



Comparative lipid and uric acid suppressing properties of four common herbs in high fat-induced obese mice with their total phenolic and flavonoid index

Tanoy Mazumder, Imran Parvez Mamun, Md. Safayat Zaman, A.K.M. Khairul Islam, Shahjala Chowdhury, Md. Sharif Reza, Md. Saddam Hussain*

Faculty of Science, Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814, Bangladesh

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ABSTRACT

Our present study was designed to investigate the comparative anti-obesity efficacy of ethanolic extract of *Azadirachta indica* A. Juss., *Trigonella foenum-graecum* L., *Allium sativum* L. and *Zingiber officinale* Roscoe in high fat-induced mice with their total phenolic and flavonoid profile. Total phenolic and flavonoid content were determined by Folin–Ciocalteu's and Aluminium chloride UV method respectively. In our study, 55 healthy mice were separated into 11 groups to take their respective treatments. Lipid and uric acid profile were estimated by using the enzymatic colourimetric method. Ethanolic extract of *A. indica* contained the highest phenolic and flavonoid content. *A. indica* normal and high fat diet group showed reduced weight gaining tendency than other extract groups. *A. indica* at a dose of 400 mg/kg body weight significantly ($p < 0.001$) reduced serum cholesterol (SC), triglyceride (TG), and uric acid (UA) level than other three extracts when compared with the control group. Thus, a considerable correlation was found between serum uric acid reducing potentials of the present experimental extracts with a lipid-lowering profile. Pathological examination revealed that the average weight of liver and kidney were significantly decreased in *A. indica* normal. Results obtained from the present study it can be concluded that ethanolic extract of *A. indica* possesses better lipid-lowering efficacy than the other three herbs.

1. Introduction

In the present context, obesity and cardiovascular diseases are the major global health issue, play a predominant role in increasing global morbidity, mortality and reduced life expectancy [1]. Obesity is a chronic metabolic disorder result from the imbalance between lipogenesis and lipolysis which is associated with excess energy intake and low energy expenditure [2]. Lipogenesis is the process where free fatty acid is stored in the form of triglyceride and lipolysis stored triglyceride is metabolized to free fatty acid and glycerol [3]. This imbalance brings about several health complications and risks like cardiovascular diseases, type 2 diabetes mellitus, osteoarthritis, dyslipidemia, asthma, fatty liver disease, congestive heart failure, stroke, gall bladder disease and development of cancer associated with insulin resistance [4,5].

With the progress of industrialization, the lifestyle has been changed and has a predominant effect on the health of people. The modernization of societies changes the food habit where people consume more saturated fats and refine sugar instead of foods with high fibre content [6].

Hyperlipidemia is characterized by elevated levels of blood total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and reduced levels of high-density lipoprotein-cholesterol (HDL-C) which is responsible for the development of atherosclerosis and other complications like ischemic heart diseases, cerebrovascular diseases, peripheral vascular diseases and these diseases contribute 29% of total death in 2005 [7,8]. A high level of serum blood cholesterol contributes to various physiological complications like heart attack, stroke, diabetes, kidney failure etc. [9]. Recent studies suggested that not only total cholesterol but also different lipoproteins like LDL and HDL play a significant role in lipid-associated disorders [10]. An elevated level of serum LDL cholesterol and reduced level HDL cholesterol are important independent risk factors of cardiovascular diseases and thus their control is important for the development of the treatment of cardiovascular diseases and in the prevention of atherosclerosis [11]. Agents used for the management of hyperlipidemia such as statins, bile acid-binding resins, fibric acid derivatives, nicotinic acid derivatives have potential side effects like hyperuricemia, diarrhea, flushing, nausea, myositis, gastric irritation, dry skin, and abnormal liver function etc. [12,13].

* Corresponding author.

E-mail address: shussain9.phar@nstu.edu.bd (Md.S. Hussain).

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List of abbreviations

SC	Serum Cholesterol
TG	triglyceride;
UA	Uric Acid
LDL-C:	Low-Density Lipoprotein-cholesterol
HDL-C:	High-Density Lipoprotein-cholesterol
HFD	High Fat Diet;
SUA	Serum Uric Acid
SEM	Standard Error of Mean
ND	Normal Diet;
PL:	Pancreatic Lipase
LDL:	Low-density lipoprotein (LDL)
HL:	Hepatic Lipase

Because of these potential side effects plant derive preparations to become a good choice for the management of hyperlipidemia because of their less toxicity, availability and rapid absorption in the body than currently used drugs [14]. Plant-derived bioactive compounds i.e. phenolic and flavonoid compounds produce potential antioxidant activity and oxidative stress has been implemented in the management of cardiovascular and neurodegenerative diseases. So plants exuberant with phenolic and flavonoid compounds can produce a positive impact on the development of anti-hyperlipidemia therapy [15].

In humans, purine catabolism ends up with the production of serum uric acid and an increase in uric acid production but decrease its excretion create hyperuricemia in the body. Various health complications including cardiovascular disease (CVD), hypertension and metabolic syndrome are closely associated with the elevated serum uric acid level as like hyperlipidemia [16]. Based on pathophysiological and metabolic researches, hyperuricemia and obesity are likely to interact with each other. Hyperuricemia accelerates hepatic and peripheral lipogenesis, which can lead to obesity through a variety of mechanisms [17]. Furthermore, it has been reported that total cholesterol, serum LDL cholesterol, triglycerides, the ratio of triglycerides to HDL cholesterol, apolipoprotein-B levels, and ratio of apolipoprotein-B to AI are significantly associated with serum uric acid levels, whereas serum HDL cholesterol levels are inversely associated [18]. On the other hand, obesity has reported to increase uric acid synthesis and impede its excretion by kidneys which promote the development of hyperuricemia [19]. It has also been reported that the elevation of serum uric acid level may be responsible for subsequent weight gain [20]. Considering these relationships between hyperlipidemia and serum uric acid level, we also investigated the uric acid reducing the efficacy of ethanolic extract of *Azadirachta indica* A. Juss., *Trigonella foenum-graecum* L., *Allium sativum* L. and *Zingiber officinale* Roscoe.

Azadirachta indica A. Juss (family: *Meliaceae*) traditionally known as neem is a fast-growing medicinal plant commonly available in India, Pakistan, Bangladesh, Nepal, Africa and America [21,22]. Different bioactive compounds like azadirachtins, nimocinol, isomeldenin, 2, 3'-dehydro-salanol gedunin, nimbin, nimolincinol, odoratone, azadironeolide, isoazadironeolide, naheedine and mahmoodine have been isolated from the leaves of this medicinal plant [22]. The plant is traditionally used in the management of cancer, hypertension, heart diseases, skin disorders and hypolipidemic, hepatoprotective and hypotensive activities and to control fever [23,24].

Fenugreek (*Trigonella foenum-graecum* L.; family: *Fabaceae*) is a medicinal herb also used for cooking purpose. Active compounds of fenugreek included soluble fibre, saponins, trigonelle, diosgenin, and 4-hydroxy isoleucine [25]. It has been reported that fenugreek seeds are exuberant with soluble dietary fibre (SDF) which is responsible for the reduction of serum and liver cholesterol level [26]. Total dietary fibre reduces cholesterol level by increasing fecal secretion of bile acids and

salts as well as by inhibiting hepatic cholesterol biosynthesis by short-chain fatty acids produced by bacterial fermentation of soluble dietary fibres in the lower parts of the large intestine [27]. In different parts of the world, fenugreek has been widely used for the management of weakness, edema, indigestion, antihyperlipidemic and stimulant [28]. The seeds of *T. foenum-graecum* L. were found to be very useful in obesity and reduce the lipid level of blood. Several studies have been reported about the hypolipidemic, hypoglycemic, antioxidant, and immunomodulatory effects of *T. foenum-graecum* L. seeds [29].

Garlic (*Allium sativum* L.) belongs to the family Liliaceae is a perennial bulb forming plant has been widely used in dietary and medicinal purpose from the ancient time [30]. It has been reported that its decomposition product diallyl disulfide, diallyl trisulfide are well known for their antihyperlipidemic properties and another compound allicin is responsible for lowering serum cholesterol level [31]. From ancient time garlic has been a cure for heart disease, headache, cancer and management of cholesterol [32].

Ginger is the rhizome of *Zingiber officinale* Roscoe belonging to the family of Zingiberaceae and is native in Asia but also grown in Africa, India and other tropical regions [33]. *Z. officinale* Roscoe is primarily used in the management of nausea, but it is also used as an anti-inflammatory, a pain remedy, a warming remedy and a cholesterol-lowering herb [34]. Several constituents have been isolated from ginger including 3-dihydroshogaols, paradols, dihydroparadols, acetylated gingerol derivatives, gingerdiols, mono and diacetyl gingerdiol derivatives, 1-dehydrogingerdiols, diaryl heptanoids, epoxide diaryl-heptanoids, methyl ether derivatives, ferulic acid derivatives and terpenes such as zingerones and zingerols [35].

The objective of our present study is to evaluate the comparative lipid-lowering efficacy of ethanolic extract of these four medicinal subjects with their total phenolic and flavonoid profile.

2. Materials and methods

2.1. Collection of the plants part

Fresh leaves of *A. indica* A. Juss were collected from Companigonj Upazila, Noakhali District, Chittagong, Bangladesh in July 2018. Fresh *A. sativum* L., *Z. officinale* Roscoe, and *T. foenum-graecum* L. seeds were brought from the local market of Noakhali. Identification and authentication of these plant parts were confirmed by the National Herbarium of Bangladesh (Authentication number: 44051(*A. indica* A. Juss); 44052 (*A. sativum* L.); 44053 (*Z. officinale* Roscoe); 44054 (*T. foenum-graecum* L.).

2.2. Drying grinding and extraction of plants part

Collected plant materials were separated from the undesired plant parts and objects. Fresh materials washed thoroughly and air-dried in the room for 14 days. The plant parts were crushed into a coarse powder with the help of a suitable blender (Panasonic MX-GX1521). The prepared powder was stored in airtight containers and kept in a cool, dry and dark place. Approximately 700 gm of the powder materials taken in a clean, flat bottomed glass container and soaked in 1.5 L of distilled ethanol. The containers were tightly sealed with aluminium foil and kept for 21 days accompanying by occasional shaking and stirring. Then the whole materials were undertaken through a coarse filtration process by filter cloth, fresh cotton plug and finally through Whatman no. 1 filter papers. The volume of the filtrate was then reduced by using a rotary evaporator at low temperature and pressure. Finally, 8.78 g of yield was obtained and stored in a conical flask tightly sealed with aluminium foil.

2.3. Materials

Atorvastatin (10 mg) was purchased from the local market which is manufactured by BEXIMCO pharmaceuticals Ltd, Bangladesh. All other

reagents and materials used in the experiment were analytical grade.

2.4. Preparation of extract and standard drug suspension

To administer, extract at a dose of 400 mg/kg body weight of mice, the extracts were measured accurately and triturated in a unidirectional way by adding a small amount of distilled water. After proper mixing of extract, distilled water was slowly added to make the final volume of the suspension up to 5 ml. Atorvastatin at the dose of 70 mg/kg body weight was prepared by trituration the tablet by mortar pestle, accurately weighing and dissolved in a small amount of water. Water was added slowly to make the final volume of the suspension up to 5 ml. To stabilize the suspension, it was stirred well by vortex mixture [12].

2.5. Determination of total phenolic content

The total phenolic contents of each sample were determined by the Folin–Ciocalteu's method [35]. The concentration of 2 mg/ml of plant extract was prepared in ethanol and 0.5 ml of each sample were introduced into test tubes, mixed with 2.5 ml of a 10-fold dilute Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with Parafilm and allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm spectrometrically. All determination was performed in triplicate. Total phenolic content was determined as mg of Gallic acid equivalent per gram using the equation obtained from a standard Gallic acid calibration curve ($R^2 = 0.889$).

2.6. Determination of total flavonoid content

The total flavonoid content of sample extracts was determined by the use of a slightly modified UV spectrometer method named the Aluminium chloride UV method [36]. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in ethanol and then diluted to 25, 50, 100 and 200 $\mu\text{g}/\text{ml}$. 1 ml of the diluted standard solutions were separately mixed with 3 ml of ethanol, 0.2 ml of aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer against a water blank. Similarly, 1 ml of ethanol extracts was reacted with aluminium chloride for the determination of flavonoid content. Total flavonoid content was determined from the calibration curve ($R^2 = 0.9947$).

2.7. Experimental animals

For this experiment, male swiss albino mice were used and collected from the animal house of Jahangirnagar University, Savar, Dhaka, Bangladesh. Feeding of mice was done by normal pellet diet and water *ad libitum* at a temperature of $25 \pm 20^\circ\text{C}$ and $55 \pm 10\%$ relative humidity. The experimental animals were kept in transparent polypropylene cages. They were allowed to acclimatize for 7 days to the laboratory conditions before experimenting [37]. The protocol used in this study in the mice model was carried out based on the guideline of the Institutional Animal Ethics Committee.

2.8. Study design

Exactly 55 healthy mice (19–20 g) were randomly selected for the experiment and separated into 11 groups while each group contains 5 mice.

Group 1 (control group): All the mice were fed a normal pellet diet.

Group 2 (HFD group): All the mice fed a high-fat diet- 20% (w/w) cow fat with normal pellet diet.

Group 3 (Standard group): All the mice were fed a high-fat diet along with atorvastatin at a dose of 70 mg/kg body weight.

Group 4: All the mice were fed a normal pellet diet along with 400 mg/kg body weight extract of *A. indica A.Juss.*

Group 5: All the mice were fed a high-fat diet along with 400 mg/kg body weight extract of *A. indica A.Juss.*

Group 6: All the mice were fed a normal pellet diet along with 400 mg/kg body weight extract of *A. sativum L.*

Group 7: All the mice were fed a high-fat diet along with 400 mg/kg body weight extract of *A. sativum L.*

Group 8: All the mice were fed a normal pellet diet along with 400 mg/kg body weight extract of *T. foenum-graecum L.*

Group 9: All the mice were fed a high-fat diet along with 400 mg/kg body weight extract of *T. foenum-graecum L.*

Group 10: All the mice were fed a normal pellet diet along with 400 mg/kg body weight extract of *Z. officinale Roscoe.*

Group 11: All the mice were fed a high-fat diet along with 400 mg/kg body weight extract of *Z. officinale Roscoe.*

All the experimental groups were treated daily with their respective foods and water for 21st days and each mouse were weighed every day.

2.9. Animal sacrifice and pathological examination of organ weight

After completing the 21st days of treatment, on the 22nd day, the experimental animals were carefully anaesthetized with a suitable dose of chloroform before sacrificing. After sacrificing liver, kidney, heart, and accumulated fat were separated from the carcasses and preserved in normal saline. After collecting the organ was weighed separately and the average weights of each group were compared statistically.

2.10. Determination of serum cholesterol, triglyceride, and uric acid level

Total serum cholesterol was determined by an enzymatic colorimetric method (endpoint) [38] whereas serum triglyceride (TG) was estimated by enzymatic colorimetric GPO-PAP method [39] by using a double beam spectrophotometer (Simadzu, Japan). Finally, serum uric acid (SUA) level was measured by using the method of Fossate et al. (enzymatic colorimetric method) with slight modification [40].

2.11. Statistical analysis

All the results were expressed as Mean \pm SEM (Standard Error of Mean). All the groups were compared with the control. $p < 0.05$ was considered to be statistically significant and $p < 0.001$ was considered to be highly significant. All the statistical evaluation was conducted using SPSS (version 21).

3. Results

3.1. Total phenolic and flavonoid content

Table 1 represents the total phenolic and flavonoid content of ethanolic extract of *A. indica A.Juss* leaves, *T. foenum-graecum L.* seeds, *A. sativum L.*, and *Z. officinale Roscoe*. The highest phenolic content was

Table 1

Total phenolic and flavonoid content of ethanolic extract of *A. indica A.Juss*, *A. sativum L.*, *T. foenum-graecum L.* and *Z. officinale Roscoe*.

Extract of	TPC (mg GAE/gm of dry extract)	TFC (quercetin equivalent/100 gm of extract)
<i>A. indica</i>	205.31 \pm 0.398	56.04 \pm 1.89
<i>T. foenum-graecum</i>	177.263 \pm 1.2	14.74 \pm 0.250
<i>A. sativum</i>	111.632 \pm 0.699	21.9 \pm 0.255
<i>Z. officinale Roscoe</i>	94.39 \pm 1.097	7.7 \pm 0.335

Values are expressed as mean \pm SEM (n = 5); TPC = Total Phenolic Content, GAE = Gallic acid equivalent, TFC = Total Flavonoid Content.

observed in *A. indica* leaves (205.31 mg GAE/gm of dry extract) followed by 177.263 in *T. foenum-graecum* L., 111.632 in *A. sativum* L. seeds and 94.39 in *Z. officinale* Roscoe. The highest level of flavonoid content was detected in ethanolic leaves extract of *A. indica* A.Juss (56.04 quercetin equivalent/100 gm of extract) followed by 21.9 in *A. sativum* L., 14.74 in seeds of *T. foenum-graecum* L. and 7.7 in *Z. officinale* Roscoe.

3.2. Bodyweight and growth

Fig. 1 represented the bodyweight variation of mice of different experimental groups after the treatment with ethanolic extracts. The average weight of the control group and the high-fat diet group showed a regular and gradual rise in weight. A noticeable downward tendency in bodyweight gaining of the mice was seen for *A. indica* A.Juss group (both normal and high-fat diet) when compared with mice of other groups. The bodyweight gaining tendency was significantly higher in *Z. officinale* Roscoe normal diet (ND) and high-fat diet (HFD) group. Standard drug atorvastatin was found to lower the bodyweight gaining tendency remarkably. All the data was found statistically significant.

3.3. Analysis of organ weight

Data obtained from Table 2 we have found that the average weight of heart was significantly ($p < 0.05$) decreased in *A. indica* A.Juss normal and high-fat diet group than other experimental groups. The average weight of the heart of *Z. officinale* Roscoe high-fat diet group was almost the same as that of the control group. The average weight of the liver also followed the same manner when compared to the control group except for *Z. officinale* Roscoe normal diet group. The average weight of the left and right kidney was also significantly ($p < 0.05$) lowered in *A. indica* A.Juss normal and high-fat diet group. In the case of *Z. officinale* Roscoe normal and, high-fat diet group the average weight was found to increase when compared to the control group.

3.4. Comparative fat deposition profile

Fig. 2 represented the comparative fat deposition profile of different treatment groups. The standard group had the lowest amount of

Table 2

Organ weight variation among the mice of different treatment groups.

Treatment group	The average weight of the heart (gm)	The average weight of the liver (gm)	The average weight of the left kidney (gm)	The average weight of the right kidney (gm)
Control	0.148 ± 0.002	1.586 ± 0.06	0.318 ± 0.03	0.316 ± 0.05
HFD Standard	0.161 ± 0.01	1.61 ± 0.1	0.331 ± 0.06*	0.336 ± 0.02*
ND + <i>A. indica</i>	0.094 ± 0.007***	1.46 ± 0.07	0.297 ± 0.01	0.301 ± 0.03
HFD + <i>A. indica</i>	0.115 ± 0.01**	1.121 ± 0.04***	0.233 ± 0.01**	0.251 ± 0.01**
ND + <i>T. foenum-graecum</i>	0.131 ± 0.004**	1.09 ± 0.07**	0.206 ± 0.2**	0.207 ± 0.09
HFD + <i>T. foenum-graecum</i>	0.12 ± 0.004**	1.116 ± 0.02***	0.267 ± 0.03*	0.273 ± 0.04
ND + <i>A. sativum</i>	0.140 ± 0.01	1.363 ± 0.04**	0.309 ± 0.01*	0.305 ± 0.03**
HFD + <i>A. sativum</i>	0.134 ± 0.004**	1.48 ± 0.05	0.249 ± 0.01*	0.245 ± 0.06**
ND + <i>Z. officinale</i>	0.141 ± 0.005	1.495 ± 0.07	0.319 ± 0.04	0.322 ± 0.21**
HFD + <i>Z. officinale</i>	0.139 ± 0.005	1.604 ± 0.07	0.321 ± 0.04**	0.326 ± 0.05
Standard	0.148 ± 0.01	1.411 ± 0.02**	0.339 ± 0.03	0.336 ± 0.04

All values are represented as mean ± SEM where, level of significance stated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with the control group.

deposited fat. *A. indica* A.Juss and *T. foenum-graecum* L. normal diet group had deposited fat slightly higher than the standard where deposited fat of *T. foenum-graecum* L. normal diet group was significantly ($p < 0.01$) reduced when compared to the control group. High-fat diet group of *T. foenum-graecum* L., *A. sativum* L. and normal diet group of *Z. officinale* Roscoe also significantly reduced the amount of deposited fat when compared to the control group. The high-fat diet group of *Z. officinale* Roscoe had the highest amount of deposited fat when compared to the control group. All the data were found statistically significant.

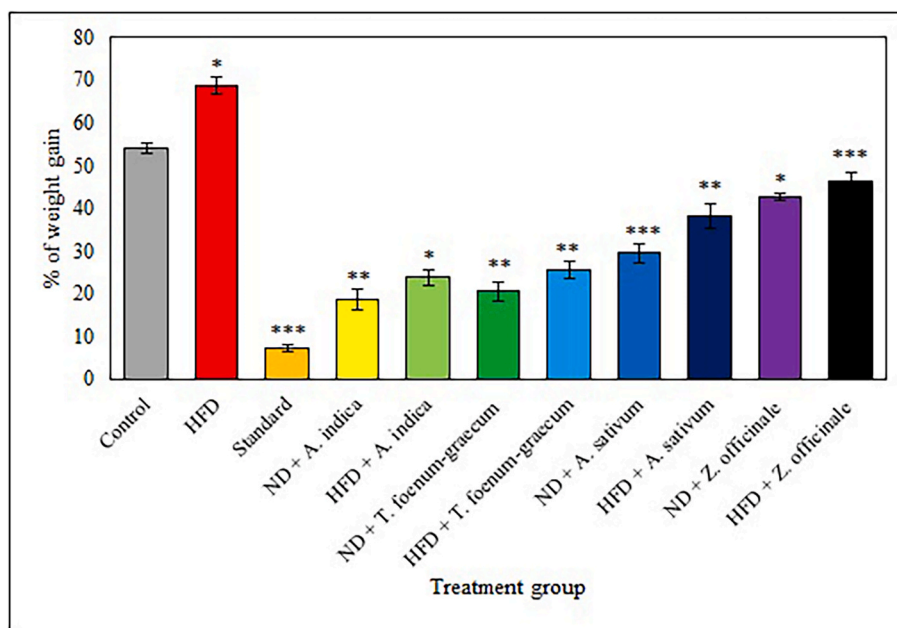


Figure-1. Bodyweight variation of mice of different experimental groups.

All values are represented as mean ± SEM where, level of significance stated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with the control group. All values are represented as mean ± SEM where, level of significance stated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with the control group.

Table 3

Analysis of serum cholesterol, triglyceride, and a uric acid level of the different experimental group.

Treatment group	CHOL level (mg/dl)	TG level (mg/dl)	UA level (mg/dl)
Control	171.56 ± 2.43	140.41 ± 2.4	4.97 ± 0.46
HFD	189.3 ± 4.41	178.98 ± 8.16***	3.63 ± 0.3
Standard	83.79 ± 2.41	72.28 ± 6.41	2.27 ± 0.3
ND + <i>A. indica</i>	124.77 ± 5.89***	90.59 ± 1.48***	2.53 ± 0.21*
HFD + <i>A. indica</i>	136.69 ± 5.05***	102.56 ± 3.38***	2.28 ± 0.51*
ND + <i>T. foenum-graecum</i>	133.02 ± 4.70***	98.16 ± 8.46**	2.37 ± 0.38*
HFD + <i>T. foenum-graecum</i>	149.23 ± 6.62**	107.69 ± 17.55	2.84 ± 2.09*
ND + <i>A. sativum</i> L.	151.37 ± 6.24**	113.79 ± 10.5**	3.58 ± 0.91
HFD + <i>A. sativum</i> L.	154.74 ± 9.13	119.41 ± 10.84	2.75 ± 0.26*
ND + <i>Z. officinale Roscoe</i>	167.58 ± 3.72	126.00 ± 4.29	3.3 ± 0.17
HFD + <i>Z. officinale Roscoe</i>	164.53 ± 10.07	129.67 ± 0.42	3.26 ± 0.26

All values are represented as mean ± SEM where, level of significance stated as ***p < 0.001, **p < 0.01, *p < 0.05 when compared with the control group; CHOL= Cholesterol, TG = Triglyceride, UA= Uric Acid.

All values are represented as mean ± SEM where, level of significance stated as ***p < 0.001, **p < 0.01, *p < 0.05 when compared with the control group.

3.5. Biochemical examination

Data obtained from the biochemical examination, we see that the serum total cholesterol (SC) and triglyceride (TG) level were reduced more significantly (p < 0.001) in *A. indica* *A.Juss* normal and high-fat diet group (Table- 3). *T. foenum-graecum* L. normal and high-fat diet group also significantly decreased serum SC and TG level when compared to the control group. Highest serum SC and TG level were observed in Normal and high-fat diet group of *Z. officinale Roscoe*. We also focused on the serum uric acid level, where we observed that the control group reveal the highest value. For all extract groups, the normal diet group showed a higher uric acid level than their respective high-fat diet group. All groups were compared with the control group.

4. Discussion

Avoidance of obesity or weight reduction in overweight individuals is likely to prevent increases in metabolic complications including diabetes, hypertension, dyslipidemia, and some cancers [41]. Consumption of fat-rich food increases the weight gaining tendency and is a key factor for the development of obesity and other metabolic complications [42]. A high-fat-diet-induced obese animal model has been commonly used for studying obesity-related changes [41].

Plant-derived bioactive compounds are responsible for the pharmacological actions of herbal medicines. It is established that various known bioactive metabolites in plant extract especially phenolic and flavonoid compounds inhibit pancreatic lipase (PL) secreted by the pancreas [42]. Pancreatic lipase is a key enzyme that is responsible for the absorption of free fatty acid and digestion of 50–70% fat into monoglyceride which results in the reduction of accumulation of fat in adipose tissue [43]. Therefore, one of the key targets for the anti-obesity agent is inhibition of PL [44]. Polyphenolic rich extract from the herbal source was reported as PL inhibitors during in vitro experiment [45]. In our study ethanolic extract of *A. indica* *A.Juss* showed the highest amount of phenolic (205.31 mg GAE/gm of dry extract) and flavonoid (56.04 quercetin equivalent/100 gm of extract) content which might be related to the high inhibitory activity of PL. The higher levels of total phenolic and flavonoid content in *A. indica* *A.Juss* are supported by other studies [46]. Phenolic content of *T. foenum-graecum* L., *A. sativum* L., and *Z. officinale Roscoe* follow descending order. The flavonoid content of *A. sativum* L. was greater than *T. foenum-graecum* L. and *Z. officinale Roscoe* had the lowest amount. These auspicious phenolics and flavonoid compounds are plant-derived secondary metabolites with various pharmacological activity that hold an aromatic ring bearing at least one hydroxyl group [47]. Since their hydroxyl groups may directly contribute to antioxidant action, phenolic compounds are good electron donors [48]. The presence of phenolic compounds demonstrate peroxide decomposition, free radical inhibition, metal inactivation or oxygen scavenging in the biological system and thus reduce the burden of oxidative disease [49]. Natural antioxidants like polyphenols and flavonoids neutralize oxygen free radicals (such as hydroxyl radicals, superoxide radicals and other active oxygen species including also singlet oxygen) and protect lipids, proteins, and DNA from oxidative damage, thus reducing the risk of coronary disease and cancer [50]. Moreover, it has been reported that natural polyphenols exert lipid-lowering effects through several mechanisms including inhibiting pancreatic lipase activity and decreasing absorption of lipids and proteins in the intestine,

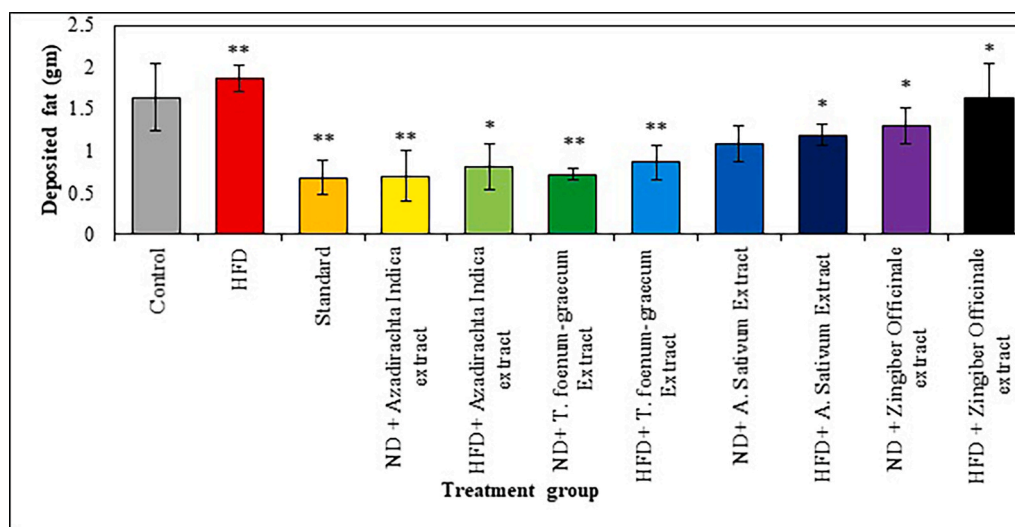


Figure-2. Comparative fat deposition profile of treatment groups.

All values are represented as mean ± SEM where, level of significance stated as ***p < 0.001, **p < 0.01, *p < 0.05 when compared with the control group.

thus reducing calorie intake, suppressing appetite, impairing adipogenesis, enhancing the activation of AMP-activated protein kinase in the skeletal muscle, liver and adipose tissues, and improving systemic inflammation, insulin resistance and hyperglycemia [51]. Based on these mechanisms, we inferred that *A. indica A.Juss* have better anti-obesity efficacy than *T. foenum-graecum L.*, *A. sativum L.*, and *Z. officinale Roscoe* may because of the presence higher phenolic and flavonoid content than others. To uncover the exact lipid lowering and uric acid reducing mechanism of these medicinal plants, isolation of bioactive compounds and molecular docking will be performed.

Mice treated with a high-fat diet the bodyweight significantly ameliorated when compared to the mice of the control group. But when they were treated with experimental extracts the weight gaining tendency was reduced. Among all the experimental group Standard atorvastatin at a dose of 70 mg/kg body weight reduced the weight gaining tendency. The weight gaining tendency of *A. indica A.Juss* normal and high-fat diet group was remarkably lower (18.71% and 23.86% respectively) when compared to the control group. Other experimental groups also showed reduced weight gaining tendency when compared to the control group. *Z. officinale Roscoe* normal and high-fat diet mice group gained weight slightly lower than the control group and highest in extract group. Data obtained from the organ weigh variations showed that the average weight of heart was decreased remarkably in all groups when compared with control except *Z. officinale Roscoe* high-fat diet group (weight was similar to control). The average weight of the liver followed the same manner. The average weight of the kidney was found to significantly decrease in *A. indica A.Juss* normal and high-fat diet group ($p < 0.01$) but increased in both group of *Z. officinale Roscoe* and *T. foenum-graecum L.* high-fat diet group. The changes in the absolute and relative organ weights indicate incurrence of toxicity in mice [52]. In our study, it is observed that ethanolic extract of *A. indica A.Juss* leaves at a dose of 400 mg/kg body weight most significantly reduced the serum cholesterol (SC) and triglyceride (TG) level ($p < 0.001$) than other experimental groups when compared with the control group. In a study by A. Zuraini et al. 2006, neem (*A. indica A.Juss*) extract at a dose of 50 and 300 mg/kg body weight prevented the rise of Total cholesterol (TC), Low-density lipoprotein (LDL) and Triglyceride (TG) in cholesterol feed rats and the study concluded that neem (*A. indica A.Juss*) extract at these conditions are the excellent lipid-lowering agent [53]. In another study, *A. indica A.Juss* leaf extract was significantly reduced the total cholesterol, LDL- and VLDL-cholesterol, triglycerides and total lipids of serum in streptozotocin-induced diabetic rats but there was no change in HDL-cholesterol levels [54]. As obesity is strongly correlated with TC, TG, LDL, HDL and serum uric acid level then it can be hypothesized that *A. indica A.Juss* possesses significant anti-obesity potential.

T. foenum-graecum L. normal diet group significantly reduced serum cholesterol ($p < 0.001$) and serum triglyceride ($p < 0.01$) level but in the case of the high-fat diet group serum cholesterol was significantly reduced ($p < 0.01$) and serum TG level insignificantly reduced. Normal diet group of *A. sativum L.* also significantly reduced serum SC and TG level ($p < 0.01$). Among the extract group, *Z. officinale Roscoe* high-fat diet group insignificantly reduced serum SC and TG level and the level of reduction is lowest than other treatment groups. The hypolipidemic activity of the ethanolic extracts found to be less effective than standard drug atorvastatin and the high-fat diet group showed the highest level of SC and TG when compared with the control group. Hepatocellular damage occurs due to the decrease of serum TG level and hepatic lipase (HL) is associated with the hydrolysis of triglyceride [52]. Our study also finds the serum uric acid (SUA) level and observed a reduction in the SUA level for all extract groups. We found that the normal diet group showed a higher uric acid level than their respective high-fat diet group but lower than the control group. Hikita et al., 2007 find a positive correlation between SUA level and visceral fat accumulation [55]. The study of Duan et al. suggested that high serum uric acid was positively associated with obesity [56]. *A. indica A.Juss* and *T. foenum-graecum L.* normal and high-fat diet group significantly reduced the serum uric acid

level ($p < 0.001$) when compared with the control group. The findings of our study endorse that ethanolic extract of *A. indica A.Juss* at a dose of 400 mg/kg body weight showed the reduced weight gaining tendency, reduced SC, TG and SUA level. The average weight of the heart, liver and kidney was decreased than other extract groups. The amount of accumulated fat also lowered.

5. Conclusions

Considering all these results it can be concluded that *A. indica A.Juss* have better anti-obesity efficacy than *T. foenum-graecum L.*, *A. sativum L.*, and *Z. officinale Roscoe* may because of the presence of higher phenolic and flavonoid content than others. Isolation of the chemical compounds and molecular docking studies will be performed to uncover the exact mechanism.

Ethical statement

Ethical committee of Noakhali Science and Technology University (NSTU-2018-067).

Consent for publication

Not applicable.

Availability of data and materials

The data used to analyse the findings of this study are available from the corresponding author upon request.

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Authors' contributions

MSH conceived and designed the experiments. TM and IPM experimented. Analysis and data interpretation were aided by MSJ, AKMKI, and SC. MSH and TM contributed reagents, materials, analysis tools and data. TM and IPM wrote the paper. MSR critically reviews the manuscript. MSH made the necessary corrections in the write-up and gave final approval for the submission of the final version. All authors read and approved the final manuscript.

Author statement

The results presented in this paper have not been published previously in whole or part. All authors have provided the consent to submit the article. I shall be grateful if you take necessary action in this regard. The data used to analyse the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no competing interests.

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