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Study of the hypolipidemic activity of Egyptian *Tropaeolum majus* L. (garden nasturtium) as a promising therapeutic plant for treatment of cardiac diseases

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Abstract
The present study was conducted to evaluate the hypolipidemic activity of Egyptian *Tropaeolum majus* L. leaves on lipid parameters (total cholesterol, triglycerides, low density lipoproteins, high density lipoproteins, atherogenic index and cardiac risk ratio) of hyperlipidemic rats as well as its effect on vital body organs (aorta, heart and liver). In addition to, the histopathological examinations and *x-vivo* relaxant test of the hyperlipidemic aortic strip. LC /MS/MS analysis was carried out to screen the biologically active compounds that may contribute to the activity. Results showed that all lipid parameters were significantly reduced after administration of the hydroalcoholic extract of *Tropaeolum* majus for 5 consecutive weeks, and the histopathological pictures were improved. While, LC /MS/MS analysis revealed that flavonoids contribute the highest percentage (91.9%) of the relative area of the hydroalcoholic extract of *Tropaeolum majus* L. leaves. Isoquercetrin was the main flavonoids (67%), in addition to isorhamntein3-o- glycoside and quinic acid derivatives. *Tropaeolum majus* L. could be used as a new promising plant against the progress of cardiovascular diseases.

Keywords: Egyptian *Tropaeolum majus* L.; hypolipidemia; LC/MS/MS, cardiovascular diseases; male Wister rats

Introduction
Cardiovascular diseases (CVD) can be defined according to the World Health Organization (WHO) as a defect of the circulatory system including heart and blood vessels. There are many types of CVD such as coronary heart disease (CHD), cerebrovascular disease, heart attacks and strokes. Deposition of fatty substances, cellular waste, cholesterol and other substances on the inner walls of blood vessels is the major cause for CVD [1].
CVD are the global causes of death in the future. They will continue to increase the mortality rate more than infectious diseases such as AIDS and tuberculosis in poor countries [2].
Hyperlipidemia is the main factor responsible for the progress of CVD. In Egypt, it is responsible for 6% of CVD when carrying a screening study on more than 6000 patients [3].
One of the most famous drugs used for treatment of hyperlipidemia is known as Statin [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors]. However, severe side effects were reported by the food and drug administration (FDA) upon long-term use of Statins as liver failure and muscle toxicity [4]. Muscle dysfunction was the most serious side effect associated with Statin therapy according to recent reports of Atherosclerosis Society in Europe [5]. Statin intolerance is a general term describes the inability of a patient to use Statin due to its adverse effect. In addition, Statins increase the occurrence of cataract and psychiatric disorders. Myositis was reported when a high dose of Statin was used alone or in combination with other drugs therefore, herbal therapy is a global demand that can serve as a natural alternative to drug therapy without side effects. Many researches were directed to find plants that can manage lipid metabolism. In a previous publication [6], many plants such as onion, garlic, ginger, guggul and nasturtium were investigated for lowering blood cholesterol, and the most potent being nasturtium or *(Tropaeolum majus* L).Nasturtium is a herbaceous plant that grows in South America, it belongs to family Tropaeolaceae. It is known as 'Garden nasturtium or Indian cress' [7]. Many bioactive compounds had been detected in nasturtium as flavonoids (quercetin, and isoquercitrin), fatty acids (oleic and linoleic), vitamin C and benzyl-isothiocyanate [8]. Both leaves and flowers are rich sources of lutein. The leaves were used as a tea bag of for treatment of many diseases as hypertension, inflammation, as well as, urinary tract infection [9,10].Isoquercitrin the main flavonoid component of the leaves was responsible for diuretic effect of the hydroalcoholic extract of *Tropaeolum majus* L (HETM) [11].

In addition, to its activity as antihypertensive activity via inhibition of production of angiotensin converting enzyme [12].While, chlorogenic acid and other ester of cinnamic acid showed anti-inflammatory activity through inhibition of hyaluronidase and cyclooxygenase1 activity [8].

Reviewing the available literature revealed that nothing was traced concerning the hypolipidemic activity of nasturtium. This encourages the author to investigate such activity in hyperlipidemic rats.

Material and methods

Drugs and Chemicals

Atorvastatin was purchased from EIPICO Company[Egyptian international pharmaceutical industries company - Egypt].Methanol; acetonotrile and water are of HPLC grade (J.T. Baker, Phillipsburg, NJ, USA). Absolute methanol and formic acid were purchased from Merck (Darmstadt, Germany).

Norepinephrine, Sodium nitroprusside and all ingredients of Krebs' solution were of analytical grade and purchased from El-Gomhorya Company for Chemicals and Pharmaceutical, Cairo, Egypt.

Composition of Krebs' solution (g/l): NaCl 6.9, KCl 0.35, and KH_2PO_4 0.16, Mg $SO_4.7H_2O$ 0.3, $CaCl_2.2H_2O$ 0.37, $NaHCO_3$ 2.1 and glucose 1.05.

Basal diet and cholesterol were purchased from El-Gomhorya Company for Chemicals and Pharmaceutical, Cairo, Egypt. The basal diet (BD) was prepared according to American Institute of Nutrition for rats (AIN-93M), whereas, the high fat diet(HFD) was made of the basal diet supplemented with 1% cholesterol,10% sheep fat and 0.5% cholic acid. Enzymatic kits ((Stanbio -USA) were used for biochemical estimation of total cholesterol (TC), triglyceride (TG) and HDL-Cwere purchased from Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Plant materials

Fresh leaves of *Tropaeolum majus* L. were collected during April and June 2012, from Experimental Station of Medicinal Plants (ESMP), Faculty of Pharmacy, Cairo University, Egypt and a voucher specimen was deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University (12.12.27e). The plant was kindly identified by Professor Dr. Ahmed EL-kaphory in Agriculture Research Center, Giza-Egypt and Mrs. Traz Labib, taxonomy specialist in El- Orman public garden- Giza-Egypt.

Preparation of hydroalcoholic extracts of *Tropaeolum majus* L. (HETM)

Two hundred and fifty grams of coarse powdered leaves were extracted by 70% ethanol; the solvent was evaporated under vacuum at about 50 °C to yield about thirty grams of dark residue (HETM), and kept at -20 °C until use. Then, it was suspended in distilled water. While; atorvastatin was dissolved in distilled water and filtered just before administration to rats.

LC /MS/MS analysis of HETM

HETM was dissolved in HPLC grade methanol in a concentration of 10 mg/ml. Aliquots of 5 µl of HETM were injected into the LC-DAD/MS analysis system. Dionex Ultimate 3000 HPLC (Germany) was used for performing the analyses. It consisted of a quaternary pump with an on line degasser, an auto sampler with thermostatted column compartment, a photodiode array detector (DAD), and Chromelon software. The separation was carried on Zobrax SB-C18 column (150 mm× 4.6 mm, 1.8 µm, Agilent Company, USA). The mobile phase consisted of (A) Acetonitrile and (B) 0.2% formic acid with gradient elution profile: 0 min, A: B 10:90; 36 min, A: B 70:30; 50 min, A: B 100:0. Flow-rate was constant at 0.8 ml/min with fixed temperature 30 °C. The sample was analyzed at 254 nm. HPLC–MS system composed of electrospray ionization (ESI) with interfaced Bruker Daltonik Esquire-LC ion trap mass spectrometer (Bremen, Germany) and an Agilent HP 1100. The ionization parameters utilized negative ion mode; capillary voltage 4000 V, end plate voltage −500 V; drying gas of 10 l / min nitrogen at 350 °C and nebulizing gas of nitrogen at 35.0 p.s.i. Scanning of mass analyzer starts from 15 to 1000 u. The amplitude of fragmentation was set to 1.0 V. MS^2 data and acquired in negative mode (Dionex Corporation, 2011).

Quantitative determination of total flavonoid, total phenolic contents

Spectrophotometric determination of total flavonoid was carried out by Aluminum chloride according to Mandal *et al*., 2013[13] using quercetin as standard. Whereas, total phenolic content was measured adopting method of Saboo *et al*., 2010[14] against gallic acid as standard. The samples were prepared in triplicate.

Antioxidant activity

The antioxidant activity of HETM was measured using DPPH reagent and ascorbic acid as standard according to Mandal *et al*., 2013[13]. The samples were prepared in triplicate.

Animals

Wister Albino male rats with an average weight (70± 10g) were obtained from Faculty of Veterinary Medicine, Cairo University-Egypt. They housed in polypropylene cages at temperature of 25± 2 °C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for a week and supplied with a standard pellet diet and water *adlibitum*. The protocol of the study was approved from Veterinary Medicine Collage Animal Ethics Committee.

High fat diet- induced hyperlipidemia

Forty rats were randomly divided into four groups (10 rats/each). Group I (negative control): was fed on basal diet for 10 weeks. Group II (HFD): was fed on a high fat diet for 10 weeks. Groups III and IV were fed on a high fat diet for5 weeks till induction of hyperlipidemia, then they were turned into basal diet, for the last 5 weeks of experiment during which they received atorvastatin 10mg/kg b.wt and 750 mg/kg b.wt of HETM respectively [6].

Measurements of body weight

The total body weights of all groups under investigation were measured weekly. Changes in weight were recorded at 2, 5, 7 and 10 weeks.

Measurements of lipid profile

Determination of total cholesterol level was carried out weekly as an indicator of hyperlipidemia after overnight fasting for 18hrs and recorded at 2, 5, 7 and 10 weeks. At the end of experiment (after 10 weeks), blood samples were

collected from retro-orbital venous plexus after anesthesia by diethyl ether. Blood samples were left for clotting for 15min then centrifugated at 3000 rpm for another 15min, then serum was separated and collected into dry clean Eppenorff tubes and stored at -20 °C until analysis.

Serum total cholesterol (TC)[15], triglyceride (TG) [16], and high density lipoprotein (HDL-C) [17] were measured using enzymatic Stanbio kits. Low-density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL) were calculated using the following equation [18].

LDL-C = total cholesterol - [HDL-C + (triglyceride / 5)].

VLDL =TG / 5.Atherogenic index (AI) and cardiac risk ratio (CRR) were calculated using the following equations [19, 20] respectively:

Atherogenic index (AI) = (total cholesterol - HDL-C / HDL-C).Cardiac risk ratio (CRR) = total cholesterol / HDL-C.

Fecal cholesterol analysis

Fecal samples were collected at the end of experiment, frozen and grinded into powder. Total cholesterol was extracted according to Folch *et al*., 1957[21] method using cholesterol enzymatic Stanbio kit.

Histopathological examination

At the end of experiment, aorta, heart and liver from each group were isolated and cleaned from blood. Heart and liver of each group were weighed, then all samples were kept in 10% formalin, cutted into sections of 4 microns thickness that stained by hematoxylin and eosin and examined of histopathological changes.

***X-vivo* relaxant test on hyperlipidemic aorta**

Rat aortic rings from each group were used to assess the vascular reactivity towards various vasoactive agents. Norepinephrine (NE) was utilized as a vasoconstrictor while, Sodium nitroprusside (SNP) was used as endothelium-independent vasodilator. Rats were killed and the thoracic aorta was isolated and cut into rings (3-5 mm width). Each ring was vertically mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates, while the upper one was attached to a force displacement transducer connected to a computer.

The isolated aortic ring was mounted in 10 ml water-jacketed automatic multi-chamber organ bath system. The organ bath contained Krebs' solution and its temperature was kept at 37 °C. Then, it was allowed to equilibrate for about 120 min under a resting tension of 2 g. Sodium nitropruside was added to the organ bath on the aortic rings pre-contracted with 10^{-6} M of NE in order to perform concentration-response relationship and the changes in isometric tension were determined. Results were expressed as percentages of achieved maximal response on a given preparation. Different concentrations of SNP were prepared to facilitate cumulative additions to the isolated tissue that gave final bath concentrations ranging from 10^{-9} to 10^{-4} M.

Statistical analysis

Statistical analyses were performed using SPSS program (Statistical Package for Social Sciences) software version 18.0. expressed as parametric mean ± SD (standard deviation) using one way ANOVA followed by Tukey-Kramer Multiple Comparisons test at a level of significance $P < 0.05$.

Results

LC/MS/MS of HETM

HPLC analysis of HETM allowed the identification of 13 different compounds including flavanol, and hydroxycinnamic acid derivatives. Results are recorded in Table (1) and presented in Fig. (1). Tentative identification was based on their mass spectra and fragmentation patterns of previously reported data. Quercetin glycosides represented the major constituents of HETM as illustrated in Fig. (1)

Identification of quercetin aglycone was confirmed by the characteristic MS^2 fragment at m/z 301 in negative mode, together with specific fragments at 179 and/or 151 [22].

While the nature of sugar could be identified by elimination of 162 amu as sugar residue, (hexose; glucose or galactose) [23].Isoquercitrin (querecetin-3-O-glucoside) compound 7with $[M-H]^-$ ion m/z 463 that cross ponds to the molecular formula $C_{21}H_{19}O_{12}$ and its characteristic fragmentation patterns due to loss of the terminal glucose moiety (162 amu) yielding the parent aglycone ion $[quercetin-H]^-$ at m/z 301 as the most abundant ion in the fragmentation spectrum [24].

Similarly, identification of Isorhamentin (methylated derivative of quercetin (compound 11), was based on presence of $[M-H]^-$ at 477.9 with specific fragment ionat m/z 314 ($C_{16}H_{11}O_7$, aglycone ion),in addition to characteristic loss of a methyl group from its methoxy group(-15Da) [25].

Several other minor derivatives of quercetin including acetyl and malonyl glycosides (compounds 8 and 13were detected[26,27].The deprotonated ion of Astragalin (Kaempferol 3-O-glucoside) compound10 was shown at m/z 447.9 that indicating a molecular formula $C_{21}H_{20}O_{11}$.Further fragmentation of the parent ion resulted in formation of kaempferol aglycone m/z 285 $[M-H-162]^-$ [8].

The main phenolic acids that found in nasturtium leaves are derivatives of hydroxyl cinnamic acid (compounds 2, 3 and 5) with the characteristic charged quasi-molecular ion $[M-H]^-$at m/z 353 and 337, (Table 1) which in agreement with the previously reported data in the literature [28]. The presence of Quinic acid moiety was confirmed by the prominent ion at m/z 191.Whereas; the characteristic fragment at m/z 179 was specific to caffeic acid [8, 28].

Total flavonoid and total phenolic contents

Concentration of total phenolic and flavonoid in HETM were 59.6 ± 0.0.14 and 41.2± 0.17 mg /g respectively. Flavonoids are important plant secondary metabolites that contribute to the anti-Atherogenic and hypolipidemic activities of many plants through their potential antioxidant effect [29, 30].

Antioxidant activity

HETM showed high antioxidant activity of (IC_{50}, 0.2mg/ml) which could be attributed to its high content of phenolic compounds.

Effect of HETM on body weight

There was significant increase in body weights in the three groups that fed on high fat diet for the first five weeks and continued to rise even after starting the administration of atorvastatin and HETM for another five weeks, (Fig 2).

Effect of HETM on serum lipid profile and fecal cholesterol

There were marked increments in TC (a marker for induction of hyperlipidemia) for rats that fed on HFD during first five weeks with marked increase in TG, LDL and decrease in

HDL. In addition, to a significant elevation of the biomarkers of CVD, Atherogenic index (AI) and Cardiac risk ratio (CRR) as compared to control group. Treatment of hyperlipidemic rats with HETM for another five weeks significantly reduced all lipid parameters (Table 2). Whereas, HDL level was highly elevated. Moreover, administration of HETM significantly reduced AI and CRR nearly reaching the normal levels of control group and very similar to standard drug atorvastatin.
The fecal cholesterol was also, increased in comparison with HFD group and superior to the effect of atorvastatin (Fig4).

Effect of HETM on histopathology of aorta, heart and liver.
Figure (5B) illustrated that the aorta of HFD showed sever pathological alteration in histopathological feature of aorta represented by focal thickening and figure like projection in some areas in the lumen. HETM prevent any changes in aorta and showed normal appearance with few vacuoles in the intima similar to atorvastatin group (Fig 5 C, D).
The heart of high fat diet showed hyaline degeneration and fatty changes as compared to normal structure of myocardium of the heart of the control negative group. HETM group protected the heart from fatty changes and degeneration induced by high fat diet and showed normal appearance similar to atorvastatin group (Fig 5 G, H). Fatty changes and hydropic degeneration of the hepatocyte were observed in liver of HFD. Whereas, HETM group showed normal appearance as did by atorvastatin group (Fig 5 K, L).

Relaxant effect of HETM on hyperlipidemic aorta
Normal relaxation reached to 174% of the precontracted value. In the HFD group, the relaxation reached only to 54% of the precontracted value. However, the treatment with HETM showed relaxation reaches about 99% (Fig 6).

Discussion
Abnormal elevation of serum lipids or hyperlipidemia is the primary metabolic disorder responsible for atherosclerosis and other associated cardiovascular diseases. Regulation of blood cholesterol and triglyceride, in addition to LDL will eventually decrease the progress of CVD [31]. Recently several researches focused on the discovery of herbal plants with hypolipidemic activity that could suppress high blood cholesterol [32, 33, 34] without severe side effects of statins [4].
The present study was conducted to evaluate the hypolipidemic activity of HETM on hyperlipidemic rats fed on HFD. High fat diet causes marked elevation of lipid parameters and fatty changes in cardiac muscle and hepatocyte.
These results are in agreement with other studies carried by Shehata *et al*., 2010 and Abdel-rahman., 2014 [35, 36] on rabbit. They reported that HFD induced cardiac injury, narrowing of aorta and necrosis of myocardium and hepatic cells. Elevated level of TG was directly linked to increase risks of heart disease [37].Importantly; the high mortality rate due to LDL was decreased by reduction of its level [38].
On the contrary, HDL markedly increased upon administration of both HETM and atorvastatin. Elevated level of HDL exerted a protective effect against further deposition of cholesterol in tissue as it promotes the reverse transport of cholesterol from tissues to liver and formation of bile acids. This elevation could be attributed to high flavonoid content of HETM [20, 11, 9].Whereas, atorvastatin as previously published data act via inhibition of HMG-CoA reductase enzyme [39].
Based on data illustrated (Table 1) leaves of nasturtium were rich in phenolic compounds especially flavonoid. Further confirmation of the presence of quercetin flavonoid was achieved by LC/MS/MS analysis. Isoquercitrin was found to be the major flavonoid in the leaves extract. These results were in agreements with previously reported by Gasparotto Junior *et al*., 2011 [11] who verified the presence of high level of quercetin glycosides in leaves.
Quercetin flavonoid exerted inhibitory effect on cyclooxygenase and lipoxygenase enzymes that are responsible for the release of arachidonic acid (inflammation mediator) and the development of atherosclerosis plaque [35].
Isoquercitrin was found to be the major flavonoid in the leaves extract. It was reported that it prevented lipid peroxidation and oxidative stress [40]. In addition to, its strong antioxidant activity that attenuate inflammatory response and inhibit progress of heart diseases [41, 42, 43].
High flavonoid content of HETM was responsible for the vasorelaxant effect on hyperlipidemic aorta. Quercetin glycosides were able to reduce progress of atherosclerosis by preventing adhesion of monocyte to the endothelium wall of blood vessel [44]. This activity could explain the powerful relaxant response of HETM on precontracted aorta reaching 99% and curative effect of histopathological pictures of cardiac muscle, aorta and hepatocyte of HETM [45].
Other phenolic compounds such as Caffeoyl-quinic acid derivatives contributed to the hypolipidemic activity as it prevented oxidation of LDL and reduced the high levels of TC and TG [46].Vizcaino and Durate, 2010 [47] reported that low doses of quercetin were more efficient than the other flavonoids (epicatechin, theaflavin) as antiatherosclerotic agent.
On the contrary, Hodgson, and Croft, 2006[48] in clinical studies suggested that increase flavonoid intake had a small effect on blood cholesterol and other associated lipoproteins.
Finally, further investigations may be needed to explore the mechanism that involved in lowering lipid parameters and the role of the phenolic compounds in establishment of HETM hypolipidemic activity. It is the first time according to our knowledge to screen this activity for *T. majus*.

Conclusion
In conclusion, our study indicates that *Tropaeolum majus* L. has a potential antihyperlipidemic activity. It significantly reduces all lipid parameters and enhances the good cholesterol. Moreover, it has a relaxant effect on aorta, enhances fecal excretion of excess cholesterol and retains the normal architecture of vital organs as atorvastatin. Polyphenolic content of HETM especially isoquercitrin and Caffeoyl-quinic acid derivatives were the main bioactive compounds responsible for such activity.

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Conflict of interest
All authors declare that absence of financial and commercial conflicts of interest concerning all data in our article.

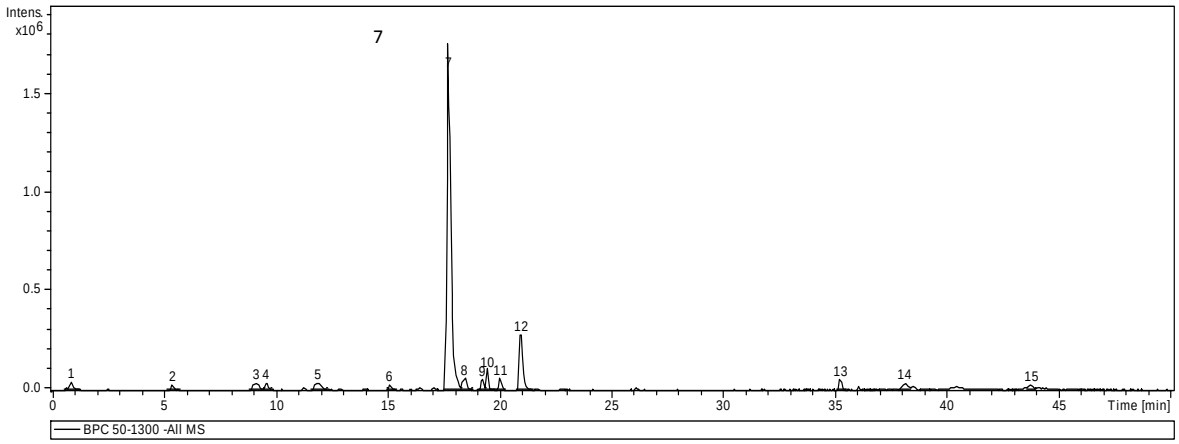

Fig 1: HPLC chromatogram of HETM at 254nm

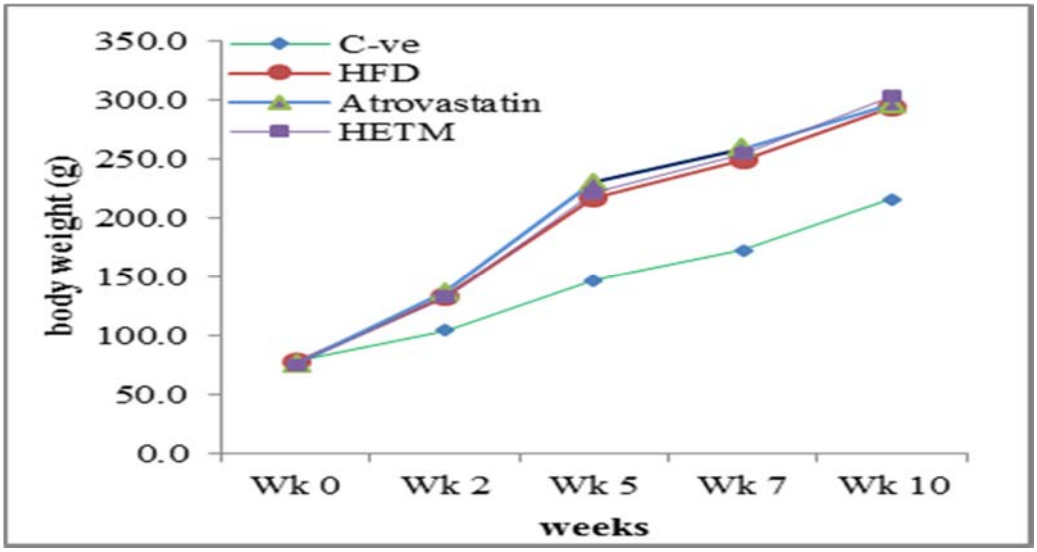

Fig 2: Effect of oral administration of HETM on body weights of hyperlipidemic rats at different weeks of experiment; Control, negative control group; HFD, high fat diet group; Atorvastatin, atorvastatin treated group; HETM, hydroalcoholic extract of *T. majus* treated group. Values are expressed as mean ± SD (n=10).

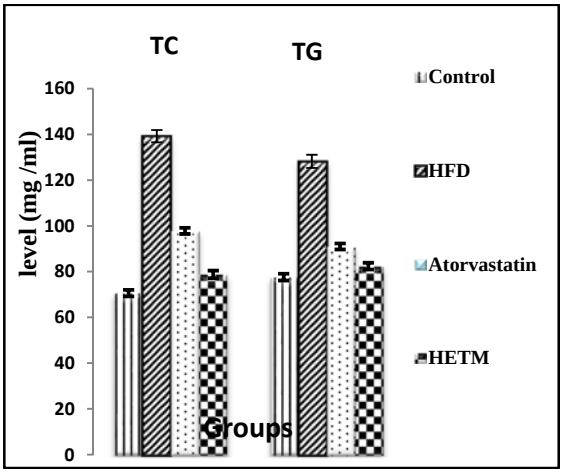

Fig 3: Effect of oral administration of HETM on total cholesterol and triglyceride of hyperlipidemic rats after 10 weeks ; Control; negative control group; HFD, high fat diet group; Atorvastatin; atorvastatin treated group; and HETM; hydroalcoholic extract of *T. majus* treated group.TC, total cholesterol, TG, total triglyceride, Values are expressed as mean ± SD (n=10).

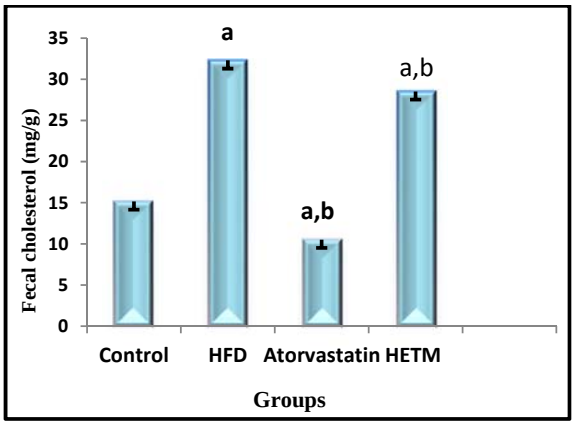

Fig 4: Effect of HETM on fecal cholesterol level of hyperlipidemic rats after10 weeks. Control; negative control group; HFD; high fat diet group; Atorvastatin; Atorvastatin treated group; and HETM; hydroalcoholic extract of *T. majus* treated group. (a) Significant difference from corresponding negative control group. (b) Significant difference from corresponding HFD group by one-way ANOVA $P \leq 0.05$.Values are mean ± SD (n=5).

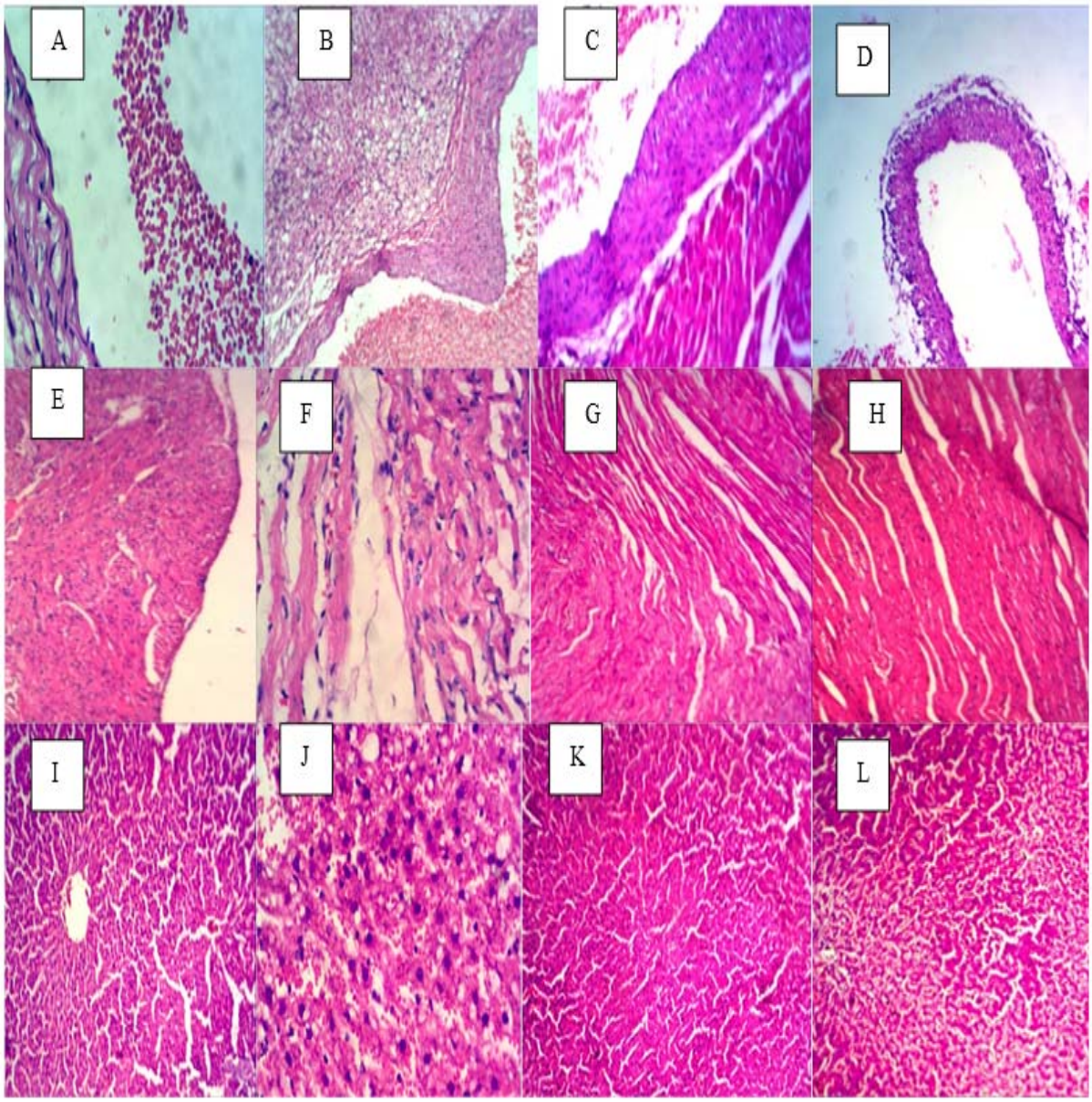

Fig 5: Effect of HETM on vital body organs. Effect on rat aorta from (A-D), rat heart from (E-H), rat liver from (I-L).
A; aorta of control negative did not reveal any pathological changes. B; aorta of high fat diet showed focal thickening in some areas represented by figure like projection in the lumen. C; aorta of atorvastatin group showed normal appearance. D; aorta of hydroalcoholic extract of *T. majus* showed normal appearance with few vacuoles in the intima. E; heart of the control negative group revealed normal structure of myocardium. F; heart of high fat diet showed hyaline degeneration and fatty changes. G; heart of atorvastatin group showed normal appearance. H; myocardium of hydroalcoholic extract of *T. majus* appeared normal. I; liver of the control negative group revealed normal structure of hepatocyte. J; liver of high fat diet showed fatty changes and hydropic degeneration of the hepatocyte. K; liver of atorvastatin showed normal appearance. L; liver of hydroalcoholic extract of *T. majus* showed normal appearance.

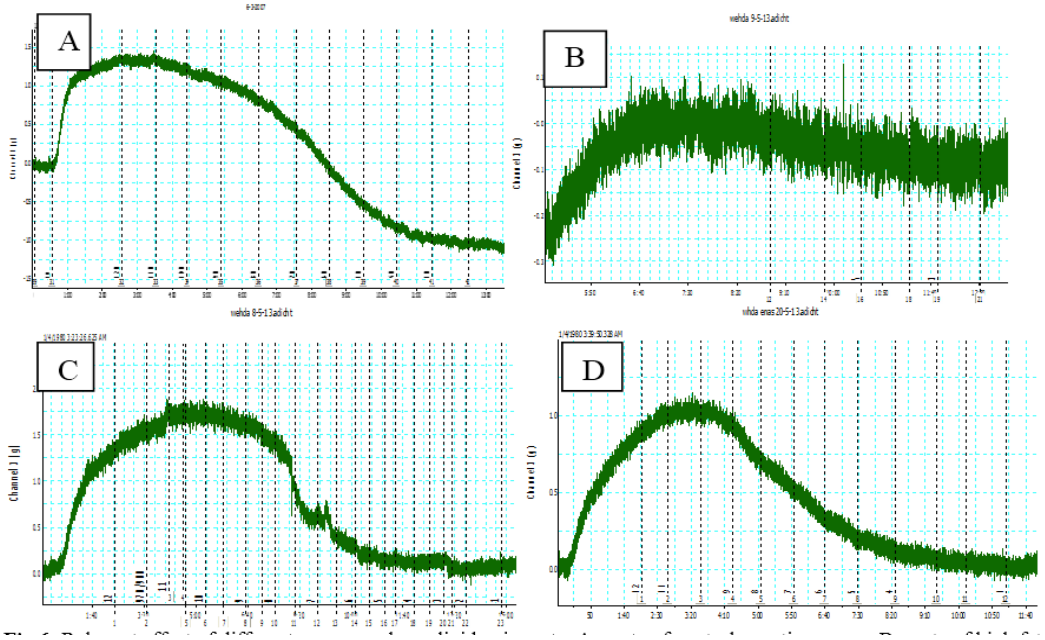

Fig 6: Relaxant effect of different groups on hyperlipidemic aorta. A; aorta of control negative group; B; aorta of high fat diet group; C; aorta of atorvastatin group; D; aorta of hydroalcoholic extract of *T. majus* group.

Table 1: LC-MS/MS identification of compounds in hydroalcoholic extract of *T. majus*

Peak no.	Rt min	Relative area %	Intensity %	$[M-H]^-$	MS^2	Identified compounds
1	0.9	1.5	3	394.5	151.5,179,301.8	n.i.*
2	5.4	1	2	353.6	178.7, 190.7	Coffeoylquinic acid derivatives
3	9.2	2.1	2	353	191 [a]	Coffeoylquinic acid derivatives
4	9.6	1.2	2	364.7	302, 151	Quercetin derivatives
5	11.9	2.3	2	337	191,173	5-Coumaroyl quinic acid
6	15.1	0.8	2	625.2	463,445, 300	Quercetin derivatives
7	17.7	67	100	463.2	300 [a]	Isoquercitrin
8	18.4	2.5	3	505.9	463,445, 301, 270.8	Quercetin acetyl glycoside
9	19.2	1.6	3	505.9	463,301,151	Quercetin acetyl glycoside
10	19.5	3	6	447.9	327,284.7,255	Astragalin
11	20	1.7	4	477.9	271,299/300,314	Isorhamntein 3-o- glucoside
12	20.9	10.8	18	531.4	463, 300.9®	Quercetin derivatives
13	35.2	1.4	3	711.6	675.1,301	Quercetin-7-o-hexoside-3-o-malonyl hexoside
14	38	1.9	2	577.4	375,300®	Quercetin derivatives
15	43.7	1.2	2	341	179, 131	n.i.*

Rt: Retention times, [a] The most abundant ion in the fragmentation spectrum.
®the identification of compound based on two main daughter ions,*n.i.: not identified.

Table 2: Effect of HETM and atorvastatin on the serum lipid levels of hyperlipidemic rats

Groups parameter	Control	HFD	Atorvastatin	HETM
TC (mg/dL)	70.6±3.2	139.2±6.0[a]	97.8±3.0 [a,b]	78.8±3.8[b]
TG (mg/dL)	77.6±3.4	128.2±6.4 [a]	91.0±2.9[b]	82.4±3.4 [b]
HDL-C (mg/dL)	39.6±3.2 [b]	26.4±2.1[a]	55.6±3.6[a,b]	46.6±3.8[a,b]
LDL-C(mg/dL)	15.5±1.5 [b]	87.2±6.5[a]	24.0±5.2 [b]	15.7±3.0 [b]
VLDL-C (mg/dL)	15.5±0.7[b]	25.6±1.3[a]	18.2±0.6[a,b]	16.5±0.7 [b]
CRR	1.79±0.082[b]	5.31±0.61[a]	1.77±0.16 [b]	1.70±0.1[b]
AI	0.79±0.081[b]	4.31±0.64[a]	0.77±0.15[b]	0.70±0.10[b]

TC; total cholesterol, TG; triglyceride, HDL-C; high density lipoprotein, LDL-C; low density lipoprotein, VLDL-C; very low density lipoprotein, CRR; cardiac risk ratio, AI; Atherogenic index. HFD; high fat diet, HETM: hydroalcoholic extract of *T. majus*. Data expressed as mean ± S.E. (n = 5). (a) Significant difference from corresponding negative control group (b) Significant difference from corresponding HFD group by one-way ANOVA at P≤0.05.

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