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Research Article

BIOINFORMATICS: INFLAMMATORY CYTOKINES AND ATTENUATION OF DIABETES HYPERCHOLESTEROLEMIA-INDUCED RENAL INJURY USING MORNING GLORY AND NECKLACE POD EXTRACTS

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ABSTRACT

Objective: The present research in bioinformatics focuses on pharmacological effects of morning glory and necklace pod ethanolic extracts (MGE and NPE) on some biochemical parameters in high fat diet-induced hypercholesterolemia and streptozotocin-induced hyperglycemia in rats.

Methods: Compared to atorvastatin; an anti-hypercholesterolemic (HC) and glibenclamide; an antidiabetic drug. Endothelium activation markers of soluble vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 were determined using enzyme-linked immunosorbent assay. Creatinine, urea, and inflammatory biomarkers; C-reactive protein (CRP) and pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and interleukin (IL)-10 levels were also measured in serum of different therapeutic groups.

Results: Significant decrease in ICAM-1 level with MGE and NPE supplemented to normal rats as compared to untreated control with percentages decrease 17.80 and 12.00% was observed. Insignificant change was detected in VCAM-1 level. Profound amelioration in CRP, total urea and creatinine levels by NPE treatment. Creatinine, urea, CRP, and TNF-α level were significantly increased in hyperglycemic (HG)-HC rats. However, IL-10 level showed a significant decrease. Meanwhile, histopathological investigation of the kidney and heart was carried out. Image recognition system for kidney and heart images was developed to diagnose their diseases. Tested extract attenuated creatinine, urea, CRP, and TNF-α level. Hyperglycemia and hypercholesterolemia linked kidney disorders were relieved.

Conclusion: *In vivo* oral administration with each extract declared suppression of cytokines mediated inflammation, vascular function leading to infiltration reduction of renal macrophage together with lowering in kidney indices and ameliorate renal tissues architectures in HG-HC rats.

Keywords: Morning glory, Necklace pod, Inflammatory, Cell adhesion, Kidney function, Statistic, Image recognition.

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INTRODUCTION

Chronic inflammation leading to immune system activation is deeply involved in the pathogenesis of diabetes [1]. It was reported that diabetic atherosclerosis is a disease of hypercholesterolemia associated with an inflammatory status involving several mediators as C-reactive protein (CRP), cytokines, tumor necrosis factor alpha (TNF-α), and interleukin (IL)-10 [2]. It was found that the treatment of monocytes with a high concentration of glucose leads to the release of several inflammatory cytokines, chemokines, and mediators. Many of them are regulated by the proinflammatory cytokine and nuclear factor-kappa β (NF-κβ) [3]. The activation of inflammatory cytokines, TNF-α and CRP in Type 2 diabetes may be relying on cytokines that inhibit insulin signaling, and glucose transport protein (GLUT)-4 [1], while IL-10 is involved in β-cell hyperactivity [4]. It was found that inflammation and the impairment of vascular function stimulated by risk factors such as hypercholesterolemia, hypertension, and smoking or diabetes are closely related. Irrespective of its cause, inflammation connected with cardiomyopathy, although, there are debatable findings referring inflammation-related atherosclerosis [5]. The choice biomarkers-related inflammation is found to be connected with various infective procedures in atherogenesis; vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and anti-inflammatory IL-10.

Medicinal plant extracts often consist of complex mixtures of active primary and secondary metabolites and their uses in medicine, against inflammation, and kidney diseases are still based on knowledge from traditional medicinal practice [4,5].

The genera of *Sophora* and *Ipomoea* are a prolific source of structurally diverse bioactive metabolites with interesting activities [6,7]. The chemical review on necklace pod ([NP], *Sophora tomentosa*, reveals the presence of alkaloids, polysaccharides, isoprenylated flavonoids, isoflavonones, flavones, flavonols, and their glycosides. Such compounds have been reported to have diverse bioactivities including antioxidant and immunoregulatory [6,8]. It was reported that the extracts of *S. tonkinensis* and *Sophora flavescens* significantly control the levels of insulin, through stimulation of glucose transporter 4 (GLUT-4) which is attenuated by AMP-activated protein kinase (AMPK) pathway while being non-toxic for mice [9].

Many phytochemical studies [7], declared that *Ipomoea* contains many bioactive secondary metabolites, such as phenolic acids, flavonoids, organic acids, and anthocyanins, with promising effects in ameliorating different disorders such as antinociceptive, antioxidative, and anti-inflammatory effects. The isolated anthocyanins decreased the atherosclerotic progress, ameliorated oxidative stress and endothelial dysfunction in mice [10].

To the last of our knowledge, the attenuation of diabetes hypercholesterolemia-induced renal injury by the extracts of morning glory (MG) nor NP have never been discussed before. *In vivo*, this research

aims to statistically investigate the action of each extract on some biochemical parameters in high fat diet-induced hypercholesterolemia, and streptozotocin (STZ)-induced hyperglycemia in rats compared to atorvastatin; an anti-hypercholesterolemic (HC) and glibenclamide; an antidiabetic drug.

In bioinformatics [11,12], a new developed image recognition system was developed to investigate renal and heart injuries images. Statistical system for extracting statistical information from the blood tests data of the rats was illustrated in another article [3].

MATERIALS AND METHODS

Plant materials

Aerial parts of the MG (*Ipomoea tricolor* Cav., Family: Convolvulaceae) and the NP (*S. tomentosa* L., Family: Fabaceae*)* (Leguminosae) were collected in May 2014 from El Qanater, Qalyubia Governorate. The identification of the plants material was performed by Treas Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex-director of Orman Garden, Giza, Egypt. A voucher sample is kept in the herbarium of National Research Centre (NRC), Egypt.

Preparation of MGE and NPE extracts

Each dried powdered aerial parts (500 mg) of MG and NP was, separately, extracted by cold percolation in ethanol for 24 hrs. Each extract was, separately, recovered and ethanol was further added to the plant material, and the extraction was continued. The process was repeated 3 times and pooled together. The extracts were, separately, concentrated under reduced pressure (22-26 mmHg) at 40°C to yield brown to greenish brown oily crude residue; 14.1 and 23.1 g of MGE and NPE, respectively. They were maintained in dark glass container, at (–4°C) until use.

Chemicals and apparatus

Reference drugs atorvastatin and glibenclamide were purchased from Novartis Pharmaceuticals Chemical Company, Cairo, Egypt. Enzyme-linked immunosorbent assay (ELISA) kits were provided by Invitrogen (U.S.A.) for IL-10, Eiaab (U.S.A.) for VCAM-1, ICAM-1, TNF-α, CRP, and IL-10. All other chemicals and reagents were purchased from Biodiagnostic Company for diagnostic and research reagents, Cairo, Egypt. Rotary evaporator (Heidolph, Germany), electric grinder, centrifugation Eppendorf S810R® (Germany), automatic biochemical analyzer Olympus AU400® (USA), and Accu-chek active® blood glucose meter device with its stripes (Germany) were used.

Experimental design

Animals

A total of 70 adult male Wister rats (16-20 weeks age days; 150±10 g) were selected for this study. They were provided by the Animal House of the NRC, Cairo, Egypt. All animals were kept in a controlled environment of air and temperature (26-29°C), with a fixed light/dark cycle for 2 weeks to acclimatize. They were allowed free access to water and diet *ad libitum*. This study had been approved by the Ethical Committee of the NRC, Egypt, which provided that the animals will not suffer at any stage of the experiment. The rats were randomly divided into seven groups of 10 animals each.

Induction of hypercholesterolemia

Hypercholesterolemia was induced in rats by feeding high fat/high cholesterol diet. Cholesterol was orally administrated at a dose of 30 mg/0.3 ml olive oil/l kg animal 5 times a week for 12 consecutive weeks [13]. Lard fat was mixed with normal diet (1 kg of animal lard was added to 5 kg of normal diet). The hypercholesterolemia was determined by measuring the lipid profile including total cholesterol, low-density lipoprotein (LDL-c), high-density lipoprotein, and triglycerides. The HC rats were only used (data not shown).

Routs of administration

HC rats received an oral dose of 2 mg/kg body weight (BW) of plants extract dissolved in distilled water of the anti-HC reference drug; atorvastatin dissolved in distilled water orally by gastric intubation for 4 weeks.

Induction of hyperglycemia

Hyperglycemia was induced by intraperitoneal injection of a single dose of streptozocin (STZ, 45 mg/kg BW dissolved in 0.01 M citrate buffer) immediately before use [5,13]. After injection, animals had free access for food, water and were given 5% glucose solution to drink overnight to encounter hypoglycemic shock. Animals were checked daily for the presence of glycosuria [14]. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and blood sugar was determined (≥300 mg/dL).

Dose regimens and route of administration

The rats were divided into the following groups; Group 1: Normal healthy control rats, Groups 2 and 3: Normal rats treated orally with 250 mg/kg BW of crude extract of MGE and NPE, respectively, for 30 consecutive days. Group 4: Rats were considered as hyperglycemic (HG)-HC which classified as follows: Groups 5 and 6: HG-HC group rats oral administered 250 mg/kg BW crude ethanol extracts of MGE and NPE for 30 days, respectively. Group 7: HG-HC rats orally administered 2 mg/kg BW of the anti-HC reference drug; atorvastatin [4,14] and the anti-HG glibenclamide reference drug at the dose of 10 mg/kg BW daily for 30 days [13,14].

Blood collection and tissue sampling

By the end of the experiment (4 weeks), the animals of different groups were fasted for 12 hrs, weighted then blood samples were collected from the sublingual vein, then left to coagulate at room temperature and centrifuged at 3000 rpm for 15 minutes. The clear, non-hemolyzed, sera were quickly removed and kept at −80°C till used for biochemical investigations, kidney function parameters, inflammatory markers, and CAMs. Then, animals sacrificed using diethyl ether anesthesia. Kidney and heart tissue were rapidly excised and accurately weighed; for subsequent histopathological investigation.

Biochemical examination

CAMs

Rat soluble VCAM-1 concentration and ICAM-1 were determined using ELISA.

Atherogenic markers

In vivo quantitative measurements of IL-10, were performed by ELISA; a sandwich enzyme Immunoassay.

Inflammatory markers

In vivo quantitative measurements of the inflammatory mediator factors, tumor necrosis factor-α (TNF-α), and CRP were estimated using diagnostic ELISA kits.

The histological study

At the end of the study, the animals were sacrificed, and their kidneys and hearts were removed, then small slices of them were fixed in 10% formalin solutions and processed routinely. Sections of 5 μL thickness were cut and stained by hematoxylin and eosin (H and E) for histological examination.

Image recognition subsystem (IRS) for rats' images

We can describe the components of image recognition and classification subsystem (IRS) [15] and the process was carried out using the following illustration (Fig. 1).

IRS input data

The main dataset contains 48 images of rat' organs (kidney and heart) magnified 200 times used for evaluating IRS. The photomicrograph images were captured by Egyptian NRC experts.

IRS subsystem development

In developing IRS, the current study passed through main steps of image recognition: Segmentation, feature extraction, and classification using digital image processing program. This procedure and the image analysis were accomplished using ImageJ v1.43u [16]. The program showed the following:

1. Preprocessing and segmentation of the images: In which, we enhance image quality and transform images in a proper format to deal with by image processing program.
2. Feature extraction and measurement: In which, the study discovered the most proper features and characteristics of the images.
3. Image classification: In which, the study combined the results of the prior measurements. The object belongs to a class of images, according to certain appurtenance mathematical criteria.

RESULTS

The effect of MGE and NPE on endothelial dysfunction (CAMs)

Table 1 showed the effect of MGE and NPE extracts on adhesion molecules in HG associated with HC module (HG-HC group) and in different therapeutic groups. The present results demonstrate a significant decrease in ICAM-1 level with MGE and NPE supplemented to normal rats as compared to untreated control by 17.80 and 12.00%, while insignificant change was detected in VCAM-1 level. HG-HC rats showed a significant increase in both VCAM-1 and ICAM-1 levels by 80.43 and 40.27%, respectively. ICAM-1 level was significantly decreased in HG-HC treated rats with MGE, NPE, and drugs as compared to control, by percentages of improvement of 68.91, 29.93, and 33.22%, respectively. VCAM-1 showed an insignificant decrease with MGE, NPE, and with amelioration percentages 31.25, 24.30, and 27.43%, respectively. From results in Table 1, the highest amelioration percentage in ICAM-1 and VCAM-1 levels was obtained with MGE, followed by drugs and finally NPE.

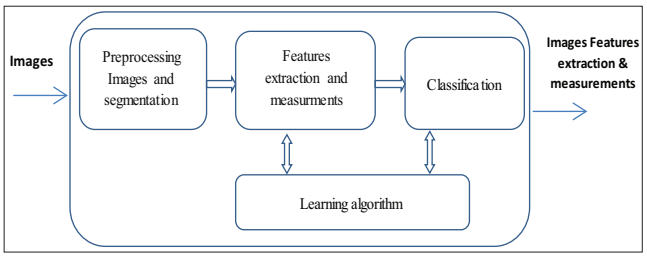

Fig. 1: Image recognition and classification process diagram

The anti-inflammatory effect of MGE and NPE

Table 2 indicated that, insignificant change in inflammatory cytokines markers; IL-10, CRP, and TNF-α in normal rat supplemented with MGE and NPE as compared to normal untreated control. HG-HC showed a significant increase in CRP and TNF-α by 85.93 and 50.99%, respectively. While a significant decrease in IL-10 was noticed in HC-HG with percentage decrease reached to 49.86% as compared to normal control rat was observed. Treatment of HG-HC rats with MGE provided insignificant change in IL-10 and TNF-α level while significant increases in CRP level as compared to normal control with percentage of amelioration 62.93. Furthermore, treatment with NPE showed the same effect of MGE as it showed insignificant change in IL-10 and TNF-α levels, while significant increase in CRP level with amelioration percentage 75.36%. In addition, treatment of HG-HC rats with hypoglycemic glibenclamide and anti-HC atorvastatin showed the same results of MGE and NPE treatments as they declared insignificant change in both IL-10 and TNF-α as compared to normal control rats, while, significant increase in CRP level with percentage of improvement 52.49%.

The effect of MGE and NPE on kidney functions

It is noticeable from Table 3 that there is no significant difference in the kidney function indices in control rats treated with each of the extract comparing to untreated normal one. With respect to the present results, HG-HC rats demonstrated marked increase in kidney function biomarkers with percentage 102.22 and 177.41%, respectively, for urea and creatinine. Urea levels were returned to a normal value, showed insignificant change in HG-HC treated groups with MGE, NPE as well as standard drugs. While creatinine levels showed insignificant change with NPE and reference drugs while significant increase (63.22%) with MGE with amelioration percentage of 114.19 (Table 3).

Histopathological investigations of the kidney

In Fig. 2a photomicrograph of control kidney section ×200 showed renal cortex of renal corpuscle with normal glomerulus. The kidney tissue section of control group treated with *S. tomentosa* (Fig. 2b) showed no change in the kidney cells. Fig. 3 showed a photomicrograph of control kidney section treated with *I. tricolor*. Fig. 3a showed no change in normal cells structure of kidney cells. While section of HC+HG group (Fig. 3b) showed a destructed epithelial lining of distal convoluted tubules stained with (H and E) ×400. Fig. 4 showed another photomicrograph of kidney tissue section of HC+HG group (Fig. 4a) revealed proximal convoluted tubules and destructed epithelial lining ×400. The kidney tissue section of HC+HG group treated with *S. tomentosa* (Fig. 4b), showed normal pattern of proximal convoluted. Fig. 5 is the photomicrograph of HC+HG group kidney

Table 1: Comparative effects of MGE and the NPE supplementation on adhesion molecules, ICAM-1 and VCAM-1 in different therapeutic groups

Groups	Parameters	ICAM-1	VCAM-1
Negative control	Mean±SD	6.08±0.23a	2.88±0.24a
Negative treated with MGE	Mean±SD	5.18±0.25b	2.43±0.41ab
	% Change to control	17.80	15.625
Negative treated with NPE	Mean±SD	5.35±0.33b	2.85±0.23a
	% Change to control	12.00	1.04
HG-HC	Mean±SD	10.97±0.37c	4.72±0.17c
	% Change to control	80.43	40.27
HG-HC treated with MGE	Mean±SD	6.78±0.31b	3.62±0.36ab
	% Change to control	21.38	9.027
	% of improvement	68.91	31.25
HG-HC treated with NPE	Mean±SD	7.09±0.28b	3.42±0.34ab
	% Change to control	21.21	15.97
	% of improvement	29.93	24.30
HG-HC treated with glibenclamide and atorvastatin	Mean±SD	5.99±0.46b	2.51±0.095ab
	% Change to control	17.92	12.84
	% of improvement	33.22	27.43

Adhesion molecules are expressed in ηg/ml. Data presented as mean±SD, n=10. Statistical analysis is performed using costate and SPSS computer programs (Version 8). Unshared letters (a, b, and c) are significant at p≤0.05. SD: Standard deviation, HG-HC: Hyperglycemia-hypercholesterolemia, MGE: Morning glory extract, NPE: Necklace pod extract, ICAM-1: Intercellular adhesion molecule-1, VCAM-1: Vascular cell adhesion molecule-1

Table 2: Comparative effects of MGE and NPE supplementation on inflammatory markers in control and different therapeutic groups

Groups	Parameters	IL-10	CRP	TNF-α
Negative control	Mean±SD	87.52±1.73cd	29.69±1.24e	49.24±0.33b
Negative treated with MGE	Mean±SD	83.2±3.23cd	29.62±0.94e	44±2.82c
	% Change to control	4.93	0.23	10.64
Negative treated with NPE	Mean±SD	82.07±6.169d	30.0.27±0.81e	42.83±0.99c
	% Change to control	6.22	1.135	1.04
HG-HC	Mean±SD	43.88±6.18a	55.205±2.68a	74.35±4.75a
	% Change to control	49.86	85.93	50.99
HG-HC treated with MGE	Mean±SD	75.60±4.26c	36.52±0.55c	52.79±2.28b
	% Change to control	9.23	23.00	7.209
	% of improvement	36.24	62.93	43.78
HG-HC treated with NPE	Mean±SD	74.17±4.33c	32.83±1.45d	51.75±3.59b
	% Change to control	13.61	10.57	5.097
	% of improvement	34.61	75.36	45.89
HG-HC treated with glibenclamide and atorvastatin	Mean±SD	77.97±12.32c	39.62±1.49b	53.83±3.75b
	% Change to control	10.91	33.44	9.32
	% of improvement	38.95	52.49	41.67

CRP is expressed in ηg/ml, TNF-α and IL-10 are expressed in ρg/ml, Data presented as mean±SD, n=10. Statistical analysis is performed using costate and SPSS computer programs (Version 7). Unshared letters (a, b, c, d, and e) are significant at p≤0.05. SD: Standard deviation, HG-HC: Hyperglycemia-hypercholesterolemia, MGE: Morning glory extract, NPE: Necklace pod extract, CRP: C-reactive protein, TNF-α: Tumor necrosis factor alpha, IL-10: Interleukin-10

Table 3: Comparative effects of MGE and NPE supplementation on kidney function in normal and different therapeutic groups

Groups	Parameters	Urea	Creatinine
Negative control	Mean±SD	22.5±2.50bc	0.155±0.013c
Negative treated with MGE	Mean±SD	23.5±2.19c	0.16±0.033c
	% Change to control	4.44	3.22
Negative treated with NPE	Mean±SD	21.00±2.83bc	0.14±0.021c
	% Change to control	6.66	9.67
HG-HC	Mean±SD	45.50±3.81a	0.43±0.021a
	% Change to control	102.22	177.41
HG-HC treated with MGE	Mean±SD	26.00±3.96b	0.253±0.10b
	% Change to control	15.55	63.22
	% of improvement	86.66	114.19
HG-HC treated with NPE	Mean±SD	25.00±1.34b	0.17±0.008c
	% Change to control	11.59	9.67
	% of improvement	91.11	167.74
HG-HC treated with glibenclamide and atorvastatin	Mean±SD	22.34±2.22bc	0.17±0.018c
	% Change to control	0.711	9.67
	% of improvement	102.93	167.74

Creatinine and total urea levels are expressed in mg/dl. Data presented as mean±SD, n=10. Statistical analysis is performed using costate and SPSS computer programs (Version 7). Unshared letters (a, b, and c) are significant at p≤0.05. SD: Standard deviation, HG-HC: Hyperglycemia-hypercholesterolemia, MGE: Morning glory extract, NPE: Necklace pod extract

section treated with *I. tricolor* (Fig. 5a), and it showed cells structure enhancement normal pattern of proximal convoluted. The kidney tissue section of HC+HG group treated with atorvastatin and glibenclamide (Fig. 5b) revealed almost normal arranged of renal corpuscle with normal glomerulus stained with (H and E) ×200.

Histopathological investigations of the heart

Fig. 6a showed a histological organization of the heart of control rats. (Fig. 6b) Control rat treated with *S. tomentosa* showed no change in cardiac muscle. Fig. 7a was the histological section of the heart of control rats treated with *I. tricolor*. Fig. 7b was the heart tissue section of HC-HG rat, and it showed degenerative changes in heart muscle. Fig. 8a histological section of the heart of diabetic rats revealed disordered cardiac myofibrils (Fig. 8b). Heart tissue section of HC-HG group treated with *S. tomentosa* showed enhancement in cardiac muscle (H and E) ×200. Fig. 9a is the histological section of the heart of HC-HG rats treated with *I. tricolor* showing restore of most cardiac histology. (Fig. 9b) Heart tissue section of HC-HG group treated with atorvastatin and glibenclamide.

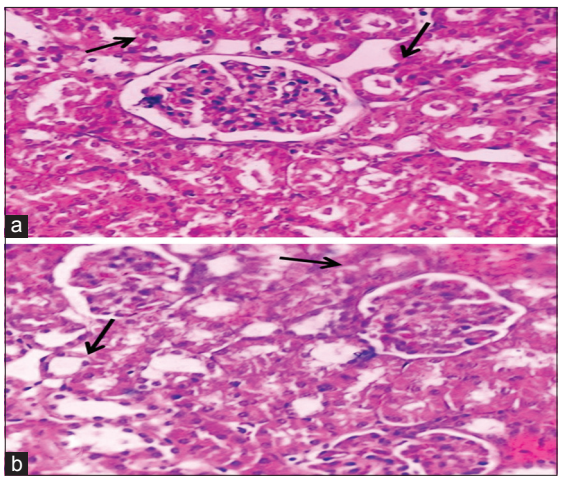

Fig. 2: (a) Photomicrograph of control kidney section ×200 showed renal cortex of renal corpuscle with normal glomerulus. (b) Kidney tissue section of control group treated with *Sophora tomentosa* showed no change in kidney cells, stained with (H and E) ×200

DISCUSSION

The present results show that, significant increase in ICAM and VCAM levels in HG-HC rats with percentages 80.43 and 40.27%, respectively.

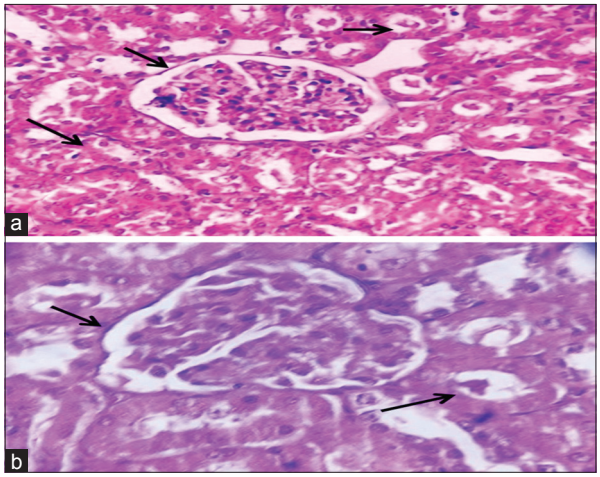

Fig. 3: (a) Photomicrograph of control kidney section treated with *Ipomoea tricolor* ×200 showed no change in normal cells structure. (b) Kidney tissue section of hypercholesterolemia+hyperglycemia group showed destructed epithelial lining of distal convoluted tubules stained with (H and E) ×400

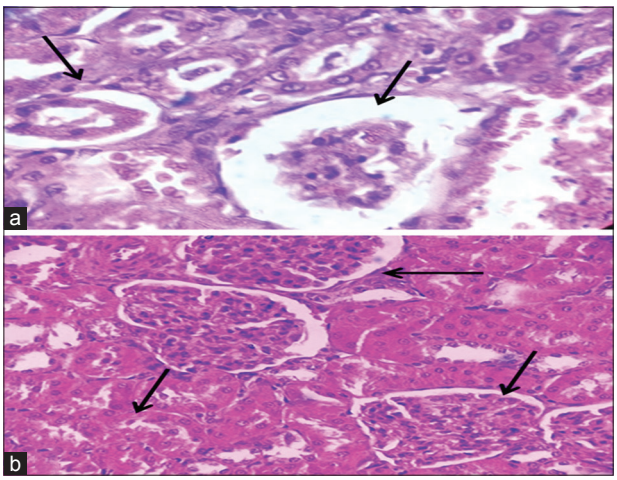

Fig. 4: (a) Another photomicrograph of kidney tissue section of hypercholesterolemia+hyperglycemia (HC+HG) group revealed proximal convoluted tubules show destructed epithelial lining ×400. (b) Kidney tissue section of HC+HG group treated with *Sophora tomentosa*, showed normal pattern of proximal convoluted (arrow) stained with (H and E) ×200

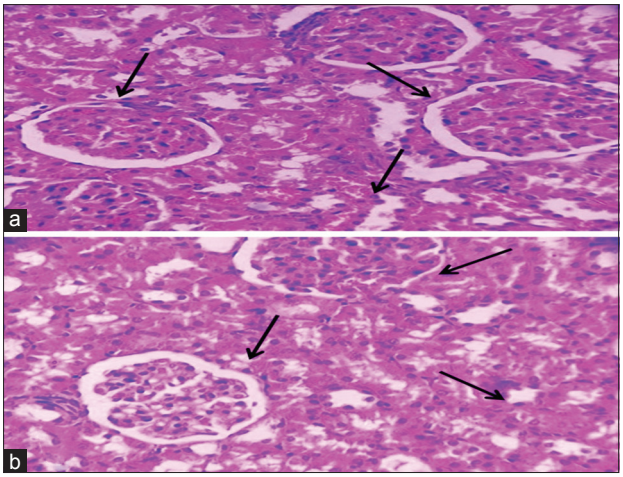

Fig. 5: (a) Photomicrograph of hypercholesterolemia + hyperglycemia (HC+HG) group kidney section treated with *Ipomoea tricolor* ×200 showed cells structure enhancement normal pattern of proximal convoluted. (b) Kidney tissue section of HC+HG group treated with atorvastatin and glibenclamide revealed almost normal arranged of renal corpuscle with normal glomerulus stained with (H and E) ×200

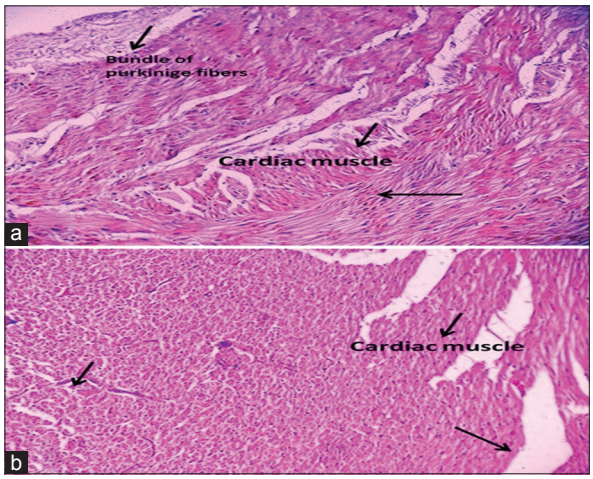

Fig. 6: (a) Histological organization of the heart of control rats ×200. (b) Control rat treated with *Sophora tomentosa* showed no change in cardiac muscle, stained with (H and E) ×200

While, a significant increase in ICAM level post MGE, NPE treatments as well as reference drugs with amelioration percentages 68.91, 29.93, and 33.22%. While, VCAM level returned to a normal value, where it detected insignificant change post-treatment of HC-HG rats with MGE, NPE as well as reference drugs.

The cytokines can be represented in two shapes including the anti-inflammatory markers such as IL-1β, IL-6, and TNF-α and the second shape is the adhesion molecules such as ICAM-1 and VCAM-1 [17]. Large evidence has shown strong associations of circulating levels of endothelial adhesion molecules with insulin resistance in non-diabetic individuals or patients with Type 2 diabetes [17]. El-Baz *et al.* [2] supported the hypothesis that insulin stimulates nitric oxide output within the endothelial cells and hence it attenuates endothelial functions as adhesion molecules. It is well known that, diabetes mellitus enhance oxidative status and hence, oxidant stress promotes increased leukocyte adhesion to vascular endothelium [2]. Free radicals are well ascertained to stimulate, cytokine expression, division of cells, and at a high level, they can imitate damage of cell and subsequent death by proteins, carbohydrates, lipid, and DNA oxidative modification [18]. This is combined with; oxidants lead to the activation of endothelial cells, VCAM-1, ICAM-1, E-selectin, and chemokines output [2].

In a good connection with the present results, the elevated levels of adhesion molecules in HG-HC condition may be explained on the basis of activation of macrophages that can stimulate several cytokines. It was induced the endothelial VCAM-1, the matter leading to macrophage accumulation and this consequently may lead to VCAM-1 expression [2,8].

Each extract-treatment improved 1CAM-1 and VCAM-1 levels, as compared to normal control rats, whereas in comparison with diseased HC-HG rats, treatment with each separate extract as well as atorvastatin and glibenclamide significantly ameliorate both CAMs. On the basis of the presented data, both extracts were observed to inhibit the expression of VCAM-1/ICAM-1 and reduced VCAM-1 expression, as they considered as a protective agent against the progression of atherosclerosis. These effects of extracts may be due to antioxidative and anti-inflammatory effects that reduced the oxidation of LDL-c to ox-LDL-c [5,18].

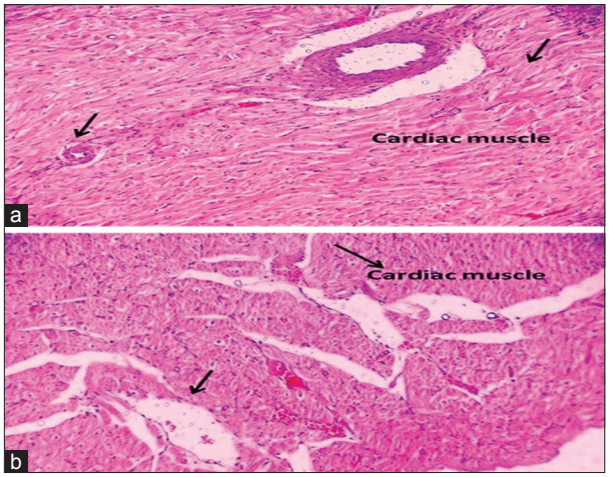

Fig. 7: (a) Histological section of the heart of control rats treated with *Ipomoea tricolor*. (b) Heart tissue section of hypercholesterolemia-hyperglycemia rat showing degenerative changes in heart muscle ×200 (H and E)

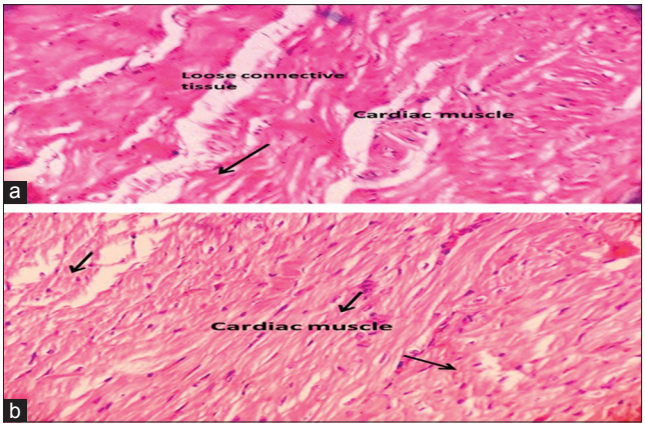

Fig. 8: (a) Histological section of the heart of diabetic rats revealed disordered cardiac myofibrils ×200. (b) Heart tissue section of hypercholesterolemia-hyperglycemia group treated with *Sophora tomentosa* showed enhancement in cardiac muscle (H and E) ×200

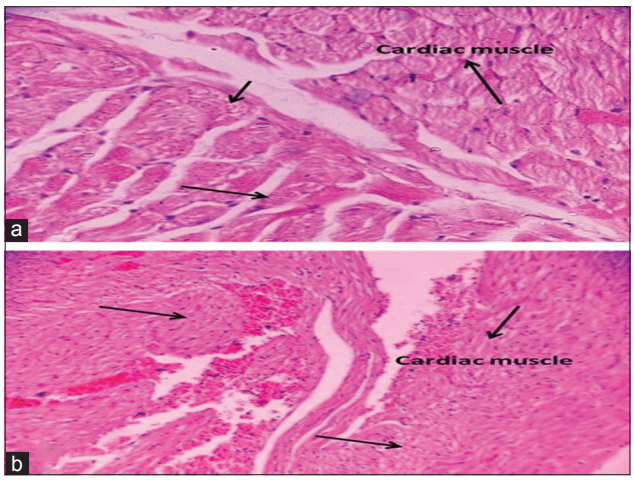

Fig. 9: (a and b) Histological section of the heart of hypercholesterolemia-hyperglycemia rats ×200 treated with *Ipomoea tricolor* and showed restore of most cardiac histology

Hyperglycemia and ox-LDL-c all are resulting from a high level of free radicle as well as elevation in adhesion molecules secretion [2,8]. CAMs act as ligands on monocytes, eliciting adhesion of monocytes to endothelial cells, monocyte winding and transferred into subendothelial matrix. In diabetes, high glucose-stimulated cytokines of inflammation leading to dysfunction of endothelial cells by oxidative stress enhancement, raising inflammatory cells leakage and inducement of adhesion molecules on endothelial cells and monocytes [19].

Plant flavonoids and their derivatives are important constituents known as natural tender drugs. Compounds other than the bioactive flavonoids as alkaloids, coumarochromones, saponins, triterpene glycosides, phospholipids, polysaccharides, oligostilbenes, and fatty acids are reported in many *Sophora* species [6].

NP showed the presence of various structural features of flavonoids as isoprenylated flavonoids, isoflavonones, flavones, and flavonols [6]. These specific classes of compounds have wide bioactivity including anti-inflammatory, antioxidant, and suppression effect on adhesion molecules [2,8,18]. These compounds like many other antioxidant compounds have an inhibiting activity toward TNF-α stimulated VCAM-1 and ICAM-1 expression through a mechanism involving NF-κB and AP-1 [20]. Furthermore, these variable compounds may strengthen capillaries and other connective tissue and some function as anti-inflammatory and antioxidant agents. Hence, the endothelial dysfunction whether acute or chronic can be counteracted by the effect of these compounds [6,20]. *Sophora* radix has been applied for different disorders such as atherosclerosis and arrhythmias. Atherosclerosis is well established by hyperglycemia which acts as a critical point in diabetes complexity [21]. The present results clearly demonstrate amelioration with *Sophora* extract treatment to HG-HC rats. The plant extract showed suppressing effect on adhesion molecules and attenuation activity of VCAM-1 and ICAM-1 expression. Recently matrine, the main active ingredient of *S. flavescens* roots was reported to decrease adhesion molecules production in stimulated vascular smooth muscle cells [22]. In parallel with our study, it was found that, the extract of S. tonkinensis root can inhibit the proliferation of melanoma cells, as well as their adhesion and movement [8]. In another study, a *Sophora* species (*S. radix*) extract showed inhibition of hyperglycemia-initiated up-regulation and production of VCAM-1 on endothelial cell line through suppressing of NF-κβ translocation [21]. These effects may be due to NPE active constituents as alkaloids and prenylated flavonoids [6,22].

Genus *Ipomoea* has different secondary metabolites of variable activity [7]. Several phenolic compounds, namely, phenolic acids, flavonoids, and organic acids have been described in several *Ipomoea* species [10,23]. In addition, *Ipomoea* species are known for the presence of anthocyanins, which may also act as antioxidants [10,20,23]. It was reported that the isolated anthocyanins inhibit the progress of atherosclerotic injury and enhancements of oxidative status and vascular adhesion molecule-1 in mice [23]. The leaves extract purple *I. batatas* showed inhibitive effect on cell adhesion and inflammatory mediation in endothelial cells of human aorta [24]. Hence; it is probable that the enhancement of endothelial dysfunction effect of MGE may be referred to its bioactive phenolic secondary metabolites as anthocyanins, flavonoids, and other flavonoids derivatives content [7].

Cytokines are classified as pro-inflammatory (TNF-a, IL-1, -6, -12, -15, -18, and -32), beside, and anti-inflammatory cytokines; IL-10 and TGF-β elicited by stimulated macrophages, implicated in the control of inflammatory reactions and IFN-γ and IL-4 from T-cells [25]. Anti-inflammatory cytokines such as IL-4, IL-10, IL-13, IFN-α, and TGF-β are involved in the downregulation of inflammation [25]. With respect to inflammatory markers CRP, TNF-α and IL-10 in control and different treated groups; CRP and TNF-α levels in hyperlipidemic diabetic rats recorded percentage increase reached to 85.93 and 50.99%, respectively, while significant decrease in IL-10 (49.86%) as compared to normal control rats. It was suggested that, inflammations

have a crucial intermediary role in pathogenesis Type 2 diabetic, thereby inflammatory mechanisms can lead to joining diabetes with a number of commonly coexisting conditions [1,17,24]. TNF-α has been demonstrated as a marker of insulin resistance [2]. The proinflammatory mediators like TNF-α secretion play a key role in the elimination of invading microorganisms besides, the inhibition of TNF-α can improve insulin sensitivity in animals [2]. The present study shows that HC-HG rats showed strong activation of TNF-α and CRP, while suppression in IL-10 levels, as they all implicated in pathophysiological alterations that may accelerate inflammatory disease development in hypercholesterolemia and hyperglycemia [5]. This enhanced cytokines may induce polymorphonuclear leukocytes adhesion to endothelial surfaces, superoxide anion production, release of lysozyme, H_2O_2, and chemotaxis, suggesting their implication in increased inflammatory response [2]. High cholesterol and glucose levels connected with increased inflammatory biomarker CRP [2,13]. CRP is linked cardiovascular inflammation. Recently, in good agreement with the present finding Rada [26], found that CRP level was significantly elevated in HC-HG rats and this elevation is markedly linked with atherosclerosis in these animals. IL-10 is an immunoregulatory cytokines, elevated during inflammatory reaction to replace proinflammatory cytokine transmission. Hence, the output of some cytokines-related inflammation was susceptible to differ with illness degree [17]. In this study, the ameliorative levels of cytokines post-treatment with each extract may be related to the presence of flavonoid compounds as flavones, which has anti-inflammatory effect and significantly attenuated VCAM-1 and IL-8 expression [26].

Many *Ipomoea* species were reported as a good source of bioactive phenolic compounds, namely, phenolic acids, flavonoids, and anthocyanins [10]. These compounds may also act as antioxidant and anti-inflammatory agent [7,10]. Many other flavone derivatives were reported to inhibit COX-2 as well as nitric oxide synthase (NOS) [7,27]. Rutin, isolated from *I. pes-caprae* and *I. fistulosa*, decreases transfer of cells which was ascertained by the reduced levels of specific cytokines [7]. Furthermore, the aqueous extract and fractions of these plants neutralize inflammation induced by dangerous scorpion venom [27]. Anthocyanins and caffeoylquinic acid derivatives, isolated from purple *I. batatas* and *I. cairica*, have been reported to suppress histamine production in *in vitro* and decrease lipid peroxidation stimulated by Fe^{2+} and ascorbic acid in brain rat [7].

Inflammatory process is a critical cause of obesity. Many *Ipomoea* species, as *I. involucrata* and *I. cairica* are traditionally used in the treatment of toothache, rheumatic pains, and other inflammatory conditions [7].

Ipomoea constitutes many bioactive compounds which have different effects as antinociceptive and anti-inflammatory. The possible explanation for the antinociception is that these compounds reduced the release of pro-nociceptive mediators such as histamine [28]. In this study, the anti-inflammatory activity and ameliorative level in adhesion molecules may be related to the presence of different bioactive compounds, viz., flavonols, flavones, chalcones, flavanones, isoflavones, isoflavonones, lavandulyl flavanones, and flavonol glycosides in the extract of MG (*I. tricolor*) [7]. Moreover, Krishna *et al*. [6] attributed the anti-inflammatory features of several active phytochemicals in *Sophora* species such as prenylated flavonoids and quinolizidine alkaloids to their abilities to moderate cell mitogen-activated protein kinases (MAPK) signaling pathways, redox balance. A prenylated flavonoid; sophoraflavanone G and isoflavone glycoside; sophoricoside, trifolirhizin, a pterocarpan flavonoid isolated from many *Sophora* roots showed anti-inflammatory and inhibition of IL-6 and cyclooxygenase-2 in inflammatory response [29]. Several prenylated flavonoids are having a C-8 lavandulyl moiety were found to inhibit cyclooxygenase-1 as well as 5-lipoxygenase, and sophoraflavanone G was the most potent inhibitor against these eicosanoid generating enzymes among 19 prenylated flavonoids tested [30]. Sophoraflavanone G showed *in vivo* anti-inflammatory activity against mouse croton oil-induced ear edema and rat carrageenan paw edema via oral (2-250 mg/kg) or topical administration (10-250 μg/ear) [31]. Flavonostilbenes, alopecuroides M–O, were isolated from the root bark of S. alopecuroides inhibit LPS-induced nitric oxide output in RAW 264.7 macrophages [21]. Arylbenzofurans and flavonoids isolated from the rhizomes and roots of *S. tonkinensis* showed IL-6 production inhibitory activity [32]. Anti-inflammatory action of sophoraflavanone G isolated from *S. flavescens* in lipopolysaccharide-stimulated mouse macrophages [33]. It has an anti-inflammatory property, reduced proinflammatory cytokines production via obstruction of the NF-κB and MAPK transmission. Production levels of interferon-gamma and TNF-α were decreased by the extract of root of *S. flavescens* (*Sophora* radix, SR) *in vivo* [34]. Finally, the release of histamine and β-hexosaminidase and migration were inhibited by the treatment with this extract. Sophocarpine, a tetracyclic quinolizidine alkaloid, isolated from *S. alopecuroides* showed anti-inflammatory effect [35] on carrageenan-induced rat hind paw edema. In this study, the anti-inflammatory activity of NPE might attribute to the lowering of iNOS and cyclooxygenase-2 expressions exerted by the quinolizidine alkaloids content [36].

Prenylated flavonoids are represented the active components that exhibit beneficial effects on human health. The prenylated apigenin, quercetin, and kaempferol, anti-inflammatory properties may be mediated by suppressing the effect of NF-κB and AP-1 transcription [7].

In this study, administration of both extracts significantly attenuated the disturbances in these cytokines; the results of this study clearly shows that the mean levels of the TNF-a and CRP were significantly decreased, while IL-10 were markedly increased in MGE and NPE-treated HC-HG rats, this is probably due to decrease in proinflammatory biomarkers, suggesting mediation of endothelial activation and amelioration in endothelial function, as it suggested by Krishna *et al*., [6] this may lead to decrease in atherosclerosis development and reduction in local production of the cytokines by inflammatory cells that have accumulated. The current study investigated expressions of TNF-α, CRP, and IL-10. These expressions were increased by STZ treatment and increased the expressions of those were decreased by extract, especially the NPE extract.

The effects of hypercholesterolemia-hyperglycemia on renal function were assessed through measuring serum total urea and creatinine; high-fat diet intake caused significantly increase in levels of serum total urea and creatinine as compared to normal rats. The elevated levels of urea may be interpreted due to stimulated protein catabolism and activation of amino acid deamination for gluconeogenesis [3]. The increase in serum urea level in HC-HG rats indicated deterioration, in the normal renal function of the animal, as the mechanism of removing urea from the blood might have been affected. It may also be signs of injury at the glomerular and tubular levels of the kidney. It is well-known that many biochemical and histopathological findings ascertained renal injury in hypercholesterolemia-hyperglycemia condition [5]. With respect to the present results, HC-HG rats show a significant increase in total urea and creatinine levels with percentage 102.22 and 177.41%, respectively. Total urea level showed insignificant change in hyperlipidemic diabetic-treated groups with MGE, NPE as well as reference drugs as compared to normal control rats. While creatinine exhibited insignificant change in hyperlipidemic diabetic-treated groups with NPE and reference drugs showed similar improvement percentages (167.74%). Although significant increase was detected in creatinine level with MGE with amelioration percentage of 114.19%. Serum creatinine and urea levels are signs of glomerular filtration rate but plasma creatinine is more sensitive index of kidney function as compared to plasma urea level because of the achieving role of creatinine in most of the requirements for a perfect filtration marker [3]. The raised content of plasma creatinine and urea levels may be an indicator on the prerenal problem such as volume depletion and may be joined with the impaired function of the nephrons [3].

In this study, the higher percentages of amelioration in ICAM, VCAM and the proinflammatory IL-10 levels were recorded by MGE

treatment. NPE showed higher percentages of amelioration in CRP, total urea and creatinine levels. The effect of many quinolizidine alkaloids, as oxymatrine isolated from different *Sophora* species, viz., *S. tonkinensis* and *S. flavescens*, markedly reduced blood glucose, serum creatinine, and blood urea nitrogen in diabetic rats and improved diabetes-induced glomerular and tubular architectures changes [37]. Moreover, plants contained significant levels of phenolic compounds have displayed high antioxidant and anti-inflammatory activities [38]. They markedly improved oxidative status and decreased glycation end products, transforming growth factor-β1, and inflammatory cytokines in diabetic rats [17]. Another study suggested that *S. flavescens* has kidney protective potential from oxidative damage by the presence of many active compounds as; sophoraflavanone G and kurarinone and the radical generator 2,2′-azobis(2-amidinopropane) dihydrochloride in renal epithelial LLC-PK_1 cells [6].

Genus *Ipomoea* is a good source of many bioactive secondary metabolites [7]. *I. batatas* (purple sweet potato) showed significant reduction of the expression level of kidney nucleotide-binding domain and leucine-rich repeat pyrin 3 domain (NLRP3) and Caspase-1, resulted in decline of IL-1β. In addition, *I. batatas* suppressed the I kappa β kinase β (IKKβ) stimulation and the nuclear translocation of NF-κB [39].

Histopathological investigation of the kidney and the heart supported by their IRS could conclude that both IRS and histopathological changes in kidney section as focal necrosis, tubular epithelial necrosis, tubular dilation, and vascular wall thickening seen in the HC+HG group regressed with *S. tomentosa* or *I. tricolor* treatment, revealed almost normal arranged of renal corpuscle with normal glomerulus and might prevent the development of diabetic nephropathy. HH+HG group treated with atorvastatin and glibenclamide, showing almost normal arranged kidney cell.

The analysis of statistical results has revealed that rats in the HC+HG group showed functional alteration of the myocardial microcirculation that may explain the left ventricular systolic dysfunction. Finally, the treatment by *S. tomentosa* or *I. tricolor* showed the decrease of myocardial perfusion reserve during the treatment, also reducing the degenerative changes in the myocardium, and restoring nuclei shape of the cardiomyocyte.

CONCLUSION

These results demonstrated that two plants extracts may be a candidate intelligent anti-inflammatory nutraceuticals and these extracts could be applied effectively to reduce renal complications in parallel to hypercholesterolemia associated diabetes. An image recognition system was useful to investigate renal injury images.

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