



SCREENING OF THE POTENTIAL ANTIHYPERCHOLESTERMIC ACTIVITY OF SOME EGYPTIAN HERBS

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ABSTRACT

Hypercholestermia is the main risk factor for development and progress of cardiovascular disease. The direct relation between high cholesterol level and mortality due to heart disease is well known. Herbs such as onion, garlic and ginger attract special interest as herbal medicine because of their potential health benefits especially as antihypercholestermic plants. Screening of the antihypercholestermic activity of the alcoholic extracts of some Egyptian herbs [red onion, garlic, ginger, and garden nasturtium] as well as guggul resin. Hypercholestermia was induced by single i.p. injection of Triton -X100 in a dose of 100 mg/kg body weight of the experimental animals. Male Wistar rats were divided into eight groups each comprising 5 animals as follows : negative control ,Triton-X100, Atorvastatin [reference standard 10 mg/kg],garden nasturtium [750 mg/kg], red onion [50 mg/kg], garlic [0.4g/ ml] , ginger[400 mg/kg] and guggul resin [200 mg/kg] groups. Doses were determined according to reported data. All groups received Triton- X 100 except the negative control. According to cholesterol biosynthesis, metabolism and excretion, blood samples were analyzed after 24, 48 and 72 hrs and at 8th day. Histopathological examination of blood vessels was carried out on the last day. Guggul and ginger showed significant reduction in cholesterol level after 24, 48 and 72 hrs only. While garden nasturtium and onion extracts showed significant protection all over the experiment schedule, where as garlic extract exerts its effect only after 72 hrs and on day 8th of Triton- X 100 treatment. The obtained results revealed that garden nasturtium and onion extracts have dual effect in reducing blood cholesterol and keep the normal appearance of blood vessel.

Key words: Egyptian red onion, Garlic, Ginger, Guggul, Nasturtium, Antihypercholesterolemic effect.

INTRODUCTION

Hypercholesterolemia is the primary metabolic risk factor of atherosclerosis and subsequent progress of cardiovascular disease according to estimation of WHO [2011] [1]. Reducing hypercholestermia is the goal of many medical treatments; among the effective synthetic drugs that took the agreement of the Food and Drug Administration [FDA] classes of drugs called hydroxymethylglutarat co-enzyme [H.M.G. CoA] reductase inhibitors, commonly known as "statins". Although they can reduce the risk of atherosclerosis, FDA reported many side effects among different statins such as

myopathy and fatal rhabdomyolysis [2] Many researches were directed to natural therapy to avoid the side effects of statins and to reduce the cost of the expensive drugs. Therefore, the present study was conducted to evaluate the antihypercholestermic properties of four Egyptian plants [onion, garlic, ginger and garden nasturtium] and guggul. The mechanism underlying the protective effects of these plants with highest effectiveness against Triton-X100 induced hypercholestermia was elucidated.

Allium species such as onion [*Allium cepa* L.] and garlic [*Allium sativa* L.] are rich source of sulfur compounds that are reported to possess antidiabetic,

antibiotic and fibrinolytic activities [3]. In addition they can protect against cardiovascular diseases due to their hypolipidemic, hypocholesteremic, antihypertensive and anti thrombotic activities [4].

Ginger [*Zingiber officinale* R.], a popular rhizome finds its way as Arabian spice and to treat many disease as gastrointestinal disorder. It was reported by Al-Azhary [5] to have anti-inflammatory, antitumor, antilipidemic and antioxidant activities. Similar activity was reported to guggul resin [*Commiphora mukul* E.] which is used for treatment of many diseases as rheumatism, atherosclerosis and hypercholesterolemia [6]. In addition, garden nasturtium [*Tropaeolum majus* L.] which is an ornamental plant that is widely used in the world, as it has many health benefits; tea bags of leaves are used for treatment of inflammation, hypertension, oedema and infection of genital and urinary system [7]. Moreover, the plant is used as expectorant, disinfectant and antibiotic [8]; besides its leaves and flowers are edible.

MATERIALS AND METHODS

Drugs and chemicals

Atrovastatin 10mg/ tablets were purchased from private pharmacies produced by EIPICO Company [Egyptian international pharmaceutical industries company -Egypt]. Triton-X-100, iso -octyl polyoxy ethylene phenol formaldehyde polymer, [Sigma-Aldrich chemical Co. USA].

Plant material and extracts

Fresh red onion and garlic bulbs were purchased from local market in Cairo-Egypt, while fresh ginger and dried guggul resin were purchased from Harazz commercial stores of Medicinal Plants, Cairo- Egypt. Fresh leaves of nasturtium were collected from the Experimental Station of Medicinal Plants [ESMP], Faculty of Pharmacy-Cairo University, Egypt. They are identified by Professor Dr. Ahmed EL-kaphory in Agriculture research center, Giza-Egypt. Voucher specimens under investigation were deposited at the Herbarium of Pharmacognosy Department- Faculty of Pharmacy - Cairo University under numbers: onion [12.12.27a], garlic [12.12.27 b], guggul [12.12.27c], ginger [12.12.27d] and nasturtium 12.12.27.

The bulbs of onion and garlic as well as ginger were peeled and cut into small pieces, dried in shade [air-dried]. Guggul that was made into fine powder, while Fresh leaves of nasturtium were allowed to air-drying .250 grams of each of the air-dried samples were separately macerated in successive aliquots of ethanol [70%] until complete extraction; the solution was filtered and the solvent evaporated at about 50° under vacuum. The yield of the alcoholic extracts were 25.2%, 7.7%,

Screening of serum total cholesterol [mg/dl] was

22.5%, 28%, 11.5% for onion, garlic, ginger, guggul and nasturtium extracts respectively .the extracts was stored at -20°C until use.

Preliminary Phytochemical Screening

Powdered samples [50 g] of the investigated dried plants were subjected to preliminary phytochemical screening for: carbohydrates, saponins, tannins, flavonoids, alkaloids, steroids, anthraquinones and cardiac glycosides [9] Results are represented in Table [1]

Animals

Forty Wister Albino adult male rats weighing 130-180g were obtained from Faculty of Veterinary Medicine- Cairo University-Egypt and housed in polypropylene cages at 25°C for 12 hrs [light and dark cycles were maintained]. The animals were left for an initial adaptation period of 7 day before any experimental adaptation and supplied with a standard pellet diet and water *ad libitum*. The experiment was carried for eight days. The protocol of the study was approved from Veterinary Medicine Collage Animal Ethics Committee.

Experimental design

Triton was freshly prepared in physiological saline [1% v/v] after overnight fasting of rats for 18 hours. All extracts were suspended in physiological saline until administered orally. The experiment was conducted according to [10 -16] procedures. Experimental animals were randomly divided into 8 groups each comprising 5 rats. Animals of the first group were given physiological saline only and kept as negative control [C-ve]. While, animals of the remaining seven groups were given Triton-X100 solution [i.p.] in a single dose equivalent to 100 mg/kg body weight to induce hypercholestermia. One group of the triton treated rats was kept as positive control [C+ve], while the remaining six groups were concurrently given different extracts under investigation as follows: Group 1 Negative control group [C-ve], Group 2 Triton group [C+ve] , Group 3 Atrovastatin group [Atro] plus Triton, Group 4 Nasturtium group [TML] plus Triton, Group 5 Onion group [ROE] plus Triton, Group 6Guggul group [GUE] plus Triton, Group 7 Garlic group [GRE] plus Triton, Group 8 Ginger group [GNE] plus Triton .

Collection of blood samples

Blood was collected after 24, 48, 72 hrs and on the 8th day of treatment by retro orbital sinus puncture, under mild diethyl ether anesthesia in heparinized capillary tubes. Serum obtained by centrifugation of blood samples [3000 rpm/5 min]. All samples were stored at [-20°C] until analysis.

Biochemical analysis

carried out using quantitative diagnostic commercial kits

[Stanbio Cholesterol LiquiColor -USA]. Quantitative enzymatic colorimetric determination of total cholesterol in serum was carried out adopting the method of Allain [17]. The optical density was read at 500 nm on ultra-violet spectrophotometer, the absorbance was proportional to the concentration of total cholesterol. Values are derived according to the following equation:

Serum Total cholesterol [mg/dL] = A sample/A standard x 200
200 is the concentration of the standard [mg/dL]. Result are illustrated in Fig.1 [A-D]

Histopathological examinations

Autopsy samples were taken from the heart of rats in different groups and fixed in 10% formalin for twenty-four hours. Washing with tap water then serial dilutions of alcohol [methyl, ethyl and absolute ethyl] were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness. Then stained by hematoxylin and eosin stain for histopathological examinations through the electric light microscope [18].

Data analysis and statistics

Data were expressed as means ± standard error of means [SEM]. Assessment of the results was performed using one-way analysis of variance [ANOVA] procedure followed by Tukey-Kramer multiple comparison post-tests. Statistical analyses were performed using Software GRAPHPAD INSTAT [Version 2] [ISI Software, Philadelphia, PA, USA]. The level of significance was set at p < 0.05.

RESULTS

Preliminary Phytochemical Screening

From table [1], it could be concluded that carbohydrates, flavonoids and steroids are present in all investigated plants except garlic and onion, which are deprived from flavonoids and steroids respectively. Tannins and saponins, which present mainly in

nasturtium. Alkaloid, anthraquinones and cardiac glycosides are absent in all plants.

Anti-hypercholestermic effect

There are two mechanisms for studying the antihypercholestermic activity Effect of different plant extracts on the cholesterol level after Triton administration are shown in figure 1 [A-D]. Guggul and ginger groups showed a significant protective effect against triton induced increase of the cholesterol level in all time point except at 8th day of Triton treatment. While nasturtium and onion extracts showed significant protective effects against Triton induced increase in cholesterol level at all time point, nasturtium showed a pronounced protective effect after the 8th day of exposure, in comparison to Atrovastatin and control negative groups. On the other hand, treatment of rats with garlic extract at dose [0.4g/ ml] showed protection only after 72 hrs and day 8th of Triton treatment.

Histopathological examination

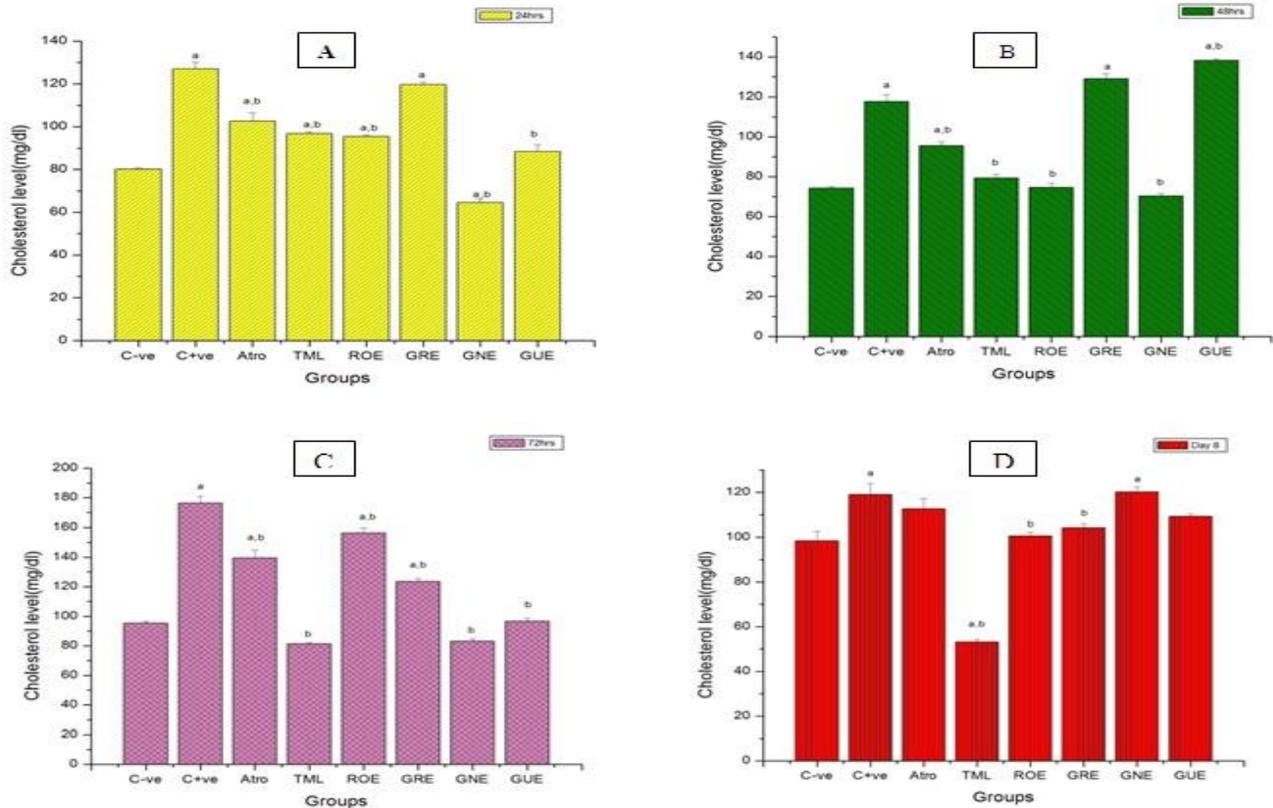
The blood vessels of the negative control [normal] rat heart showing normal appearance with no histopathological lesions [Fig. 2 A], while in triton group there is thickening in the wall of large blood vessels with the presence of large fat vacuoles in sub endothelial layer [Fig. 2 B]. The onion extract, treated group revealed no pathological changes in blood vessels [Fig. 2 D]. Whereas, the blood vessels treated with of nasturtium leaves, [TML] appeared more or less normal with thin wall and intact endothelial lining [Fig. 2 E] as compared to Atrovastatin treated group that showed slight thickening of blood vessel wall [Fig. 2 C], Zenker's necrosis of some arteries, and severe congestion of small blood vessels in the myocardium .Similarly the blood vessels treated with garlic extract showed little thickening of the walls [Fig. 2 F]. There was no effect on the blood vessels when treated with either ginger or guggul but there were necrosis and edema in cardiac muscles [Fig. 2 G, H] respectively.

Table 1.Preliminary phytochemical screening of the plants.

Name of Active ingredient	Onion	Garlic	Ginger	Guggul	Nasturtium
Carbohydrates	+	+	+	+	+
Saponins	-	+	-	-	++
Tannins	-	-	-	-	++
flavonoids	+	-	+	+	++
Steroids	-	+	+	+	+
Alkaloid	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-

[++] moderately present, [+] present, [-] absent

Fig 1. Effects of different plant extracts [onion, garlic, ginger, and garden nasturtium] and guggul resin on total cholesterol level in hypercholestermic rats.



[A]: Cholesterol level after 24 hrs of treatment; [B]: Cholesterol level after 48 hrs of treatment; [C]: Cholesterol level after 72 hrs of treatment; [D]: Cholesterol level after 8 days of treatment.

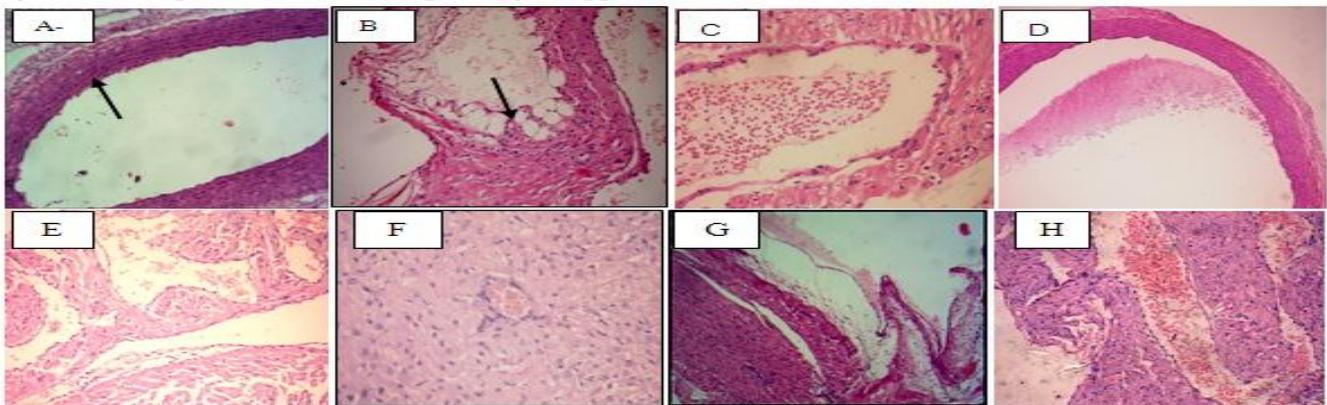
Data expressed as mean ± S.E. [n = 5 animals].

[a] Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$.

[b] Significant different from corresponding Triton group by one-way ANOVA at $P \leq 0.05$.

*[C-ve]: Rats were received physiological saline, [C+ve]: Triton-x100 [Atro]: Atrovastatin, [TML]: Nasturtium leaves extract, [ROE]: Red onion extract, [GUE]: Guggul extract, [GRE]: Garlic extract, [GNE]: Ginger extract.

Fig 2. Effects of plant extracts on histopathological appearance of rat heart and blood vessels.



[A]: blood vessels of the control rat heart showing normal appearance; [B]: Triton treated rat showed thickening in the walls of large blood vessels in heart and Vacuolation of tunica media with many large fat vacuoles in sub endothelial layer; [C]: Atrovastatin treated rat showed thickening of the heart blood vessels wall; [D]: Blood vessel of rat treated with onion extract showing no pathological changes; [E]: Blood vessel revealed normal appearance when treated with nasturtium extract; [F]: Blood vessels of heart treated with garlic extract revealed little thickening of walls; [G], [H] No effect on the walls of the blood vessels when treated with ginger and guggul extracts respectively, but showing necrosis and edema in cardiac muscles.

DISCUSSION

There is a direct relation between cholesterol level and Coronary heart disease [CHD] mortality. It is the primary killer in the growing countries so, management of hypercholestermia can be considered the first line of defense against development of coronary diseases and a global demand to decrease the risk of CHD [19].

Triton X-100 is a non-ionic surfactant that belongs to same category of drugs as Triton X -1339 and Triton WR 1339. It is excessively used to induce acute hyperlipidemia and considered a good model to screen the antihypercholestermic activity of drugs of natural origin [20]. Intraperitoneal injection of Triton X-100 causes two phases of elevation of serum cholesterol level. In the phase I, triton causes increasing the hepatic biosynthesis of cholesterol after 24 hrs of injection then the level decline within 48 hrs of injection due to absorption of the cholesterol from plasma into the tissue. Hepatic synthesis will continue to produce more cholesterol as triton is not completely removed from body. A second sharp elevation of cholesterol level reaching maximum within 72 hrs of injection [phase II], then cholesterol level decline due to metabolism and excretion. On the 8th day, triton will be eliminated completely from the body and the histopathological lesions due to triton could be elucidated. Therefore; plants that affect biosynthesis of cholesterol will be active in phase I, while the plants that influence cholesterol excretion or metabolism will be active in phase II [21, 22].

In the present study, both ginger and guggul extracts provide protection against triton induced hypercholestermia after 24, 72 hrs so they seem to affect both cholesterol biosynthesis and excretion. Their protective effect was better than did by standard Atrovastatin as shown in Fig 1[A, C]. This result was in agreement with Abd-El raheem [23] study who reported that ginger contains [[E]-8 beta, 17-epoxylabeled-ene-15, 16-dial] that interfere with cholesterol biosynthesis in liver of mice and with Heeba study that reported the Antihyperlipidemic activity of ginger was due to activation of the conversion of cholesterol into bile acids.

Whereas, the result of the present investigation are in agreement with those reported with Anurekha [24] who reported that blocking of cholesterol synthesis in liver is the mechanism by which guggul exert its effect as

well as improvement of cholesterol excretion by decreasing its absorption in the intestine.

Red onion extract is found to be more effective in cholesterol reduction than garlic, A fact that can be attributed to the predominant presence of flavonoid in onion than garlic [25] Also Fig. [A, B, D] show better reduction of serum cholesterol exerted by onion than the standard Atrovastatin as well as decreasing in the thickness of the blood vessels as indicated in Fig.2 [D], while garlic was only better than Atrovastatin only on 72 hrs and 8th day. However, other studies carried by Emmanuel [26] on aqueous extract of onion and garlic confirms that there were no significant differences between both onion and garlic, which may be attributed to the presence of sulfated compounds in both species.

There is a significant reduction in cholesterol level exerted by garden nasturtium being more prominent in the 8th day that could be attributed to the presence of high concentration of saponins and flavonoids as indicated in phytochemical screening of the leaves [table 1]. Both saponins [27] and quercetin flavonoid [28] exert hypocholestermic effect.

Histopathological examinations indicated that only nasturtium and onion extract showed no pathological changes on heart and normal appearance of blood vessel. Although garlic, ginger and guggul lower cholesterol levels, but their histopathological examinations showed little thickening of blood vessels with garlic, necrosis and oedema in ginger and oedema in guggul treated groups. Therefore, we can conclude that nasturtium and onion have dual benefit in reducing blood cholesterol and keep the normal appearance of blood vessel.

In conclusion, the present study showed that garden nasturtium as well as onion could be a new promising drug for protection against development of hypercholestermia. Further studies are in progress.

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