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Original Research Article

Antihyperlipidemic activity of a unani formulation in high fat diet-induced obese murine model

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ABSTRACT

Arq Zeera (AZ), a poly herbal unani formulation has been used traditionally as a remedy for reducing body fat and gastric disorder. The current study was designed to investigate the antihyperlipidemic activity of AZ against rat model of high fat diet (HFD)-induced obesity. AZ was prepared and administered orally 7.75 mL/kg/twice a day for 4 weeks to HFD-induced obese rat. Body weight and serum biomarkers were evaluated. Stability testing of AZ was also carried out. At the end of study, HFD significantly (p<0.001) increased body weight, cholesterol, triglycerides, pancreatic lipase activity and malondialdehyde (MDA) levels as compared to normal diet control group. AZ-treated rats significantly (p<0.001) reduced body weights, cholesterol, triglycerides, pancreatic lipase activity and MDA levels as compared to HFD control. These results suggest that AZ has an antihyperlipidemic action against HFD-induced obesity in rats, possibly through lipid lowering action, reduction of intestinal absorption of dietary fat, and increased antioxidant defense.

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1. Introduction

Obesity is a multi-factorial disorder, which is often associated with many other unrelieved diseases such as hyperlipidemia, diabetes mellitus, hypertension, coronary artery disease, and certain cancers. On a global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese (Neuhofer et al., 2014). Therefore, impediment and management of obesity are important for a healthy life. Even though, a number of pharmacological approaches to the management of obesity have been recently investigated, only a few drugs have been approved for clinical usage. Most of the drugs have failed to treat and control obesity either due to effectiveness or adverse effects (Kang and Park, 2012). However, due to the undesirable effects associated with many allopathic drugs, more recent trials have focused on screening natural sources that have been reported to reduce body weight with minimal side effects. Numerous animal studies and clinical studies with various herbal medicines have been performed, and some studies reported significant improvements in controlling body weight without any noticeable adverse effects (Gupta et al., 2012; Cardile et al., 2015). It is reported that plant containing essential oil is responsible for various biological activities such as antioxidant and antimicrobial activity etc. (Mohammadhosseini et al., 2017; Mohammadhosseini, 2017).

AZ is a distillate product and is prepared from four various herbs, namely ajwain (*Trachyspermum ammi* L., Apiaceae), ginger (*Zingiber officinale* Roxb., Zingiberaceae), black caraway (*Carum carvi* L., Apiaceae) and cumin (*Cuminum cyminum* L., Apiaceae). This formulation is considered safe at the dose of 75 mL when administered twice a day to the adult patient by a Unani physician for reducing gastric disorder (Anonymous, 2008; Haque et al., 2012). All four ingredients of same formulation are known to have various biological

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activities due to the presence of essential oil. It is investigated that ajwain oil mainly contains thymol (Haque et al., 2012) and it has good antiobesity action against HFD induced obese rat (Haque et al., 2014). Ajwain oil containing thymol (as major component) is known to possess a number of activities, namely antifungal, antioxidant, and antihyperlipidemic activity (Soni et al., 2016; Sharifzadeh and Shokri, 2016; Singh and Ahmad, 2017; Saleem et al., 2017). The extract of this plant has been reported to significantly reduce blood lipid in obese rabbits and hyperlipidemic patients (Javed et al., 2006).

Ginger extract has been investigated to possess various pharmacological activities such as anti-inflammatory, antiplatelet, antioxidant, and antihypercholesterolemia, antihyperlipidemic and hepatoprotective effects (Chan et al., 2008; Liao et al., 2012; Al-Noory et al., 2013; Shivashankara et al., 2013; Ebrahimzadeh Attari et al., 2017; Kumar et al., 2018; Montserrat-de la Paz et al., 2018). It has been reported that cumin oil containing cuminaldehyde, as a major component, possesses numerous activities including antifungal, antibacterial, antioxidant, hypolipidemic, hypoglycaemic and antiobesity activity (Romagnoli et al., 2010; Hassan et al., 2010; Iacobellis et al., 2010; Ardekani et al., 2010; Haque et al., 2013). Various data reported that cumin fruit has antidiabetic action in alloxan/streptozotocin induced diabetic rats (Jagtap and Patil, 2010). Many data indicated the *Carum carvi* fruits possessed antioxidant, anticonvulsant, antibacterial, antidiabetic and antiobesity activities (Kazemipoor et al., 2013; Thippeswamy et al., 2013; Sadjadi et al., 2014; Showraki et al., 2016; Mahmoudzadeh et al., 2016). Samojlik et al. (2010) found that caraway oil containing cuminaldehyde, 1-phenyl propanol and 4-ethyl-3-nonen-5-yne compound possesses strong antioxidant and hepatoprotective potentials. These functions of each component of AZ suggest that AZ can serve as an effective antihyperlipidemic agent to improve bad blood circulation, adjust blood lipid profiles to normal, recovery of damaged liver and kidney, and remove the excessive accumulation of body fat induced by obesity. Based on the observations, the antihyperlipidemic of AZ in rats fed with HFD was investigated through changes in body weight, serum lipid profile, MDA, and pancreatic lipase activity.

2. Experimental

2.1. Instruments, kits and chemicals

The analytical instruments like pH meter (Orion digital pH meter), Ostwald viscometer (Sigma Aldrich, M.O. USA), Abee's refractometer (Cole-Parmer, India), polarimeter (Sigma Aldrich, M.O. USA) Stalagmometer (Kocour, US), and Pycnometer (Chemkind, India) were used. Total cholesterol (TC), and total triglycerides (TG) kits were obtained from Span Diagnostics Ltd, Surratt, Gujarat, India and high-density lipoprotein-cholesterol (HDL-C) from Reckon Diagnostics Pvt Ltd, Baroda, Gujarat, India. Quanti ChromTM Lipase Assay Kit (DLPS-100), (Corporate Place, Hayward, CA 94545, USA), thiobarbituric acid reactive substances (TBARS) assay kit (Cayman, Ann Arbor, MI, USA) and orlistat (purity>99.5%) were obtained from Labex corporation, vasant kunj, New Delhi. Normal pellet diet (NPD) were purchased from Pranav Agro Industries Ltd (Amrut rat feed, Maharashtra, India) and HFD from NIN (Research Diets Inc, Hyderabad, India).

2.2. Plant materials

The entire herbal ingredients were procured from Shamsi dawakhana (raw drug supplier), Ballimaran, New Delhi, (India) with the knowledge of Unani physician in the month December. All the four ingredients were identified and authenticated by Dr. H. B. Singh (Scientist F), from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen and identification certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 were obtained and kept in the department for future reference. All the samples were rinsed with tap water to remove salt and dried in an air dryer at 37 ºC for 40 h. The dried sample was ground and the coarse powder was stored at -20 ºC until used.

2.3. Preparation of AZ

The dried coarse powder of *Zingiber officinale* (125 g), *Carum carvi* (125 g), *Cuminum cyminum* (375 g) and *Trachyspermum ammi* (250 g) was soaked in purified water and transferred to the distillation plant along with purified water (12 L). This was distilled at 100 ºC for about 5:30 h and 7.5 L of distillate was collected (Haque et al., 2012).

2.4. Accelerated stability testing of AZ

To establish the stability of AZ, we had prepared three samples of polyherbal formulation and the parameters like pH, viscosity, refractive index (R.I), surface tension, density, optical rotation, free radical scavenging activity and microbiological load were assessed at an interval of 0, 48 and 120 h, maintaining the packs of formulations at 30 ± 2 ºC and at 65% relative humidity (Salunkhe and Bhise, 2001). The analytical instruments like pH meter, Ostwald viscometer, Abee's refractometer, polarimeter, Stalagmometer and pycnometer were used for the measurement of pH value, viscosity, refractive index (R.I), optical rotation, surface tension and specific density respectively.

2.5. Experimental animals and diet

The study was approved by the Institutional Animal

Ethics Committee (IAEC) of Hamdard University (Letter number-607, 13/6/11), New Delhi, which is registered with Committee for the purpose of Control & Supervision of Experiments on Animals (CPCSEA), Government of India. Wistar male rats, weighing 150-200 g, were procured from the Central Animal House Facility, Hamdard University, New Delhi, and acclimatized under standard laboratory conditions at 25 ± 2 ºC, and relative humidity (50% ± 15%) and normal. The animals were kept in polypropylene cages under standard laboratory conditions (12 h light and 12 h dark: day: night cycle) and had a free access to tap water *ad libitum*. After 7 days of acclimation, animals were randomly divided into four groups (n=6): one normal control group, one HFD control and remaining 3-4 as treatment groups. Animals in normal control group were fed with normal pellet diet (NPD) consisting of 12.5% lipids, 62.3% carbohydrate and 24.3% protein, while the other groups were fed with HFD consisting of 60% fat, 20% protein, and 20% carbohydrate (in g/kg) *ad libitum*, respectively, throughout the experiment.

The dose needed for rats was calculated on the basis of its human dose informed by Unani Physician using the formula (Gupta et al., 2012) (Eqn. 1):

$$\text{Animal dose}\left(\frac{\text{mL}}{\text{kg}}\right) = \frac{\text{Human dose (75 mL twice a day}}{\text{Average body wt (60 kg)}} \times 6.2 \text{ (Conversion factor)} \quad \text{(Eqn. 1)}$$

2.6. Treatments

Treatments were started from 15th day and continued for four weeks. The treatment groups HFD + AZ and HFD + orlistat were given AZ and orlistat respectively at 7.75 mL/kg, twice per day and 30 mg/kg of the body weight once per day of body weight by oral root. During the course of treatment, the treatment groups were fed with HFD continuously. Normal control group received 0.5% carboxy methyl cellulose (CMC) sodium aqueous solution. Orlistat drugs were suspended in 0.5% carboxymethyl cellulose sodium aqueous solution for animal's treatment. AZ was also dissolved in 0.5% CMC sodium aqueous solution to form suitable dose. Body weights were monitored every week. At the end of the treatment period, rats were subjected to fasting for 12 h.

2.7. Serum biomarkers analysis

Blood was collected from the retro-orbital plexus of the all groups of overnight fasted rats using micro capillary tubes containing heparin on day 43. Serum was separated by centrifugation (4000 rpm, 10 min) and transferred to Eppendorf tubes. The concentrations of total cholesterol (TC), triglycerides and high-density lipoprotein-cholesterol (HDL-C) in serum were measured with commercial kits. The concentrations of pancreatic lipase in the serum were measured with quanti chromTM lipase assay kit. All other chemicals used were of analytical grade. Double distilled water was used for all biochemical assays. Estimations of serum low-density lipoprotein-cholesterol (LDL-cholesterol) were measured by using of Friedewalds equation (Friedewald et al., 1972). The serum malondialdehyde (MDA) level was measured using the thiobarbituric acid reactive substances (TBARS) assay kit.

2.8. Hepatic tissue study

The considered rats were scarified and four rat hepatic tissue samples were collected, fixed in 10% formalin buffered solution, cut into 5-µm sections and stained with haematoxylin/eosin. The sections of hepatic tissues were studied to determine the level of tissue injure or steatosis by HFD.

2.9. Toxicity studies of AZ

Evaluation of oral acute toxicity of AZ was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals (425) (Anonymous., 2001). A limit test (120 mL/kg body weight) was performed using five male Wistar rats (150-200 g) from our breeding stock. All the animals were observed for behavioral changes and mortality till 14 days after administration of the dose.

Evaluation of oral 28 day toxicity of AZ was carried out according to the OECD guidelines for testing of chemicals (407) (Anonymous, 2008).

2.10. Data analysis

Data analysis was carried out using Graphpad Prism 3.0 (Graphpad software, San Diego, California, USA). All of the data were expressed as mean ± SEM. Groups of data were compared with the analysis of variance (ANOVA) followed by Dennett's t-test to identify significance among groups. Values were considered statistically significant when $p<0.05$.

3. Results and Discussion

3.1. Stability study of AZ

It is shown in Table 1 that accelerated stability data followed a linear pattern throughout the stability testing. Physical parameters such as color, odor etc. did not produce significant changes. Furthermore, the harmful microbes were absent throughout the accelerated stability studies. The above stability studies indicate that AZ is stable at room temperature for quite a longer period of time. Stability testing study of AZ revealed that it is a stable and safety formulation at room temperature without showing any contamination. It may be due to the presence of essential oil. Thus, the present study indicates that AZ is a stable formulation

Table 1
Accelerated stability data of AZ.

S .no	Parameters	Observations and time tables			
		Time (hrs)(0)	**Time(hrs)(48)**	**Time (hrs)(120)**	**Mean ± SD**
1	Colour	Watery	Watery	Watery	
2	Odour	Aromatic	Aromatic	Aromatic	
4	External appearance	Clear liquid	Clear liquid	Clear liquid	
5	Density	0.9896 g/mL	0.9896 g/mL	0.9896 g/mL	0.9896 g/ml
6	Viscosity	1.02 cST	1.02 cST	1.02 cST	1.02 cST
7	Refractive index	1.467	1.467	1.467	1.467
8	Optical rotation	+ 0.4	+ 0.4	+ 0.4	+ 0.4
9	pH	6.83	6.83	6.83	6.83
10	Surface tension	51.97 mN/m	51.86 mN/m	51.57 mN/m	51.57 mN/m
11	Free radical scavenging activity	87.25 ± 0.22%	86.75 ± 0.22%	88.05 ± 0.22%	87.35 ± 0.65 %
11	**Microbiological Load**				
11.1	Total Bacterial Count, cfu/mL	<1.0	<1.0	<1.0	
11.2	Total Fungal Count, cfu /mL	<1.0	<1.0	<1.0	
11.3	*Escherichia coli*, cfu/mL	absent	absent	absent	
11.4	*Salmonella enterica* cfu/25mL	absent	absent	absent	
11.5	*Staphylococcus aureus*, cfu/mL	absent	absent	absent	

cfu=Colony Forming Unit.

Table 2
The results of the effect of AZ on body weight.

Groups	Initial wt (g)	14th day	43rd day	Weight gain during treatment (g)
Control	157.2 ± 2.166	178.87 ± 4.34	250.08 ± 3.12	71.21 ± 5.12
HFD	157. 32 ± 1.261	212.56 ± 3.56	340.45 ±10.2	127.89 ± 6.02$^{\$\$}$
HFD + AZ	181.1 ± 5.432	246.75 ± 3.67	307. 97 ± 4.31	61.22 ± 5.90**
HFD + O	166.21 ± 2.321	221.45 ± 5.84	285.64 ± 4.37	64.19 ± 5.12**

All values were expressed as mean ± SEM for six rats in each group. * $p<0.05$ as compared to HFD group. $^{\$\$}$ $p<0.001$ as compared to control group. ** $p<0.001$ as compared to HFD group.

Table 3
The results of the effect of AZ on lipid profile.

Groups	Total triglycerides level (mg/dl)	Total cholesterol level (mg/dl)	HDL-cholesterol level (mg/dl)	LDL-cholesterol level (mg/dl)
Control	49.67 ± 2.51	61.87 ± 2.18	33.18 ± 0.89	18.956 ± 0.81
HFD	107.15 ± 4.37$^{\$\$}$	98.59 ± 2.88$^{\$\$}$	25.07 ± 2.59$^{\$\$}$	52.89 ± 3.81$^{\$\$}$
HFD + AZ	51.80 ± 1.89**	64.02 ± 1.39**	37.11 ±1 .60**	18.12 ± 0.80**
HFD + O	44.20 ± 3.31**	73.98 ± 1.174$^{\$\$}$	29.6 6 ± 1.09**	35.09 ± 1.81**

All values were expressed as mean ± SEM for six rats in each group. * $p<0.05$ as compared to HFD group. $^{\$\$}$ $p<0.001$ as compared to control group. ** $p<0.001$ as compared to HFD group.

which may be beneficial for obese patients.

3.2. Effect of AZ on body weight

The final mean body weight and body weight gain of the HFD group was significantly ($p<0.001$) higher than those of the control group (Table 2). Four weeks after AZ treatment, the body weight gain of rat was significantly ($p<0.001$) lower than those of rats in the non treated HFD group. High-energy diets are widely used in nutritional experiments as a strategy to induce overweight conditions and fat deposition in animals (Madsen et al., 2010). We also observed that final body weight and body-weight gain in the HFD group were greater as compared to the NPD group. Administration of AZ or orlistat for 4 weeks remarkably decreased the body weight gain compared with that of the HFD group.

3.3. Effect of AZ on serum lipid levels

Rats in the HFD group exhibited significantly ($p<0.001$) higher TG, TC, and LDL-C and lower HDL-C as compared to rats in the NPD group (Table 3). However, AZ or orlistat treatment led to a reversal of the aforementioned parameters to the levels similar to those of the NPD group. AZ treatment significantly ($p<0.001$) decreases serum TG, TC and LDL-C and increases HDL-C compared with no treatment (HFD group). In general, a high-fat diet significantly increases the TC and TG levels in serum (El Ayed et al., 2017). Our data also showed that rats in the HFD group exhibited significantly higher TG, TC and LDL-C levels, and lower HDL-C. However, the administration of AZ or orlistat reduced these parameters to near normal levels in serum. These results indicate that oral administration of

Table 4
The results of the effect of AZ on pancreatic lipase activity.

Groups	Pancreatic lipase activity (U/L)
Control	165.03 ± 13.55
HFD	558.18 ± 36.02$^{\$\$}$
HFD + AZ	180.38 ±5.54**
HFD + O	132.46 ± 4.58**

All values were expressed as mean ± SEM for six rats in each group. * $p<0.05$ as compared to HFD group. $^{\$\$}$ $p<0.001$ as compared to control group. ** $p<0.001$ as compared to HFD group.

Table 5
The results of the effect of AZ on MDA.

Groups	MDA (μM)
Control	4.51 ± 0.05
HFD	8.03 ± 0.16$^{\$\$}$
HFD + AZ	5.04 ± 0.25**
HFD + O	6.09 ± 0.15**

All values were expressed as mean ± SEM for six rats in each group. * $p<0.05$ as compared to HFD group. $^{\$\$}$ $p<0.001$ as compared to control group. ** $p<0.001$ as compared to HFD group.

AZ suppresses the accumulation of body fat, resulting in improved lipid profiles in serum.

3.4. Effect of AZ on pancreatic lipase activity

HFD-fed rat exhibited significantly ($p<0.001$) an increased serum pancreatic lipase activity compared to the levels observed in NPD-fed rat. However, AZ or orlistat administration showed decreased pancreatic lipase activity in blood compared to the HFD group. AZ administration significantly ($p<0.001$) decreased pancreatic lipase activity levels in serum compared to the HFD group (Table 4). Han et al. (2001) have suggested that high fat diet for a long time may result in increased pancreatic lipase activity. Our data also showed that rats in the HFD group exhibited significantly higher pancreatic lipase activity. Interestingly, AZ treatment significantly reduced the serum pancreatic lipase activity levels in obese rats. Pancreatic lipase inhibitor which helps to limit intestinal fat absorption at the initial stage, has been proved as useful medications for the treatment of hyperlipidemia. These results suggest that AZ can be considered to be useful as a medication for the treatment of hyperlipidemia.

3.5. Effect of AZ on serum MDA

Lipid peroxidation was measured by the TBARS assay, which evaluates oxidative stress by assaying levels of MDA, a product of lipid breakdown. Serum MDA was significantly ($p<0.001$) higher in HFD-fed rat than in NPD-fed rat, whereas HFD + AZ and HFD + orlistat treatment significantly ($p<0.001$) inhibited the HFD-induced increase in MDA levels (Table 5). AZ treatment significantly reduced MDA level as compared to HFD group. Accumulation of body fat is correlated with an increase in the lipid peroxidation product, MDA. The quantity of TBARS is an important indicator of lipid

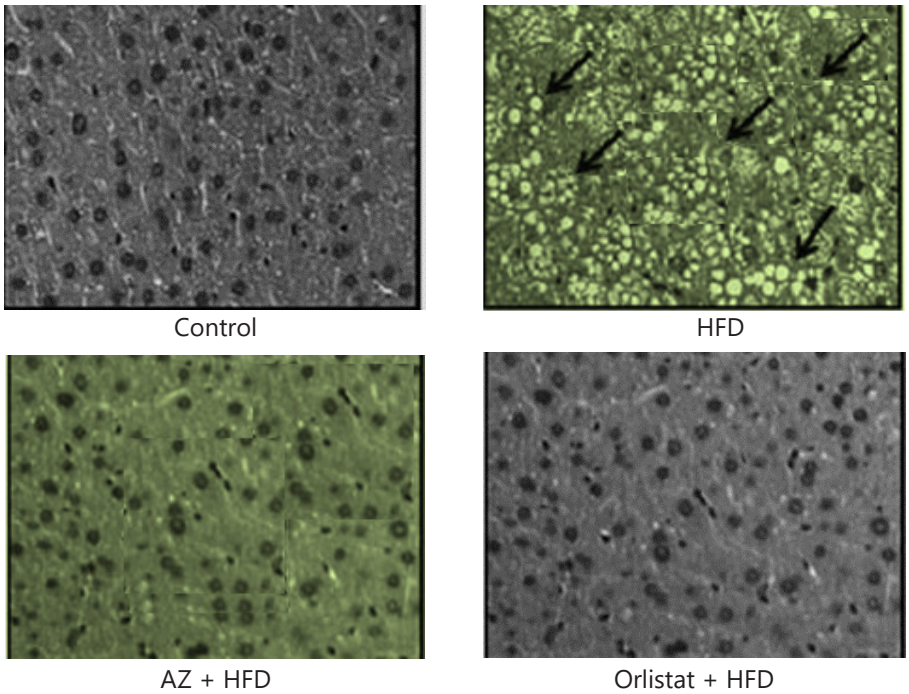

Fig. 1. Fat accumulation (indicated by the arrow) in the form of large fat droplets present in liver of HFD-fed rats. Representative pictures of hematoxylin and eosin–stained sections of liver tissue from AZ or orlistat treatment show smaller-sized adipocytes than in HFD-fed rats. Magnification X 200.

peroxide accumulation in the body. Malondialdehyde is a by-product of lipid peroxidation and reflects the degree of oxidation in the body (Ohkawa et al., 1979). The MDA level in serum decreased significantly in the group treated with AZ. The reduction in the lipid peroxidation could be related to the antioxidant and free radical scavenging properties of AZ. AZ formulation exhibited highly scavenging of free radicals property (Table 1). Thus, this result suggests that the antioxidant activity of AZ may, at least partly, contributes to the reduction of blood lipid level.

3.6. Histopathological studies of liver

Our histological examination of liver demonstrated the inflammation or steatosis in liver tissues of the HFD group as compared to control group (Fig. 1). AZ or orlistat treatment noticeably attenuated the extent of steatosis or inflammation in liver tissues in comparison with HFD group. HFD is known to increase the synthesis of fatty acids or TG accumulation in the liver resulting in macro vesicular hepatic steatosis (Hasan et al., 2014). Our histological examinations also revealed that macro vesicular steatosis in liver tissues of the HFD group were higher than the NPD group (Fig. 1). Moreever, AZ or orlistat administration noticeably attenuated the extent of steatosis, suggesting that AZ maybe a potential promising candidate for the protection of nonalcoholic steatohepatitis.

3.7. Toxicity profile of AZ

Administration of AZ in a dose of 120 mL/kg body weight did not produce any behavioral abnormalities in the animals. As all tested animals survived, the oral LD_{50} of AZ in rats was found to be >120 mL/kg per day, body weight.

4. Concluding remarks

In conclusion, the administration of AZ to HFD-induced obese rat significantly reduced body weight gain, serum lipid level, MDA and pancreatic lipase activity. These findings demonstrate that AZ treatment has an antihyperlipidemic effect against a high-fat-diet-induced obesity in rats possibly through lipid lowering action, reduction of intestinal absorption of dietary fat and increased antioxidant activity. The antihyperlipidemic effects of AZ support its potential as a therapeutic agent or a source of therapeutic substances; it's non-toxicity in rat and historical use for other indications in humans indicate it may be safer than antihyperlipidemic pharmaceuticals currently available. However, further study is needed to clarify the pharmacological mechanisms of AZ and to identify the active components responsible for its antihyperlipidemic and antioxidant effects.

Conflict of interest

The authors declare that there is no conflict of interest.

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