and 3.74 (2H, *m*, CH2-10). A sharp doublet appeared at δ 4.26 (1H, *d*, *J* = 7.6 Hz, H-1´) corresponding to the anomeric proton of a hexose moiety identified to be glucose, the remaining of the hexose protons overlapped at δ 2.91 – 3.88 (5H, *m*).

13C-NMR spectrum revealed the presence of 15 signals appeared at *δC* 94.14 (C-1), 140.44 (C-3), 104.16 (C-4), 36.50 (C-5), 76.84 (C-6), 60.27 (C-7), 64.70 (C-8), 42.41 (C 9), 58.97 (C-10), 97.70 (C-1´), 73.89 (C-2´), 77.96 (C-3´), 69.90 (C-4´), 77.29 (C-5´), 61.24 (C-6´). Mass spectral analysis *m/z* of compound (**1**) its molecular formula was determined to be C15H22O10 using HRMALDI/MS (+ve) revealed the presence of quasimolecular ion peak at *m/z* 363.1286 [M+H] + (calcd 363.1291 for C15H23O10). Hence, according to the above mentioned data and by comparison with the literature[9] compound (**1**) was established and assigned to catalpol.



Catalpol

Compound 2

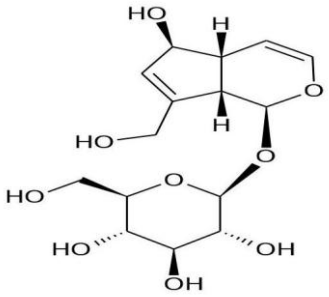
It was isolated as white crystalline powder by preparative TLC from fractions (31-45) CHCl3-MeOH-H20 (80:20:2) then purified and crystallized from methanol, its m.p. =181°C, soluble in water, insoluble in chloroform, ether, petroleum ether and Rf values (0.5 & 0.8) in two different solvent systems *n*-butanol-H2O (9:1) & *n*-butanol-MeOHH2O (7:2:1).

Spectral analyses of compound 2

1H-NMR and 1H-1H COSY spectra revealed the presence a contiguous sequence of cross peaks, which can be explained as follow; two cross peaks represent the correlation of H-3 at *δ* 5.98 (1H, brs, H-3) with H-4 appeared at *δ* 5.30 (1H, brs), a broad singlet signal appeared at *δ* 5.48 (1H, brs) corresponding to H-7. Furthermore, H-5 at *δ* 2.26 (1H, brt, *J* = 7.6 Hz) appeared to correlate with H-6 at *δ* 3.99 (1H, brt, *J* = 5.2 Hz) and H-9 at *δ* 2.94 (1H, brt, *J* = 8.4 Hz). H-1 appeared at *δ* 5.19 (1H, brs). Moreover, two doublets appeared at *δ* 4.05 and 3.79 (2H, *d*, *J* = 7.6 Hz, CH2-10). A doublet signal appeared at *δ* 4.48 (1H, *d*, *J* = 8 Hz, H-1´) corresponding to the anomeric proton of a glucose moiety, the remaining of its protons overlapped at *δ* 3.10 – 3.77 (5H, *m*). Mass spectral analysis *m/z* of compound 2 molecular

formula is C15H22O9 using HRMALDI/MS (+ve) revealed the presence of quasi-molecular ion peak at *m/z* 369.1156 [M+Na]+ (calcd 369.1161 for C15H22O9Na).

Hence, according to the above mentioned data and by comparison with the literature,[10] also by applying different chromatographic techniques using authentic aucubin, compound (**2**) was established and assigned to aucubin.



Aucubin

Compound 3

It was isolated as white crystalline powder by preparative TLC from fractions (31-45) CHCl3-MeOH-H2O (80:20:2) then purified and crystallized from methanol; its m.p. is 308°C (decomposed) soluble in water, methanol and insoluble in chloroform & Rf values (0.38 & 0.68) in two different solvent systems *n*-butanol-H2O (9:1) & *n*-butanol-MeOH H2O (7:2:1) as shown.

Spectral analyses of compound 3

1H-NMR spectrum revealed the presence of a broad singlet signal at *δH* 5.96 (1H, brs, H- 1), two sets of doublet of doublet signal appeared at *δH* 6.35 (1H, *dd*, *J*= 6, 2 Hz, H-3) and at *δH* 5.00 (1H, *dd*, *J* = 6, 1 Hz, H-4), H-5 appeared at *δH*2.11 (1H, *m*), H-6 appeared at *δH* 3.76 (1H, br*d*, *J* = 8.6 Hz) and H-9 at *δ* 2.31 (1H, br*t*, *J* = 9.5 Hz). Moreover, two doublets correlated with each other appeared at *δ* 3.86 and 3.66 (2H, *d*, *J* = 13 Hz, CH2- 10). A doublet signal appeared at *δ* 4.89 (1H, *d*, *J* =5 Hz, H-1´) corresponding to the anomeric proton of a hexose moiety identified to be glucose from 13C-NMR and HMQC spectra, H-2´ appeared at *δ* 3.20 (1H, *m*), the rest of the hexose protons overlapped at *δ* 3.10-3.85 (5H, *m*).

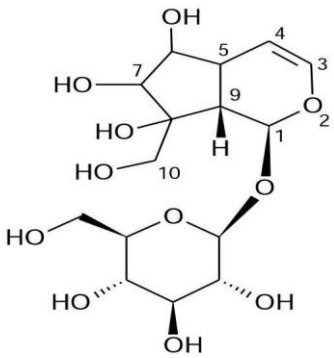
1H-1H COSY spectra revealed the presence a contiguous sequence of cross peaks, which can be explained as follow; two cross peaks represent the correlation of H-3 at *δ* 6.35 (1H, *dd*, *J*= 6, 2 Hz, H-3) with H-4 at *δ* 5.00 (1H, *dd*, *J* = 6, 1 Hz), moreover H-4 correlated by allylic

coupling with H-5 which appeared at δ 2.11 (1H, *m*). Furthermore, H-5 appeared to correlate with H-6 at δ 3.76 (1H, br*d*, *J* = 8.6 Hz) and H-9 at δ 2.31 (1H, br*t*, *J* = 9.5 Hz). Moreover, the two doublets corresponding to the methylene hydroxyl (CH2-10) correlated with each other. A cross peak represented the correlation of the anomeric proton of the hexose with H-2´. A contiguous sequence of cross peaks represented the correlations of the rest of the hexose protons at δ 3.10 – 3.85 (5H, *m*).

13C-NMR and HSQC spectra showed the presence of two hydroxy methylenes at δC 61.14 (6´-CH2OH) and at δC 64.92 (10-CH2OH), twelve methines appeared at δC93.40 (C-1), 140.43(C-3), 103.51 (C-4), 37.47 (C-5), 76.48 (C-6), 77.26 (C-7), 42.17 (C-9), 97.96 (C-1´), 73.49 (C-2´), 76.75 (C-3´), 70.16 (C-4´), 76.95 (C-5´) and one quaternary carbon at δC 77.54 (C-8).

The HMBC experiment clarified the long rang correlations (*2J* &*3J*), and confirmed the attachment of the glucose moiety at the C-1 via the long range correlation between its anomeric proton H-1´ and C-1 at δ 93.40. Mass spectral analysis *m/z* its molecular formula is C15H24O11 using HRMALDI/MS (+ve) revealed the presence of quasi-molecular ion peak at *m/z* 403.1216 [M+Na] + (calcd 403.1211 for C15H24O11Na).

Hence, according to the above mentioned data compound (**3**) was established and assigned to paulownioside.[11]



Paulownioside

Compound 4

It was isolated as white amorphous powder from the choroformic fractions (20 fractions). Crystallized by methanol, its m.p. = 201, soluble in chloroform, insoluble in methanol and Rf value (0.75) in solvent system *n*-butanol-H2O (9:1).

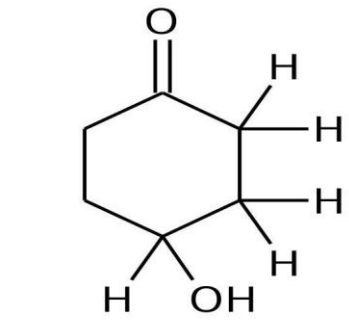
Spectral analyses of compound 4

1H-NMR (DMSO-*d6* at *δ*H 2.50) spectrum showed the presence of a broad doublet signal at *δ* 5.04 (1H, br*d*, *J* = 5.6 Hz, H-4), a broad multiplet at *δ* 2.03-2.23 (4H, br*m*, H-3 & -5), and a broad triplet at *δ* 1.81 (4H, br*m*, H-2 & -6), one broad signal appeared at *δH* 8.58 characteristic for hydroxy proton.

1H-1H COSY spectrum revealed the presence of a cross peak representing the correlation between H-4 and H-3, furthermore H-3 correlated with H-2 with an overlapped cross peaks. 13C-NMR (DMSO-*d6* at *δ*C 39.51) spectrum showed the presence of four signals, two of which appeared at *δ*C 27.97 and 28.12 characteristic for aliphatic carbons (C-C), at *δ*C 82.56 characteristic for hydroxy carbons (HO-C), and finally at *δ*C 177.33 characteristic for carbonyl carbon's region.

DEPT (135o angle) and HSQC spectra confirmed the presence of two methylenes (CH2) appeared at *δ*C 27.97 and 28.12, and confirmed that the hydroxy carbon at *δ*C 82.56 could be (CH or CH3). Moreover, the disappearance of the signal at *δ*C 177.33 confirmed its carbonyl nature.

HMBC experiment confirmed the proposed structure by clarifying the long range correlations of H-4 with C-3 and C-2, H-2 correlated with C-3, C-4 and with the carbonyl carbon, H-3 showed long range correlations with C-2, C-4 and with C-1 (carbonyl carbon). Mass spectral analysis *m/z* revealed its molecular formula was C6H10O2 using DART/MS (+ve), which revealed the presence of quasi-molecular ion peak at *m/z* 229.1434 [2M+H] + (calcd 229.1390 for C12H21O4). Hence, and according to the above mentioned data compound (**4**) was established and assigned to 4-hydroxycyclohexanone which reported here to be isolated for the first time from nature.



4-hydroxycyclohexanone

Chemicals and biochemical kits

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), Merck (Germany), BDH (England), Alloxan (Sigma Co.) was used for the induction of diabetes in rats. Glibenclamide (Daonil) (Sanofi Aventis) was used as standard antidiabetic drug.

Kits used for the quantitative determination of different parameters were purchased from Bio-diagnostic (Egypt).

Animals

Male albino rats weighting (120-150g), supplied from the animal house of National Research Centre (Dokki, Giza, Egypt) were used for experimental investigation. The rats were kept in our laboratory under controlled environmental conditions. Anesthetic procedures complied with the legal ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA and were approved by the ethical committee of the National Research Centre in Egypt with registration No. 13-015.

Experimental Design

Fifty six male Wister albino rats, weighing 100-150g will be used in the present study. Animals will be divided into 7 groups each group will be of eight rats where; Group (1) will be normal healthy control rats, Groups (2) and (3) will be normal healthy rat's orally administrated methanolic extracts of leaves and fruits respectively. Group (4) diabetic rats (Alloxan 150 mg/ Kg body weight).Groups (5) and (6) will be diabetic rats' orally administrated methanolic extracts of leaves and fruits (500mg/kg) respectively. Group (7) orally administrated glibenclamide as standard drug.

Alloxan-induced diabetes

Male Wister albino rats were injected intraperitoneal with Alloxan (150 mg/ Kg body weight) to induce diabetes mellitus.[12] After alloxan application, the pancreas secretes insulin at high levels. As a consequence, fatal hypoglycemia can occur. To prevent this adverse effect, 5 ml 20% glucose solution were injected intraperitoneal 4–6 h after alloxan injection. Hyperglycemia was assessed after 72 hrs. by measuring blood glucose level using blood glucose glucometer apparatus after one week, two weeks, three and four week's intervals.

Sample Preparation

Liver tissue was homogenized in 30% NaOH for estimation glycogen content. Blood sample was collected by puncture of the sublingual vein, left 10 min to clot and centrifuged at 3000 rpm for serum separation, the separated serum was used for determination of glucose liver function test and lipid profile.

Biochemical assay

Glucose in serum was determined calorimetrically at 505nm by the method of Trinder.[13] Total protein was estimated by the method of Bradford,[14] where Bovine serum albumin was used as a standard and the color developed was read calorimetrically at 595 nm. Albumin level was determined according to Dumas *et al.*,[15] Using bromocresel green (BCG) inn citrate buffer, the developed color was read at 550nm. Aminotransferases (AST and ALT) activities were determined according to the method of Rietman and Frankle[16] Using 2, 4-dinitrorphenyl hydrazine in phosphate buffer color developed was read calorimetrically at 505 nm. Alkaline phosphatase (ALP) activity was estimated by the method of Babson et al.[17] Using phenolphthalein mono phosphate and the colored developed measured at 550 nm.

Histopathological Examination

For light microscopic examination, liver tissue from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5 μl thickness and stained with haematoxylin and eosin & Masson's trichome.[18]

Statistical Analysis

The results of biochemical analysis were analyzed using one-way analysis of variance (ANOVA) followed by Co-stat computer program. Values of less than ≤ 0.05 were regarded as statistically significant.

RESULTS

Chemical characterization of isolated compounds

Four compounds (1-4) were isolated and identified as catalpol (1), aucubin (2), paulownioside (3) and 4-hydroxy cyclohexanone (4) using different spectral analysis (1- H-NMR, 1H-1-H COSY spectrum, 13-CNMR, Dept. 135^{o}, HSQC, HMBC and mass spectrometry) as in Figure 1.

Biochemical determinations

The results demonstrated the use of the methanolic extracts of both leaves and fruits of *P. tomentosa* (Thunb.) Steud.(500 mg/kg) for investigation of the anti-diabetic activity against the standard drug glibenclamide (10mg/kg).The methanolic extracts of both leaves and fruits of *P. tomentosa* (Thunb.)Steud, effectively reduce the blood glucose level of alloxan induced diabetic rats which increased between 500 to 600 mg/dl.

According to leaves extract there was significant reduction in blood glucose level starting from week 1 and 2 with percentage 230.8% and 98.28% respectively, while highly significant results after 3 and 4 weeks with percentage 49.39% and 5.77% respectively in comparison to control group and glibenclamide treated group with percentage 169%, 60.68%, 35.75% and 32.55% respectively. The methanol extract of the fruits produced significant reduction in blood glucose level starting from week 1 and 2 with percentage 275%, 152.45% respectively, while highly significant results after 3 and 4 weeks with percentage 53.44% and 29.97% respectively in comparison to control group and glibenclamide treated group with percentage 169%, 60.68%, 35.75% and 32.55% respectively as shown in table 1 & figure 1.

In case of alloxan diabetic rats there was significant reduction in glycogen, albumin and total protein during this study with different percentages 68.92, 29.32 and 55.76 % respectively, with respect to control group. Therapeutic treatment of rats with *P. tomentosa* (Thunb.) Steud., Leaves, fruits and glibenclamide drug ameliorated these disturbances by different percentages as follows: Albumin recorded 40.22% and 31.39% elevation in groups treated with methanolic extracts of *P. tomentosa* (Thunb.) Steud., Leaves and fruits respectively, while group treated with drug showed decrease with 32.33% with respect to control group. Leaves and fruits extracts showed enhancement when compared with control group while total protein results close to control group after treatment with these extracts. As for glycogen results leaves, fruits extracts and glibenclamide drug ameliorated this disturbance by different percentages as follows 25.49%, 27.26 % and 25.49% respectively with respect to control group showing that group treated leaves ameliorated glycogen disturbance by same percentage as that of Glibenclamide drug. As shown in table 2 & figure 2.

According to hepatic marker enzymes, significant increase in AST, ALT and ALP by 185.4%, 255% and 305.37% respectively as a result of alloxan induction with respect to control group and therapeutic treatment with *P. tomentosa* (Thunb.)Steud., Leaves enhanced the hepatic marker enzymes by 27.45%, 68.35% and 71.61% respectively. In rats given fruits

extract 28.67%, 61.65% and 55.47% while in glibenclamide group 18.23, 3.55 and 48.24% respectively, compared to control groups. On the other hand, control groups treated with either leaves or fruits extracts showed no significant change of AST, ALT and ALP by 9.71, 3.73 and 18.16% respectively for leaves extract, while for fruits by 11.10, 7.04 and 17.59% respectively with respect to control, as shown in table 3 & figure 3. The lipid profile levels, revealed a significant elevation in total cholesterol, triglyceride and total lipid with respect to normal control groups due to alloxan induction by 219%, 853.85% and 967.4% as compared to control. Therapeutic treatment with *P. tomentosa* (Thunb.)Steud., leaves extracts reduced their increase with respect to control by 10.65%, 58.19% and 249.26% respectively, and according to group treated fruits reduce triglyceride level & significant reduction in total lipid by 74.05% and 73.37% respectively, while total cholesterol results were close to control group control groups were not significant if compared with normal control´ in control leaves extract changed with 25.52%, 23.05% and 39.83% respectively, while fruits group recorded 17.83%, 19.67% and 19.60% respectively as shown in table 4 & figure 4.

Table 5 & Figure 5 showed the changes in rats liver weight, body weight & relative liver weight due to alloxan induction, where liver weight is increased with percentage change 34.53% as compared to control group, while body weight record reduction with 18.38 %, relative liver weight were highly significant increase in alloxan group compared to control group with percentage change 65.59%.

Histopathological Study

Liver histology in rats (Alloxan): **Figure 6(A)** Liver sections showed the normal structure of control rats H&E stain **(A*)**. Normal hepatocytes cell with normal collagen deposit Masson' trichome stain; **(B)** Liver section of rats treated with alloxan resulted in the damage of liver structure with abnormal cells along with disarrangement of hepatic strands with sinusoidal dilatation and congestion showing moderate to marked hepatocytes balloon in stained with H&E while **(B*)** Masson's trichome stains revealed abnormal collagen deposit around hepatic strands with fibrosis cells; **(**C) leaves given to control rats showing no change in hepatic normal cells H&E stain and confirmed with **(C*)** showing normal collagen distribution.

Figure 7 (D) Liver sections revealed that leaves, treatments for alloxan group, brought back the cellular arrangement around the central vein and reduced necrosis, hepatocytes almost normal arranged in thin plates stained with H&E and Masson's trichome stains as in**(D*)**;

(E) fruits extract given to control rats revealed normal hepatic cells H&E stain and confirmed with section **(E*)** showing no change in collagen distribution; **(F)**Fruits extract treatments for alloxan group showing nearly normal cells, around the central vein necrosis disappeared in the hepatic cells which stained with H&E and Masson' trichome stains as in section**(F*)**; **(G)**Glibenclamide drug treatments for alloxan group showing enhancement in most hepatic cells and reduced necrosis, stained with H&E and Masson's trichome stains as in section**(G*)**.

Table 1: Effect of Glibenclamide drug and methanol extracts of leaves & fruits of *Paulownia tomentosa* (Thunb.) steud. on normal and diabetic rats for 4 weeks.

Duration	Fasting blood sugar concentrations		
	Group treated leaves	Group treated fruits	Treated glibenclamide drug
Control	101.75 ± 9.6	101.75 ± 9.6	101.75 ± 9.6
Alloxan group	585.75 ± 29.8	553.3 ± 53.0	493.13 ± 149.9
Alloxan group	585.75 ± 29.8	553.3 ± 53.0	493.13 ± 149.9
1week	336.63 ± 47.6	381.87 ± 109.7	274.0 ± 66.5
2 weeks	201.75 ± 38.1	256.87 ± 72.8	163.5 ± 51.4
3 weeks	152.0 ± 24.6	156.13 ± 54.1	138.13 ± 47.8
4 weeks	107.63 ± 26.4	71.25 ± 9.4	68.63 ± 14.5

Data are means ± SD of eight rats in each group.

Table 2: Effect of methanol extracts of leaves and fruits *Paulownia tomentosa* (Thunb.) Steud. on glycogen, albumin & total protein in control and diabetic rats.

Groups	Treatment	Glycogen	Albumin	Total protein	P≤
1	-ve control	60.05 ± 4.73^{a}	5.32 ± 0.58^{bc}	17.7 ± 1.42^{abc}	0.0001
2	+ve control	18.66 ± 4.22^{d}	3.76 ± 0.46^{d}	7.83 ± 0.93^{f}	0.0001
3	-ve treated leaves	47.49 ± 3.69^{b}	3.86 ± 0.81^{d}	18.32 ± 0.83^{ab}	0.0001
4	+ve treated leaves	44.74 ± 4.82^{bc}	7.46 ± 1.02^{a}	16.05 ± 1.09^{d}	0.0001
5	-ve treated fruits	56.55 ± 6.27^{a}	3.62 ± 0.55^{d}	18.6 ± 1.97^{a}	0.0001
6	+ve treated fruits	43.68 ± 7.52^{bc}	6.99 ± 1.02^{a}	16.55 ± 1.13^{cd}	0.0001
7	+ve treated Drug	44.74 ± 4.82^{a}	3.60 ± 0.56^{d}	18.65 ± 1.91^{a}	0.0001

- Data are means ± SD of eight rats in each group.
- Data are expressed as mg/g tissue for glycogen.
- Data are expressed as g/dl for serum protein and albumin as g/l
- Unshared letters between groups are significance value at $p<0.0001$.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) by Co Stat Computer Program.

Table 3: Effect of the methanol extracts of leaves & fruits *Paulownia tomentosa* Thunb.) Steud. on liver enzymes ALT, AST & ALP in control and diabetic rats.

Groups	Treatment	AST	ALT	ALP	P ≤
1	-ve control	48.37 ± 5.34^{ef}	28.95 ± 2.67^{c}	247.03 ± 28.95^{f}	0.0001
2	+ve control	138.06 ± 13.11^{a}	102.79 ±13.78^{a}	1001.4 ± 170.1^{a}	0.0001
3	-ve treated leaves	53.07 ± 9.02^{de}	27.87 ± 2.18^{c}	291.9 ± 46.4^{ef}	0.0001
4	+ve treated leaves	61.65 ± 9.91^{bc}	48.74 ± 4.83^{b}	423.94 ± 46.1^{bc}	0.0001
5	-ve treated fruits	53.74 ± 9.57^{cde}	30.99 ± 3.79^{c}	290.5 ± 43.34^{ef}	0.0001
6	+ve treated fruits	62.24 ± 13.39^{b}	46.80 ± 2.18^{b}	384.07 ± 70.7^{cd}	0.0001
7	+ve treated Drug	57.19 ± 5.70^{bcd}	29.98 ± 4.45^{c}	366.21 ± 44.4^{cde}	0.0001

- Data are means ± SD of eight rats in each group
- Data are expressed as U/ml
- Unshared letters between groups are significance value at p<0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) by Co Stat Computer Program.

Table 4: Effect of methanolic extracts of leaves and fruits of *Paulownia tomentosa* (Thunb.) Steud. on total cholesterol, triglyceride and total lipid in control and diabetic rats.

Groups	Treatment	Total cholesterol	Triacylglyceride	Total lipid	P ≤
1	-ve control	173.67 ± 20.3^{c}	86.47 ± 18.75^{f}	75.34 ± 12.3^{e}	0.0001
2	+ve control	554.09 ± 65.77^{a}	824.8 ± 100.4^{a}	804.19 ± 79.7^{a}	0.0001
3	-ve treated leaves	129.34 ± 13.37^{e}	106.4 ± 17.1**e**^{f}	105.35 ± 14.5^{de}	0.0001
4	+ve treated leaves	192.18 ± 21.7^{bc}	136.79 ± 24.07^{cde}	263.13 ± 56.7^{b}	0.0001
5	-ve treated fruits	142.7 ± 9.35^{de}	103.48 ± 15.6^{ef}	90.11 ± 19.3^{e}	0.0001
6	+ve treated fruits	172.5 ± 30.6^{cd}	150.5 ± 19.15^{c}	130.62 ± 15.6^{d}	0.0001
7	+ve treated Drug	126.9 ± 22.4^{e}	141.88 ± 21.11^{cd}	92.81 ± 12.22^{e}	0.0001

- Data are expressed as mg/dl.
- Data are means ± SD of eight rats in each group.
- Unshared letters between groups are significance value at p<0.0001.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) by Co Stat Computer Program.

Table 5: Effect of methanol leaves and fruits extracts of *Paulownia tomentosa* (Thunb.) Steud. on liver weight, body weight and liver ratio in control and diabetic rats.

Groups	Treatment	Liver wt.	Body wt.	Liver ratio	P ≤
1	-ve control	5.82 ± 0.64^{cde}	187.91 ± 12.27^{ab}	3.11 ± 0.36^{d}	0.0001
2	+ve control	7.83 ± 1.40^{a}	153.37 ± 22.39^{c}	5.15 ± 0.88^{a}	0.0001
3	-ve treated leaves	8.33 ± 0.74^{a}	180.13 ± 5.33^{ab}	4.62 ± 0.39^{ab}	0.0001

4	+ve treated leaves	5.12 ± 0.21^{e}	154.49 ± 7.91^{cd}	3.52 ± 0.11^{d}	0.0001
5	-ve treated fruits	7.44 ± 1.20^{ab}	178.50 ± 8.54^{ab}	4.15 ± 0.54^{bc}	0.0001
6	+ve treated fruits	5.63 ± 0.98^{de}	143.65 ± 10.54^{d}	4.18 ± 0.64^{bc}	0.0001
7	+ve treated Drug	6.45 ± 0.69^{cd}	142.5 ± 9.07^{cd}	4.53 ± 0.45^{b}	0.0001

- Data are means ± SD of eight rats in each group.
- Data are expressed as grams.
- Unshared letters between groups are significance value at $p<0.0001$.
- Statistical analysis is carried out using one way analysis of variance (ANOVA) by Co Stat Computer Program.

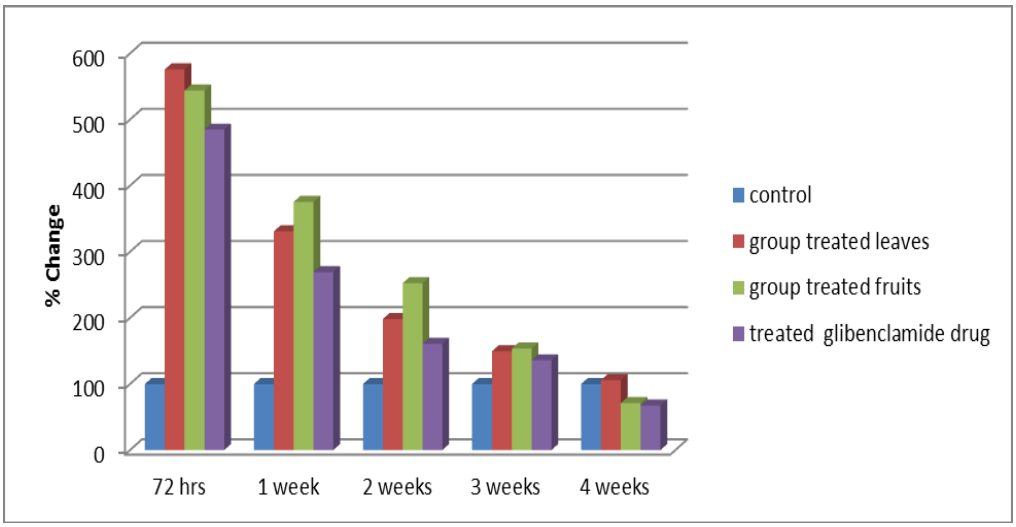


Figure 1

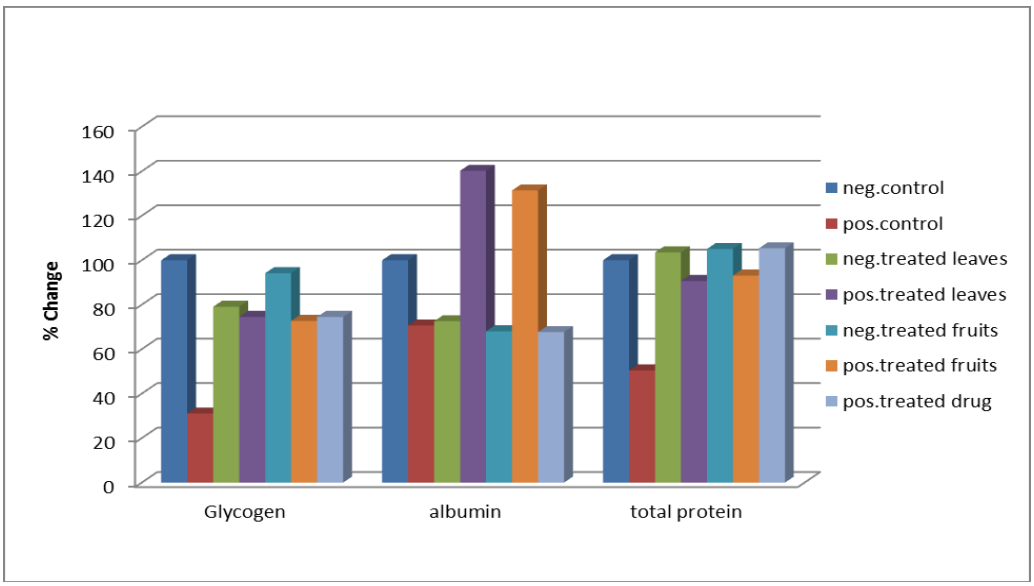
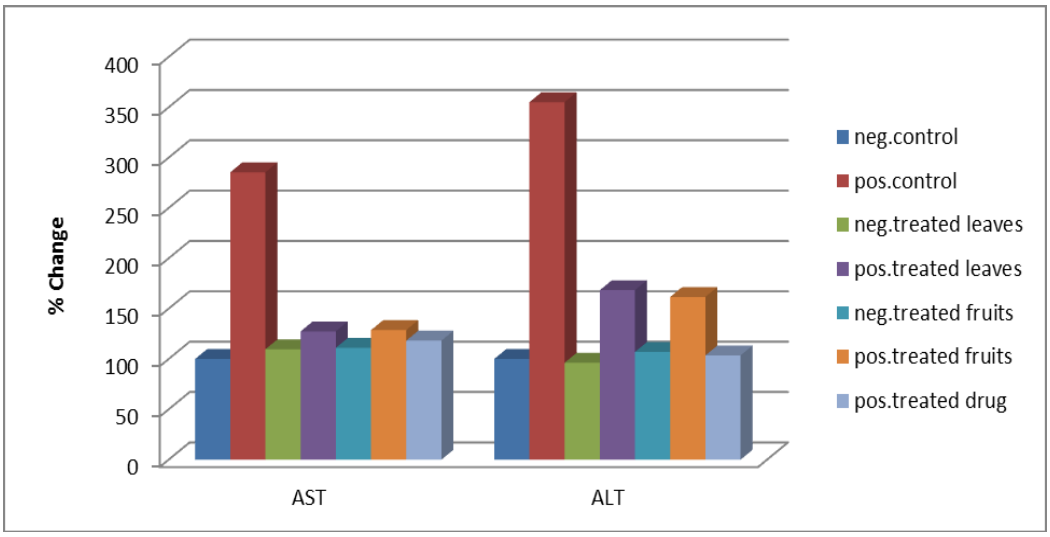


Figure 2



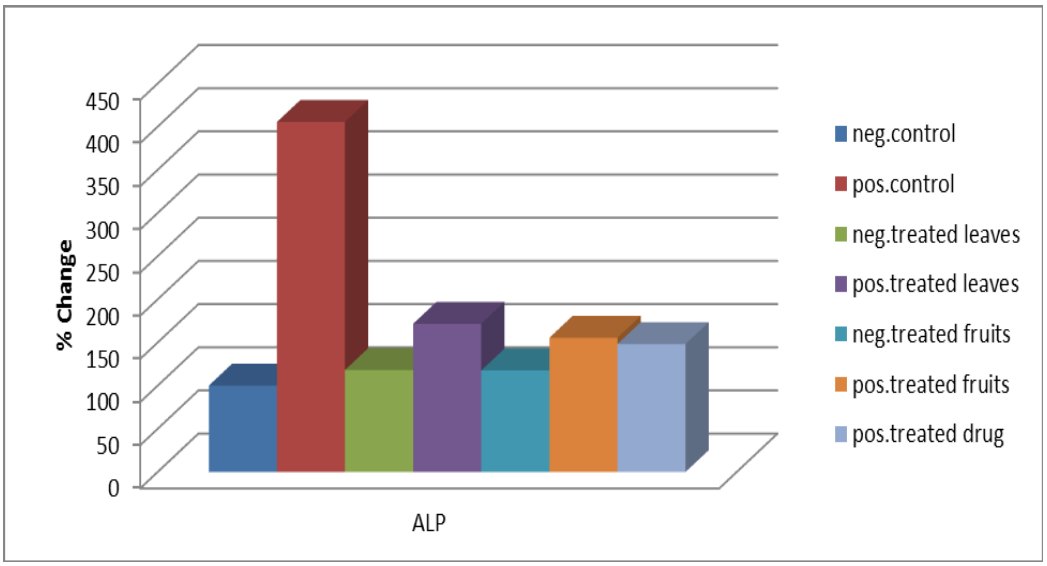


Figure 3

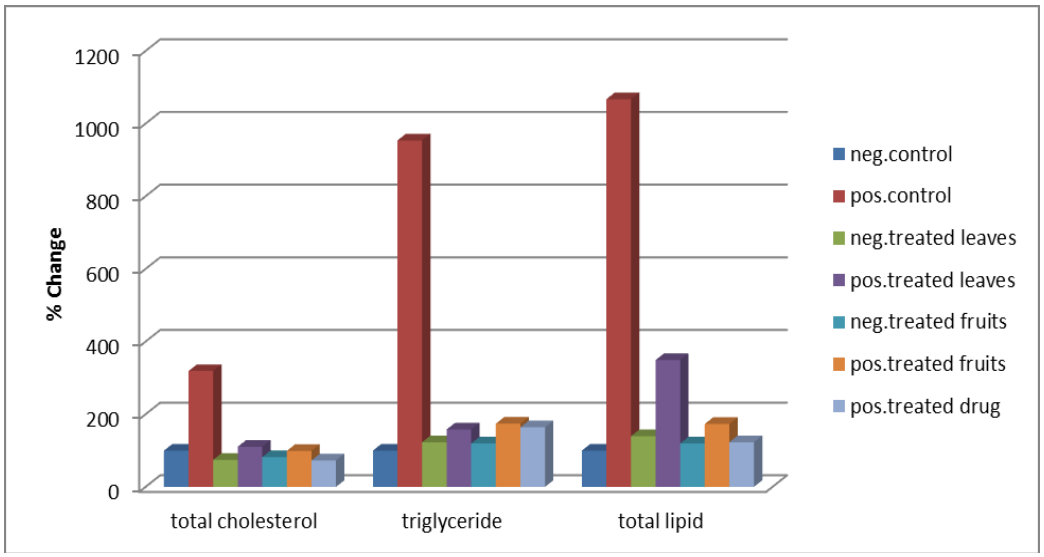


Figure 4

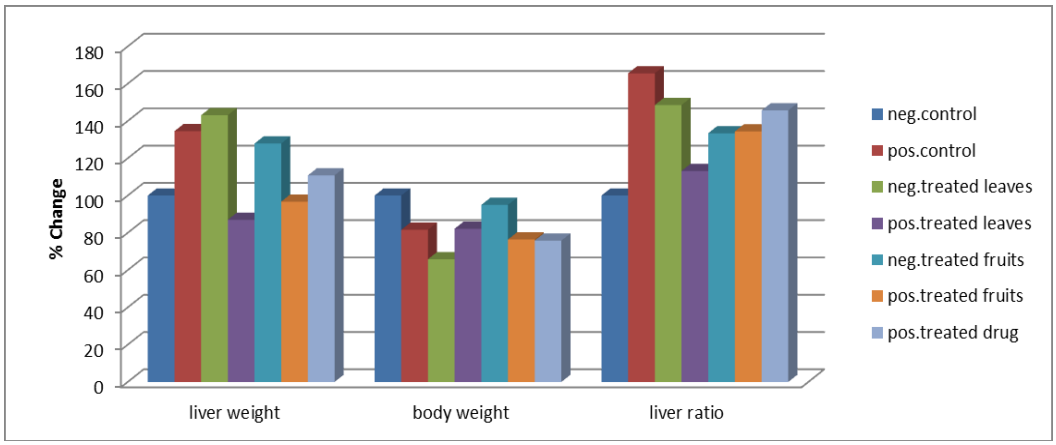


Figure 5

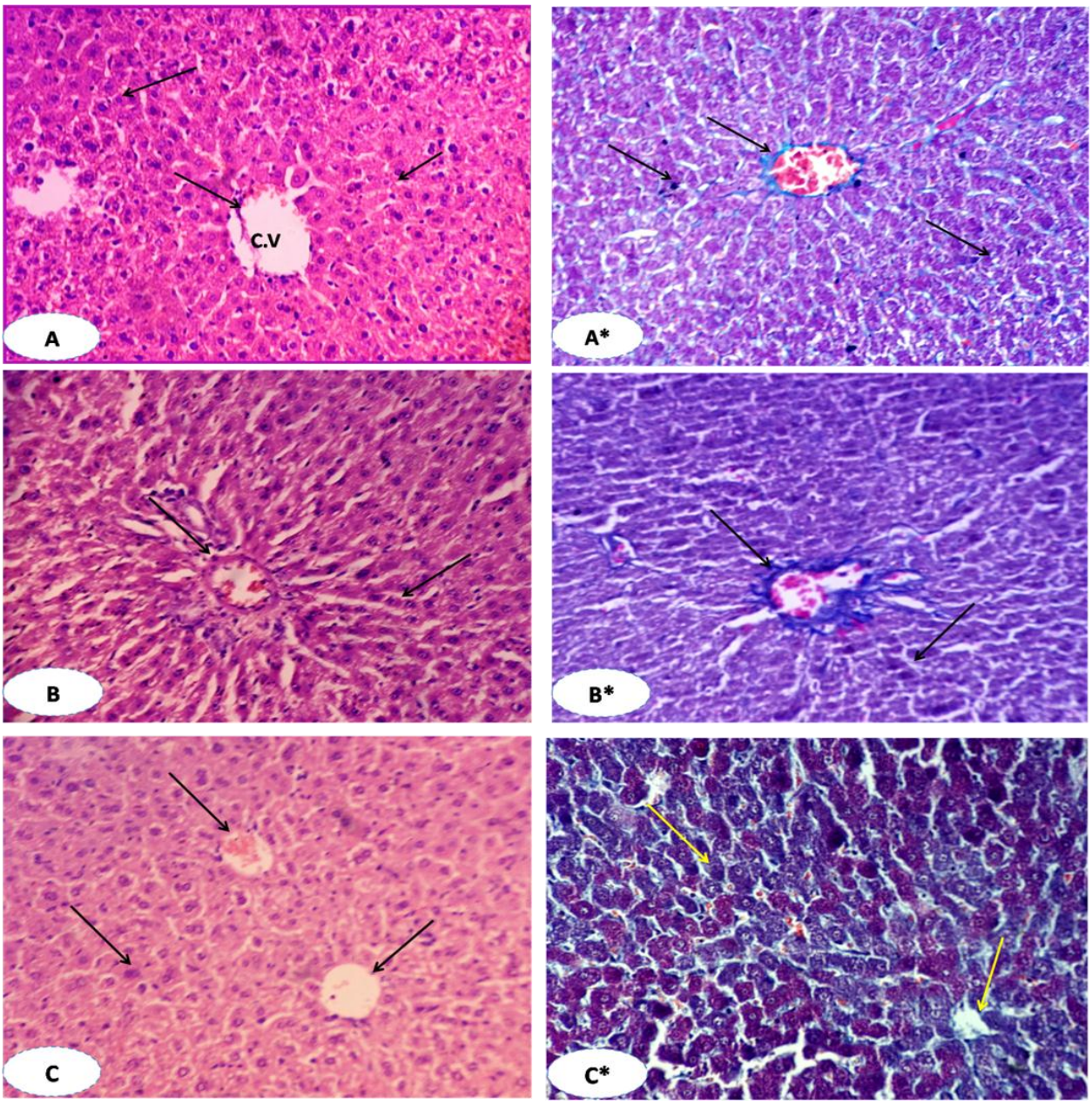


Figure 6

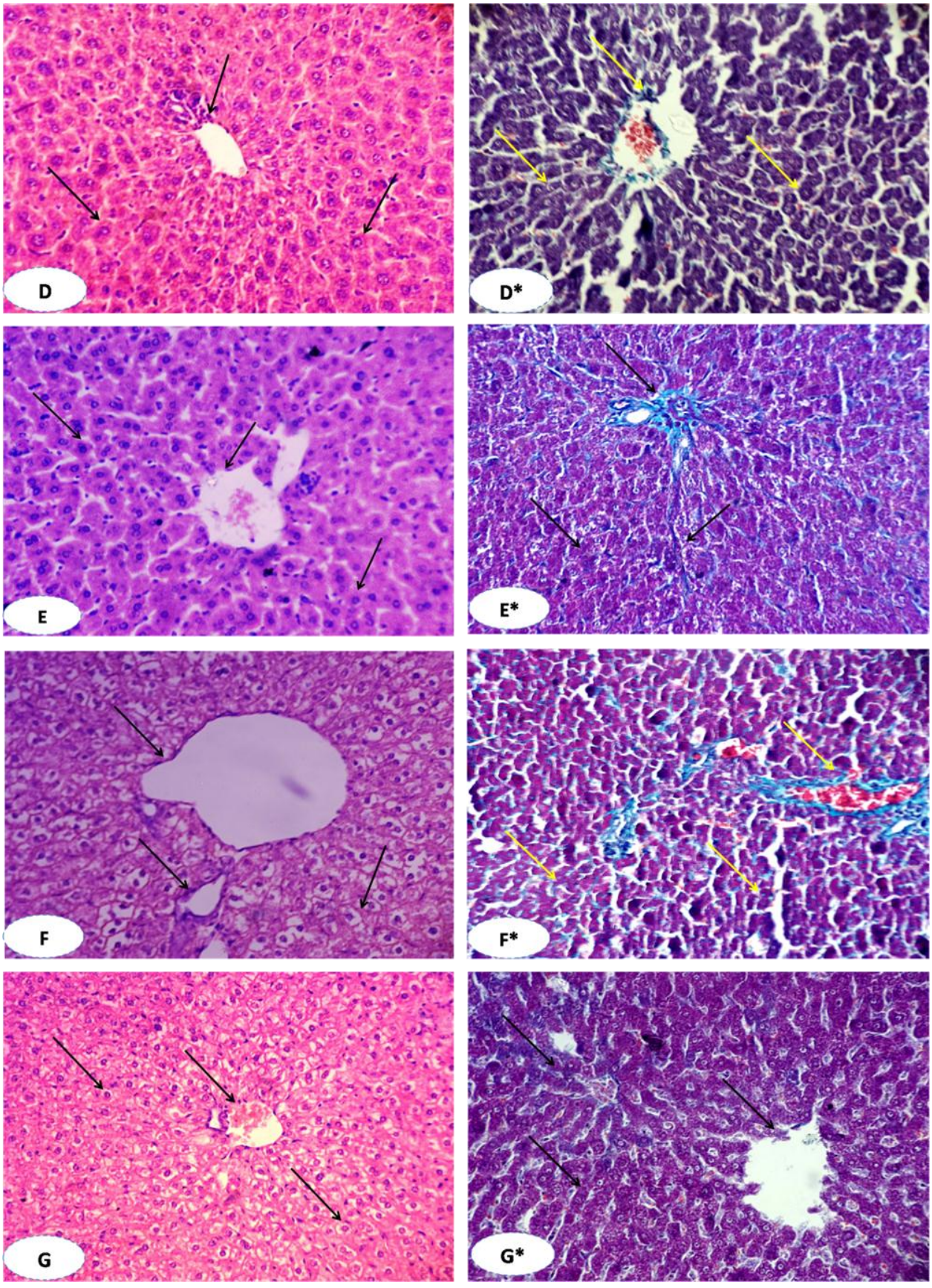


Figure 7

DISCUSSION

This study was undertaken to evaluate the anti-diabetic and hepatoprotective activity of *Paulownia tomentosa* (Thunb.) Steud. In alloxan-induced diabetic rats. The increase in the activities of serum AST and ALT indicated that diabetes may have induced hepatic dysfunction. Ali[19] Supporting the finding that liver cells were necrotized in diabetic patient. An abnormality in glucose metabolism influences lipid metabolism.[20] Clinical knowledge of the level of serum lipids is an important biochemical tool in the toxicity or beneficial effects of foreign compounds. Serum lipids are predominantly resident in body tissue.

The hypoglycemic effect of the leaves and fruits of *Paulownia tomentosa* (Thunb.)Steud., on normoglycemic and hyperglycemic rats was carried out for the first time, where the hypoglycemic activity of methanol fruits of *P. tomentosa* (Thunb.) Steud., was higher than that of leaves in alloxan induced diabetic rats in comparison to control and even more than glibenclamide reference drug during 4 weeks of treatment in this study. As shown in Table 1& Figure1.Therapeutic treatment with leaves and fruits extracts ameliorated the disturbances in glycogen, total protein & albumin, which as a result of alloxan induction and blood glucose elevation. Also the decrease of AST, ALT, and ALP activities observed in the rats treated with leaves or fruits, when compared to control group suggests a possible protective effect of *P. tomentosa* (Thunb.) Steud., treatment in the liver function disturbance condition. According to lipid profile levels there is significant elevation in total cholesterol, triglyceride and total lipid as a result of alloxan induction and blood glucose level elevation. Treatment with leaves and fruits extracts of *P. tomentosa* (Thunb.) Steud., ameliorated this disturbance in which cholesterol level in fruits treated group was close to control. Therapeutic treatment with *P. tomentosa* (Thunb.)Steud. leaves recorded close result to control group in liver & body weights by 12% &17.78% while relative liver weight record 13.18% percent change.

Group given *P. tomentosa* (Thunb.)Steud., fruits ameliorate the effect of alloxan by 3.26, 23.55 and 34.4% respectively. In case of group treated glibenclamide record 10.82, 24.16% and 45.65% respectively with respect to control. The physiological and pathological state of body tissues is highly associated with metabolism and level of serum lipids. In situation where there is high activity of these lipids in body tissues due to oxidative damage, associated with lipid metabolism,[21] the administration of an antioxidant such as polyphenols may ameliorate tissue dysfunction since antioxidant are known to improve tissue integrity. From this study, it was observed that administration of *P. tomentosa* (Thunb.) Steud., MeOH

extracts to alloxan induced diabetic rats, revealed preserving cellular architecture, reappearance and cellular restoration, vascular congestion, re-appearance of hepatocytes with pyknotic nuclei migrating from the sinusoidal lining layer in group 4& 6 as complete regeneration in the liver tissues when compared to non-diabetic and diabetic control groups.

Several studies have shown that oxidative free radicals generated by alloxan administration being the most common etiology for the destruction of vital organs of the body. Liver is one of the organs damaged by free radicals.[22] In the present study iridoid glycosides were isolated from *P. tomentosa* (Thunb.) Steud., Fruits. Iridoids are large group of cyclopenta[c] pyran monoterpenoids, widely distributed in family Scrophulariaceae and in other families like Apocynaceae, Diervillaceae, Lamiaceae, Loganiaceae and Rubiaceae. Recently, more extensive studies revealed that iridoids exhibit a wide range of bioactivities, such as hypoglycaemic, neuroprotective, antiinflammatory, immunomodulatory, hepatoprotective, antioxidant, hypolipidemic and antispasmodic properties were also reported. Bioactivities like antibacterial, anticoagulant and antifungal are highlighted.[10] Also, iridoid glycosides successfully decreased the hyperglycemic state and improve renal function. Iridoid glycosides and polyphenols improved the metabolic parameters associated with the development of diabetic renal damage.[23]

In the present study, three iridoids were isolated from *P. tomentosa* (Thunb.) Steud., Fruits and identified as catalpol, aucubin and paulownioside using different spectral analysis described previously.[24] Catalpolwas isolated previously from the roots of Chinese herb *Rahmannia glutinosa* and showed antihyperglycemic activity in streptozotocin (STZ)-induced diabetic rats. It was found to increase glycogen synthesis in STZ-diabetic rats. The STZ-diabetic rats treated with catalpol increased the glucose utilization in skeletal muscle. These results suggested that catalpol could increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin and might become a suitable adjuvant for the treatment of diabetic patients in the future.[25]

Histopathological examination of liver in diabetic rats showed a marked degeneration of the liver parenchyma correlated with other study.[19, 26] The underlying mechanism might be due to the presence of antioxidant polyphenolic components in the fruit, which could be beneficial in treatment of liver damage in diabetes.[27] Findings indicated possible hepatoprotective and antidiabetic role played by the methanolic extracts of leaves and fruits of *P. tomentosa* (Thunb.) Steud.

CONCLUSION

In conclusion, administration of the methanolic extracts of leaves and fruits of *P. tomentosa* (Thunb.) Steud., Significantly reduced blood glucose levels in alloxan induced diabetic rats. It also showed hypolipidemia as well as hepatoprotective effects. Further identification and isolation of active phytochemical constituents of *P. tomentosa* (Thunb.)Steud., Leaves and fruits and their underlying mechanisms responsible for glucose lowering and hepatoprotective activity may be useful in developing a new drug for the treatment of diabetes complications in human beings in near future.

DISCLOSURE STATEMENT

The authors have nothing to disclose.

Authors' contributions

SAA performed the histological and biochemical experimental Procedures, and participate in wrote the manuscript. EER participated in the experimental procedures and writing of the manuscript. NAI, SSE and MMDM participated in the experimental procedures. All of authors participated in delineated the study, participated in interpreting the data, and edited the manuscript. All of the authors read and approved the final manuscript.

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