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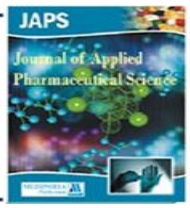

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Chemical constituents of *Argyreia speciosa Fam.* Convolvulaceae and its role against hyperglycemia

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ABSTRACT

The present work on the aerial parts of *Argyreia speciosa* Sweet (Convolvulaceae) has led to the isolation of a new lipid ester (**1**) and four coumarin compounds (**2-5**). The new compound has been characterized as stigmasteryl formate. The coumarins were identified as 7-hydroxy-*6-methoxycoumarin; 6, 7-dihydroxycoumarin; furanocoumarin and scopoletin-7-O-β-*glucopyranoside. Isolation of these constituents was performed using chromatographic techniques. Their structures were established through chemical and spectral analysis. Our aim was to demonstrate the antidiabetic activity of the ethanolic extract obtained from *Argyreia speciosa*, and investigated in normal and alloxan-induced diabetic rats. Glibenclamide was used as a reference drug. Hypoglycemic activity was assessed by a significant reduction in blood glucose and elevation of Liver glycogen content in diabetic rats, a significant decrease in LDH, while glucose-6-phosphatase enzyme showed significant increase. The lipid profile of diabetic group revealed a significant increase in total cholesterol, triglycerides and LDL-C, while HDL-C showed significant decrease. Antioxidant status in liver was determined by measuring the activity of lipid peroxides which revealed a significant rise in it's level, while glutathione level was depleted. *Argyreia speciosa* showed recovery in hyperglycemia and successfully prevented the alterations of other biochemical parameters. Histopathological study of liver, kidney, pancreas and spleen was also carried out to confirm the protection offered by the ethanolic extract. Data showed that *Argyreia speciosa* possessed strong antioxidant activity, remarkable antidiabetogenic effect comparable to glibenclamide, a well known liver protecting herbal formula.

Keywords: Coumarins, Stigmasteryl formate , *Argyreia speciosa*, Alloxan, Enzymes.

INTRODUCTION

Argyreia speciosa (Convolvulaceae), commonly known as elephant creeper is a woody climber distributed mainly in the tropical and subtropical regions, up to an attitude of 300m (Shukla et al., 1999). Phytochemical screening of the plant has shown the presence of lipids (Batra and Mehta, 1985), triterpenes (Bachhav et al., 2009), flavonoids (Ahmad and Jain, 1993), two aryl esters and a coumarin (Srivastava and Shukla, 1998). A lipid ester and a disubstituted tetrahydrofuran were isolated from the roots and characterized as tetradecanyl palmitate and 5, 8-oxidotetracosan-10-one (Rani and Shukla, 1997). Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by pancreas, or by ineffectiveness in the production of insulin. Such a deficiency results in increased concentration of glucose in blood which, in turn damages many body systems and in particular the blood vessels and nerves (Ogihara et al.,2002). Diabetes mellitus is one of the most prevalent chronic diseases in the world. Chronic liver disease and diabetes mellitus can co-exist in a single individual, and the

liver is a frequent site of unrecognized injury in diabetes mellitus patients (Skyler, 2004). The mortality rate in diabetes mellitus patients is generally not due to classical diabetes-related complications (micro and/or macro vascular complications), but rather to an increased risk of hepatocellular failure (Grundy et al., 2005). Antidiabetic medicinal plants are in general known to exert their beneficial effect (s) on diabetes via various modes and mechanisms depending on the phytochemicals and bioactive agents endowed in such plants or a collection of plants (Hemet et al., 2008).

Whereas, the previous reports had postulated the role of *Argyreia spesioca* as antifungal (Shukla et al., 1999), anti-inflammatory (Gokhale et al., 2003), immunomodulatory (Gokhale et al., 2003), hepatoprotective and antioxidant effects (Galani and Patel, 2009), no published data discuss the role of plant aerial part as hypoglycemic and hypolipidemic effect.

Our study was conducted to assess the influence of the ethanolic extract of *A. speciosa* as antidiabetic and antioxidant activities in alloxan-diabetic rats. The isolation, purification and structure elucidation of coumarins present in the aerial parts of the plant were reported for the first time.

MATERIALS AND METHODS

Plant material

The aerials parts of *Argyreia speciosa* Sweet were collected from Suez-Safaga road, red sea costal region. The plant was identified by Prof. I. El Garf, Faculty of Science, Cairo University. Voucher speciment (ASF-2010) was kept at Phytochemistry and Plant Systematic Department, National Research Center as a reference.

Extraction & Isolation

Air dried aerials parts of *Argyreia speciosa* (1000 g) were subjected to successive extractions, using n-hexane, chloroform and methanol. The $CHCl_3$ extract was subjected to silica gel column chromatography (CC) eluted by $CHCl_3$ /ethyl acetate step gradient. The eluted fractions were purified on sephadex LH-20 columns, eluting with CHCl3-MeOH (1:1) to give 12 mg of stigmasteryl formate and 8 mg of 7-hydroxy-6-methoxycoumarin (**2**). The methanol extract was fractionated on a polyamide S6 CC, eluted with water / methanol step gradient. The obtained fractions were subjected to repetitive separation and purification on preparative paper chromatography (PPC) on whatman 3 MM and sephadex LH-20 columns to give 10 mg of psoralen (**3**), 7 mg of 6, 7-dihydroxycoumarin (aesculetin **4**), 13 mg of scopoletin-7-O-glucopyranoside (**5**).

Chemicals

All chemicals used in the present study were, Riedal de Haën (Germany), Fluka (Switzerland), Randox (United Kingdom), and Bio-diagnostic (Egypt).

Animals

Adult female Wistar albino rats weighing 180-200g supplied from the animal house of National Research Center, Dokki, Egypt were used for experimental investigation. The rats were kept in our laboratory under controlled environmental conditions. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt. (Approval no: 09210).

Experimental design

Administration regimen was three times/ week for thirty days. Thirty rats were separated into five groups. Group (1) was non-diabetic healthy group. Group (2) was healthy rats orally administered with *Argeriea speciosa* (200 mg/kg) 3 times /week for 30 days. Diabetic group (3): intraperitoneally injected with alloxan at a single dose of 150 mg/kg b.w. Group 4 was diabetic *Argeriea speciosa* group. Group 5 was diabetic glibenclamide (100 mg/kg) group.

Alloxan-induced diabetes

Diabetes was induced in 12 hours fasted male Wistar albino rats by intraperitoneal administration of aqueous alloxan monohydrate (Sigma-Aldrich Co.) at a single dose of 150 mg/kg body weight dissolved in 0.9% NaCl (Halici et al., 2009). Vehicle group intraperitoneally injected with 0.9% NaCl. 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 intraperitoneally 4–6 h after alloxan injection.

Sample Preparation

Liver tissue was homogenized in 0.9% NaCl (1:9 w/v) for estimation of lactate dehydrogenase (LDH), glucose-6-phosphatase, total protein, and glycogen content. Further dilution of liver homogenate to 20% was prepared for determination of lipid peroxide, glutathione, vitamin C and E. 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 and lipid profile.

Biochemical assay

Glucose in serum was determined colorimetrically at 505 nm by the method of Trinder (1969). Glycogen content was estimated by the method of Nichoals et al. (1956), the green color formed was read at 610 nm against blank. Lactate dehydrogenase: the reduction of NAD coupled with the reduction of tetrazolium salt and the resulted formazan of INT was measured colorimetrically at 503 nm. (Babson & Babson, 1973). Glucose-6-phosphatase (G-6-Pase) was measured colorimetrically at 660 nm through measuring the inorganic phosphorus release (Swanson, 1955). Total protein was estimated by the method of Bradford (1976), where Bovine serum albumin was used as a standard and the colour developed was read colourimetrically at 595 nm. Cholesterol was determined by the method of Meiattini et al. (1987), where free and esterified cholesterol formed a coloured complex that can be measured spectrophotometrically at 500 nm. Cholesterol- HDL (Bustein et al.,1980) was done through precipitation of low density lipoprotein with phosphotungstate and

magnesium ions. The supernatant contains high density lipoprotein was then measured spectrophotometrically at 500 nm. Cholesterol-LDL assayed by the method of Assmann et al. (2004) and based on precipitation of LDL with polyvinylsulphate. Its concentration was calculated from the difference between the serum total cholesterol and cholesterol in the supernatant at 500 nm. Triglycerides originated by means of a coupled reaction that can be measured against blank at 500 nm (Fossati& Prencipe, 1982). Lipid peroxide was determined as malondialdehyde. Its concentration was calculated using the extinction coefficient value $1.56 < 10^5 M^{-1} cm^{-1}$ and read calourimetrically at 535 nm by the method of Buege and Aust (1978). Glutathione was estimated by the method of Moron et al. (1979) using pithiobis-2-nitrobenzoic acid (DTNB) in phosphate buffer. The developed colour was read at 412 nm. The method adapted by Jogata & Dani (1982) was used colourimetrically for estimation of vitamin C using Folin reagent and the developed colour was read at 760 nm. Vitamin E was measured by the colourimetric assay of Angustin et al. (1985). The method based on the oxidation of xylene -extracted tocopherols of the liver homogenate by ferric chloride and the pink complex of ferrous ions is measured at 536 nm.

Histopathological Examination

For light microscopic examination, liver, kidney, spleen and pancreas tissues from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5 μm thickness and stained with haematoxylin and eosin (Hirsch et al., 1997).

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

Five compounds (Figure1) were isolated and identified as stigmasteryl formate (1) ; three coumarin aglycones, 7-hydroxy-6-methoxycoumarin (scopoletin) (2), 6,7-dihydroxycoumarin(aesculetin) (3), furanocoumarin (psoralen) (4) and one coumarin glycoside, 6-methoxycoumarin-7-O-β-glucopyranoside (5) . These compounds, except scopoletin (2), were isolated for the first time from *Argyreia speciosa* plant.

The structures of the isolated compounds were established by means of a UV-visible spectrophotometer (Shimadzu model UV-240 and 2401 PC), MS and NMR spectrometer (Jeol ECA 500),NRC, Egypt as follows:

Stigmasteryl formate (1): Solid, mp:82˚C (12mg), IR: 2919, 2849, 1735, 1620, 1465, 1380, 1284, 1180, 981, 840, 724 cm^{-1}, EIMS m/z (rel. int.) : $440M^+$ (2),412[M^+ -acid moiety]$^+$(20), 397 (2), 394 (5),), 380 [M^+ - 63]$^+$(17), 365 [412- 46]$^+$ (7), 275 [443-168]$^+$ (4), 272 [412- side chain]$^+$ (5), 254 [412- side chain-H_2O]$^+$ (6), 196 (11), 168 (6), 78 (70), 63 (100), 46 (58).^{1}HNMR: δ 0.70 (3H,s,Me-18), 0.82 (6H,d, j=6Hz,Me-26&Me-27), 0.84 (3H,t, j=6Hz,Me-29), 1.02 (3H,s,Me-19), 1.04 (3H,d, j=3Hz,Me-21), 1.30 [br.s, $(CH_2)n$], 4.18 (1H,t, j=6Hz,H-3), 3.63 (1H,t, j=6Hz,H-C=O), 5.35 (1H,m,H-6), 5.15 (2H,m,H-22&H-23).Alkaline hydrolysis of **1:** compound **1** (5mg) was hydrolysed with ethanolic KOH (5%, 5 hrs). The mixture was diluted with water and shaked with $CHCl_3$. The $CHCl_3$ extract afforded stigmasterol. Its identity was confirmed by direct comparison with authentic sample (Co-TLC on silica gel 60G, m.p, IR, MS). 7-hydroxy-6-methoxycoumarin **2** (scopoletin): Needles, m.p. 205˚C (8mg), EIMS m/z (rel. int.) : $192M^+$, U.V: λ_{max} (MeOH) 260, 300, 345. ^{1}HNMR: δ 3.60 (3H,s, OMe-6), 6.16 (1H,d, j=9Hz,H-3), 6.73 (1H,s, H-8), 7.13 (1H,s,H-5), 7.90 (1H,d, j=9Hz,H-4).

6,7-dihydroxycoumarin 3(aesculetin): Needles, (7mg), EIMS m/z (rel. int.): $178M^+$, U.V: λ_{max} (MeOH) 260, 303, 350. ^{1}HNMR: δ 6.11 (1H,d, j=9Hz,H-3), 6.70 (1H,s, H-8), 6.95 (1H,s,H-5), 7.82 (1H,d, j=9Hz,H-4).

Furanocoumarin 4 (psoralen): Needles, (10 mg), EIMS m/z (rel. int): $186M^+$, U.V: λ_{max} (MeOH) 251, 259, 267, 308. ^{1}HNMR: δ 6.20 (1H,d, j=9Hz,H-3), 6.87 (1H,s, H-8), 7.00 (1H,d, j=2.3Hz,H-6 furan), 7.21 (1H,s,H-5), 7.50(1H,d, j=2.3Hz,H-7 furan),8.00(1H,d, j=9Hz,H-4).

Scopoletin-7-O-β-glucopyranoside 5: (13mg), U.V: λ_{max} (MeOH): 255, 289, 339. ^{1}HNMR: δ 3.79(3H,s,OCH_3-6) ,6.19 (1H,d, j=9Hz,H-3), 6.84 (1H,s, H-8),7.20 (1H,s,H-5), 7.90(1H,d, j=9,H-4), 5.00(1H,d, j=7.5Hz,O-glucose-7-position).

Acid hydrolysis of compound **5:** compound **5** was hydrolysed with 1NHCl for 5 hrs. The mixture was diluted with water and shaked with ether. The ethereal extract was chromatographed on P.C.to yield scopoletin (R_f, U.V and M.S). The aqueous extract (containing glucose) was chromatographed on P.C with authentic glucose solution (Co-chromatography, R_f).

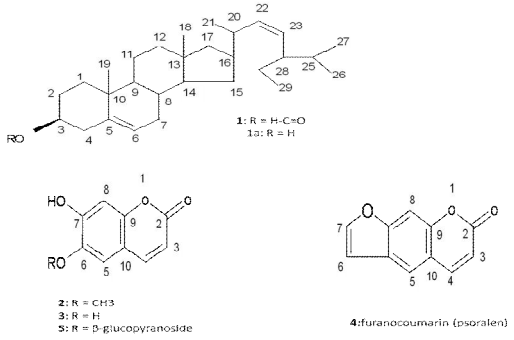

Fig 1 Five chemical constituents of the aerial parts of *Argyreia speciosa* Sweet (Convolvulaceae), lipid ester (**1)** and four coumarin compounds (**2-5**).

Biochemical determinations

Glucose level in diabetic rats showed elevation by 398 % while significant decrease in glycogen content (79.95%) was observed,compared to control group. Lactate dehydrogenase in the present study revealed significant decrease (37.75%) in diabetic rats liver as compared to control, while glucose-6-phosphatase increased by 86.48% and body weight by 8.62%.

Table 1: Glycolysis, gluconeogenesis pathways and body weight in diabetic rats trated with *Argyreia speciosa* ethanol extract and glibenclamide drug..

Parameters	Control	Control-treated plant	Diabetes	Diabetic- treated plant (A)	Diabetic-treated drug (B)	Improvement %	
						(A)	(B)
Glucose	100 ±7.9^{b}	90 ± 7.9^{b}	498 ± 34^{a}	102 ± 13.2^{b}	97.0 ± 11.5^{b}	396	401
Glycogen	8.83 ± 1.1^{a}	6.99 ± 0.82^{b}	1.77 ± 0.58^{c}	7.4 ± 1.11^{b}	9.7 ± 0.9^{a}	63.76	89.8
Lactate dehydrogenase	203.08±11.41^{b}	192.15±6.93^{c}	169.15±6.02^{a}	192.22±16.23^{b}	195.60±11.26^{b}	11.36	13.02
Glucose-6-phosphatase	0.37±0.02^{a}	0.30±0.03^{a}	0.69±0.03^{b}	0.49±0.02^{a}	0.42±0.02^{a}	54.05	72.97
Body weight	150.80±3.83^{a}	144.40±4.20^{ab}	137.80±8.14^{b}	141.40±5.54^{b}	143.40±9.60^{ab}	6.23	4.90

Data are means ± SD of six rats in each group. Data are expressed as Lactate dehydrogenase; Glucose-6-phosphatase µmol/min/mg protein. Body weight in grams. Glucose serum level as g/dl; glycogen as mg/g tissue. Improvement % = mean treated- mean diabetic/ mean control x 100. Unshared superscript letters between groups are the significance values at p< 0.001. Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.

Table 2: Lipid profile in diabetic rats treated with *Argyreia speciosa* ethanol extract and glibenclamide drug.

Parameters	Control	Control-treated plant	Diabetes	Diabetic- treated plant (A)	Diabetic-treated drug (B)	Improvement %	
						(A)	(B)
Total cholesterol	84.80±3.70^{b}	62.00±5.70^{c}	103.00±8.36^{a}	89.40±6.38^{b}	85.80±2.38^{b}	16.03	20.28
HDL-C	42.54±6.65^{b}	51.73±5.02^{a}	32.20±3.23^{c}	38.03±2.29^{b}	40.09±2.32^{b}	14.33	18.54
LDL-C	39.41±4.06^{b}	19.90±2.54^{c}	56.66±8.34^{a}	41.13±4.58^{b}	40.20±4.49^{b}	39.40	41.76
Triglycerides	52.87±3.8^{b}	38.63±5.67^{c}	73.85±13.38^{a}	62.36±8.84^{b}	60.40±5.17^{b}	21.73	25.43

Data are means ± SD of six rats in each group. Data are expressed as mg/dL. Improvement % = mean treated- mean diabetic/ mean control x 100. Unshared superscript letters between groups are the significance values at p < 0.0001. Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.

Table 3: Antioxidants and total protein levels in diabetic rats treated with *Argyreia speciosa* ethanol extract and glibenclamide drug.

Parameters	Control	Control-treated plant	Diabetes	Diabetic- treated plant (A)	Diabetic-treated drug (B)	Improvement %	
						(A)	(B)
Lipid peroxide	0.89±0.16^{d}	1.12±0.07^{e}	2.25±0.29^{a}	1.61±0.23^{b}	1.23±0.07^{c}	71.91	114.60
Glutathione	25.77± 2.11^{a}	17.82±1.87^{c}	19.62±3.44^{bc}	22.57±3.01^{ab}	23.13±1.88^{bc}	11.44	13.62
Vit. C	4.15±0.66^{a}	3.74±0.48^{ab}	3.51±0.24^{ab}	3.92±0.55^{b}	4.03±0.19^{ab}	9.87	12.53
Vit.E	0.24±0.02^{a}	0.20±0.03^{b}	0.16±0.02^{c}	0.21±0.01^{ab}	0.22±0.01^{ab}	20.83	25.00
Total protein	153.0±18.41^{a}	123.20±6.09^{b}	104.80±4.60^{c}	111.20±7.29^{bc}	109.60±3.28^{c}	4.18	3.13

Data are means ± SD of six rats in each group. Data are expressed as µg/mg protein for glutathione, Vit. C and Vit.E, µmol/mg protein for lipid peroxides and µg protein/g liver tissue in total protin. Improvement % = mean treated- mean diabetic/ mean control x 100. Unshared superscript letters between groups are the significance values at p< 0.0001 for lipid peroxides, p< 0.001for glutathione and Vit.E. Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.

In addition, total hepatic protein showed reduction by 31.50% (Table 1 & Fig. 2). LDL-C, triglycerides and total cholesterol levels in diabetic rats recorded significant increase by 43.77, 39.68 and 21.46%, respectively. The recorded inhibition of HDL-C in diabetic rats was24.30% (Table 2 & Fig. 3), while lipid peroxide elevation was 52.80%. Glutathione level showed significant decrease by 23.86%. In case of Vit. C and E significant decreases in diabetic rats by 15.42 and 33.33% were recorded, respectively (Table 3& Fig 4).

Treatment of diabetic rats with ethanol extract of *Argyreia spesioca* recorded improvement percentages in glucose level, glycogen content , LDH, G-6-Pase, total protein and body weight by 396, 63.76, 11.36, 54.05, 4.18, 6.23%, respectively (Tables 1&3). In case of lipid profile, improvement amounted 16.03, 14.33, 29.40 and 31.73% for total cholesterol, HDL-C, LDL-C and TG, respectively (Table 2). In addition, LP, GSH, Vit. C and Vit. E levels were enhaced by 71.91, 11.44, 9.87 and 20.83%, respectively (Table 3).

Glibenclamide drug used in the present work reduced blood glucose by 401%. It also improves glycogen content, LDH and G-6-Pase activities by 89.8, 13.02 and 72.97%, respectively (Table 1). It plays a role in enhances lipid profile by 20.28, 18.54, 41.76 and 25.43% for TC, HDL-C, LDL-C and TG, respectively (Table 2). Improvement in LP, GSH, Vit. C and Vit. E reached 114.60, 13.62, 12.53 and 25.00%, respectively (Table 3).

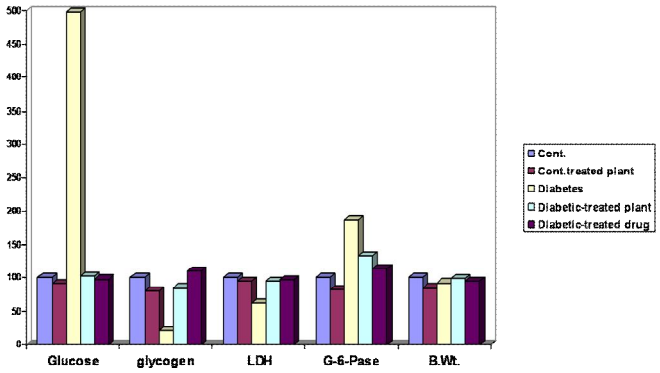

Fig 2 Percentage changes in glucose, glycogen, lactate dehydrogenase, glucose-6-phospatase levels and body weight in the different groups of experimental rats as compared to control group.

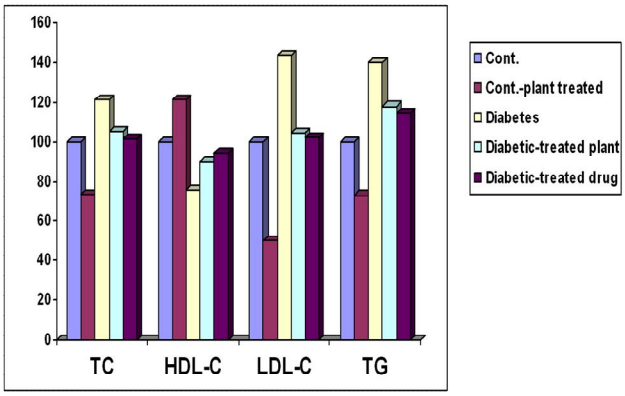

Fig 3 Percentage changes in lipid profile in the different groups of experimental rats as compared to control group.

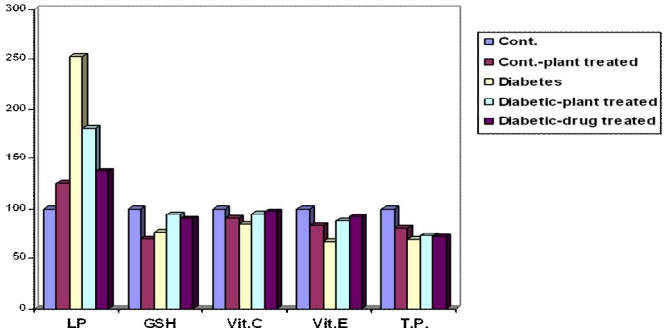

Fig 4 Percentage changes in antioxidants and protein levels in the different groups of experimental rats as compared to control group.

Histopathological studies

Photomicrographs of liver (H&E×200) showed the normal structure of control rats (Fig.5A). Rats treated with alloxan resulted in the damage of liver structure along with disarrangement of hepatic strands (Fig.5B). *Argeria Speciosa* and drug treatment brought back the cellular arrangement around the central vein and reduced necrosis (Fig.5C &D) respectively.

Photomicrographs of kidney (H&E×200) showed the normal renal corpuscle consists of a tuft of capillaries, the glomerulus, surrounded by a double-walled epithelial capsule called Bowman's capsule. Between the two layers of the capsule is the urinary or Bowman's space (Fig.5E). Alloxan dosed rats, showing highly degeneration of glomeruli, Bowman's capsules and associated tubules structure (Fig.5F). *Argeria Speciosa* reversed abnormal histology of renal cortex areas induced by alloxan (Fig.5G). Renal corpuscles were appeared more as normal when treated with glibenclamide drug (Fig.5H). Photomicrographs of pancreas (H&E×200) showed normal acini and normal cellular in the islets of langerhans in the pancreas of normal control (Fig.6 I). Extensive damage to islets of langerhans and reduced dimensions

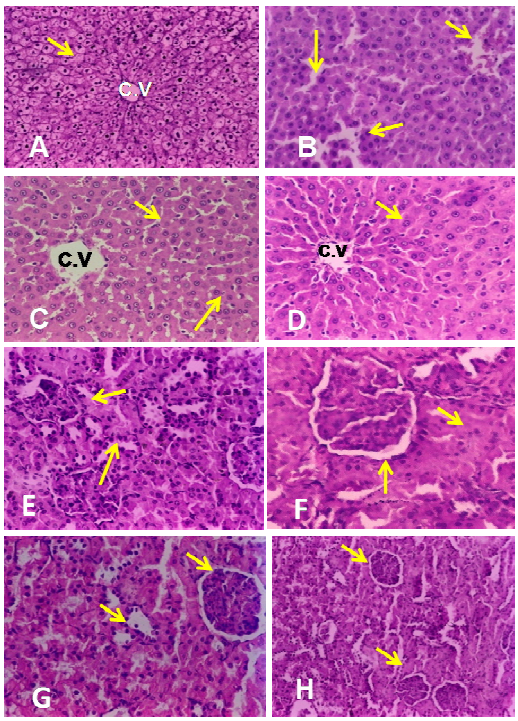

Fig 5: Liver Sections (H&E×200) showed the normal structure **(A)**. Hepatic structure alloxan treated **(B).** *Argeria Speciosa* and drug treatment (**C &D)** respectively. Kidney sections (H&E×200) showed the normal renal corpuscle **(E).** Alloxan dosed rats **(F).** *Argeria Speciosa* reversed abnormal histology feature **(G)**. Renal corpuscles after treated with glibenclamide drug **(H).**

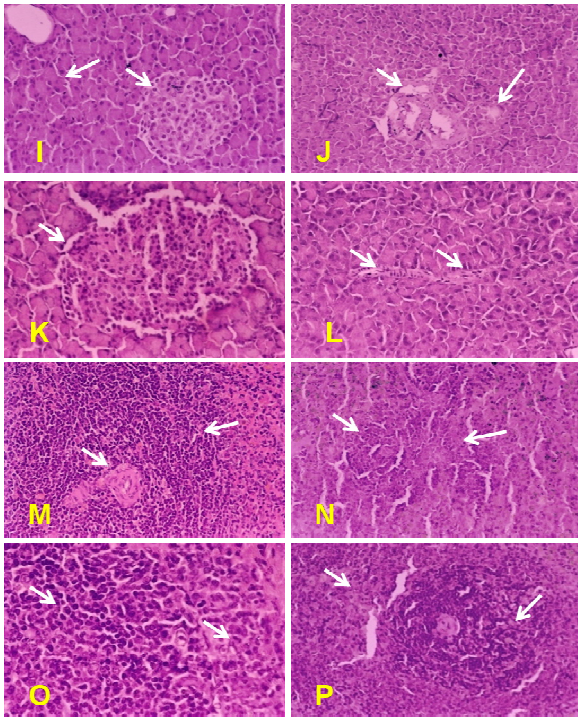

Fig 6: pancreas sections (H&E×200) showed normal normal cellular **(I)**. showed damage to islets of langerhans **(J).** Sections after *Argyreia speciosa* and glibenclamide drug treated **(K&L)** respectively. Spleen sections (H&E×200) showed the normal structure **(M)**. Diabetic rats revealed abnormal histology feature **(N)**. There were pronounced changes in the histological feature after treated with *Argeria Speciosa* and glibenclamide drug **(O&P)** respectively.

of islets (Fig.6 J). Restoration of normal cellular population size of islets with hyperplasia by *Argyreia speciosa* and glibenclamide drug (Fig.6 K&L) respectively. Photomicrographs of spleen (H&E×200) showed the normal structure red pulps of control rats (Fig.6 M). Diabetic rats revealed severe hyperemia in the red pulps and sinusoids with distorted lymphoid nodules and atrophy of the other parts (Fig.6 N). There were pronounced changes in the structure of spleen, no detectable histological differences are observed by the light microscope between control rats and rats supplemented with *Argeria Speciosa* and glibenclamide drug (Fig.6 O&P).

DISCUSSION

Silica gel column chromatography of the $CHCl_3$ extract afforded compouds 1 and 2 while the polyamide column chromatography of the EtOH extract provided compounds 3, 4 and 5. Compound (1), mp 82°, indicated the presence of ester carbonyl (1735 cm^{-1}), methyl (1380 cm^{-1}) and double bond functions (1620 cm^{-1}) in its IR spectrum, it showed positive Lieberman-Burchard test. A molecular ion peak was observed in the mass spectrum of 1 at m/z 440 suggested the molecular formula as $C_{30}H_{48}O_2$. It showed an ion at m/z 412 corresponding to stigmasterol moiety. The characteristic ions in the mass spectrum, suggesting a stigmastane skeleton (Clarck-Lewis and Dainis, 1967) were observed at m/z 272 [412-side chain]$^+$, 254 [412-side chain-H_2O]$^+$.

The 500 MHz ^{1}HNMR spectrum displayed one triplet at 4.18 (1H, t, j=6Hz) for O-CH at 3 position of stigmasteryl moiety, another triplet at 3.63 (1H, t,j=6Hz)assigned for proton of the formate moiety.Other signals corresponding to the remaining protons of stigmasteryl moiety. The presence of ester group was evident by the bands at 1735 cm^{-1}, and δ 4.18. Alkaline hydrolysis of 1 afforded stigmasterol 1a indicating the structure of 1 as stigmasteryl formate, a new compound isolated from the first time from nature. The identity of compound (2) was established as 7-hydroxy-6-methoxycoumarin (scopoletin) by comparison with literature data (mp, UV, MS and ^{1}HNMR) (Shukla et al., 1999).

Compound (3) exhibited ^{1}HNMR spectrum similar to compound (2) except that it lacked the O-CH3 signal at 3.60. It was established as 6,7-dihydroxycoumarin(aesculetin) by comparison with literature data (UV, MS and ^{1}HNMR). Compound (4) was identified as furanocoumarin (psoralen) based on direct comparison with literature data (UV, MS and ^{1}HNMR) (Jiangning et al., 2005). Compound (5) was identified as 6-methoxycoumarin-7-O-β-glucopyranoside. It showed ^{1}HNMR spectrum similar to scopoletin in its aglycone parts and a doublet signal at δ 5.00 j =7.5Hz corresponding to glycosylation at position 7. It gave scopoletin and glucose upon acid hydrolysis (co-chromatography with authentic scopoletin and glucose). Thus compound (5) was identified as 6-methoxycoumarin-7-O-β-glucopyranoside (scopolin), by direct comparison with literature data.

Hyperglycemia can generate a redox imbalance inside the cells, especially in the liver (Gallou et al., 1993). Hepatic glycolysis and gluconeogenesis are the major contributing factors of hyperglycemia in the fasting and postprandial states in type 2 diabetes mellitus (Erion et al., 2009). Decrease of hepatic lactate dehydrogenase in diabetic group was in accordance with Elük et al., (2002), they observed a significant increase in plasma LDH of diabetes mellitus rabbits and attributed this increase in enzyme activity to the influence of insulin on liver and muscle tissue, malnutrition and hepatic anoxia. Raju et al. (2001) also recorded the same observation in inhibition of glycolytic enzymes in hepatic tissue of diabetic rats. Kohl et al. (2002) explained the disturbance in fat metabolism in diabetic rats to the abnormalities in glucose metabolism in the liver and skeletal muscles that stimulates hepatic gluconeogenesis, this gives an additional support of the observed increase in glucose-6-phosphatase (Hemet et al., 2008). The elevation of glucose level in diabetic rats 398% was in agreement with Samuel et al. (2009) who added that fasting hyperglycemia in patients with type 2 diabetes mellitus is associated with increase hepatic gluconeogenesis, which has been ascribed to increase transcriptional expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase genes.

Raju et al. (2001) recorded a 0.7-fold lower body weight in diabetic rats compared to controls. In parallel with this observation, our results revealed a reduction in body weight and total hepatic protein, may be due to disturbance in hepatic metabolism as a result of diabetes, malnutrition and hepatic anoxia which lead to leakage of protein into circulation and excreted to urine which give an additional support to the kidneypathy associated with diabetes (Watanabe et al., 2010).

Type 2 diabetes and familial combined hyperlipidaemia had been observed by many investigators (van Deursen et al., 2008), where elevated hepatic lipase and upstream stimulatory factors gene are responsible for this association. In the present study and in accordance with this fact LDL-C, triglyceride and total cholesterol levels in diabetic rats recorded significant increase. Kohl et al. (2002) confirmed the present results by the observed elevation of low density lipoprotein and triglycerides levels in diabetes mellitus rats. In accordance with the recorded inhibition of HDL-C in diabetic rats (24.30%), Song et al. (2010) showed reduction in HDL-C in insulin-resistant humans.

During oxidative stress, auto-oxidation and the presence of an excess of hydroxyl radical damaged carbohydrates, is well evidenced by the production of thiobarbituric acid-reactive material, reactive carbonyl compound and malonaldehyde (Morelli et al., 2003). These effects are regarded as an important risk factor in the acceleration of chronic diseases including diabetes (Ceriello et al., 2000). Overproduction of free radicals in diabetes could be due to a persistent chronic increase in blood glucose levels. In the present study, glutathione level showed significant decrease in diabetic group, this was in accordance with Parveen et al. (2010) who recorded the same results.

Lipids and proteins, the major structural and functional components of the cell membrane, are the target of oxidative modification by free radicals. There is extensive evidence that lipid peroxidation and protein oxidation lead to loss of membrane integrity, an important factor in acceleration of diabetes (Maritim

et al., 2003). Free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation (Ramesh et al., 2007). Lipid peroxide is frequently used as an index of tissue oxidative stress, in which oxygen interacts with polyunsaturated fatty acids and leads to the formation of lipid products such as malonaldehyde and increase generation of free radicals, which then leads to damage membrane components of the cell, cell necrosis and inflammation (Parveen et al., 2010). This is in accordance with the present finding of elevation lipid peroxide in diabetic rats

Palsamy et al. (2010) observed significant reduction in Vit. C and E in diabetic patients and rats. In accordance of our study Frei et al. (1988) reported that peroxyl radicals are trapped by ascorbate thus, the level of the enzyme and vitamin C decreased during the free radical scavenging process. Also, the reduction of vitamin E in diabetes occurs since the vitamin adds as a soluble antioxidant to protect biological membranes against oxidative stress which leads to distribution of cell function. Also, Sokal et al. (1998) reported that vitamin E protect hepatocytes against lipid peroxidation and toxic injury.

Glibenclamide inhibiting ATP-sensitive potassium channels in pancreatic beta cells, this inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, causes an increase in intracellular calcium in the beta cell and stimulates insulin release (Serrano-Martín et al., 2006). This let us to consider that *Argyreia spesioca* may work by the same mode of action of glibenclamide drug beside it's richen with flavonoids, triterpenes and phenoles that recorded hypoglycemic, hypolipidemic effects.

Liver, kidney, pancreas and spleen histopathology in diabetic rats showed fatty changes surrounding portal triad of liver; enlargement of lining cells of tubules of kidney, fatty infiltration and large area of hemorrhage of pancreas and lymphocyte infiltration of spleen. *Argyreia speciosa* normalized theses observations (Fig.5&6). Results showed that *Argyreia speciosa* has a protective effect on membrane fatty acid composition of liver and kidney as supported by antioxidant and antihyperlipidemic effects. It has evidence to improve histopathological changes, hepatic and nephritic markers indicating recovery from the risk of diabetic complications.

Management of diabetes without any side effect is still a challenge; this has led to an increasing demand for natural products with anti-diabetic activity and no side effects (Kameswara et al., 1999). It has been suggested that bioactive compounds from plants sources having anti-hyperglycemic activities might act by several mechanisms such as stimulating insulin secretion, increasing repair or proliferation of β-cells and enhancing the effects of insulin and adrenalin (Adeyemi et al., 2009).

This is the first study to show the hypoglycemic activity of *Argyreia spesioca*, on rats alloxan-induced diabetes. The antidiabetic activity of the ethanol extract was evaluated by measuring glucose, glycogen, LDH and G6Pase levels. It resembles glibenclamide drug action, as it reduced blood glucose level.

The antioxidant activity of *Argyreia spesioca*, might be due to the presence of phenolic compounds such as scopoletin, aesculetin and scopolin, which are believed to neutralize the free radicals in lipid chain by donating a hydrogen atom, usually from phenolic hydroxyl groups (Larson, 1988), which in turn converts phenolic groups into free radicals that do not initiate further oxidation of lipids. *Argyreia spesioca* significantly increased the hepatic glutathione. These results suggest that a significantly higher content of glutathione in liver would offer the tissue a better protection against an oxidative stress, thus indicating the hepatoprotective effect of the extract. In addition, *Argyreia spesioca* has a favorable effect to inhibit the histopathological changes of the liver, kidney, pancreas and spleen, in alloxan induced diabetes.

Therefore, *Argyreia spesioca* may provide new alternatives for the clinical management of diabetes and the consumption of *A. spesioca* can prevent hyperglycemic complications associated with this disease.

CONCLUSION

These results provide confirmatory evidence of oxidative stress in diabetic liver and kidney and point towards the possible anti-oxidative mechanism being responsible for the hepato-renal protective actions of *A. spesioca Fam*.

Authors' statements

Competing interests: The authors declare no conflict of interest.

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