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EFFECT OF *COSMOS CAUDATUS* KUNTH LEAVES ON THE LIPID PROFILE OF A HYPERLIPIDEMIA-INDUCED ANIMAL MODEL

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A B S T R A C T

Hyperlipidemia, a metabolic disorder closely associated with the modern life-style and eating habits, is increasingly prevalent among the world population. This study was conducted on obese rats to evaluate the efficacy of *Cosmos caudatus* leaves as a treatment of hyperlipidemia. Hyperlipidemic conditions were induced in rats by submitting them to a high-fat diet during 3 months. After this, the rats were administered 200 mg/kg body weight of an ethanolic *C. caudatus* leaf extract or 35 mg/kg body weight atorvastatin for four weeks. The rats treated with the *C. caudatus* extract showed a significant ($P<0.05$) reduction of plasma triglycerides, total cholesterol, low density lipoprotein-cholesterol and glucose, and a significant ($P< 0.05$) increase in high density lipoprotein-cholesterol and Atherogenic Index values. The study indicated that supplementation with *C. caudatus* has a potential for the treatment of hyperlipidemia.

Keywords: Hyperlipidemia; High Fat Diet; *Cosmos caudatus*; Atorvastatin; Rats; Obesity.

ABBREVIATIONS

C. caudatus; Cosmos caudatus
CHD; Coronory heart disease
CMC; Carboxymethylcellulose
HFD; High fat diet
ACUC; Animal care and use committee
SD; Standard deviation; HDL; High density lipoprotein
LDL; Low density lipoprotein
HMG-CoA; 3-hydroxy-3-methyl glutaryl coenzyme A
AI; Atherogenic index
IDL; Intermediate density lipoprotein
BW; Body weight
Kg; Kilogram TC; Total cholesterol
mg; Milligram
LC-MS; Liquid chromatography-mass spectrometry
GC-MS; Gas chromatography-mass spectrometry
FTIR; Fourier transform infrared spectroscopy
^{1}H-NMR; Proton nuclear magnetic resonance
w/ v; weight per volume; RT; Room temperature
hr; Hour
ANOVA; Analysis of variance
AMP; Adenosine monophosphate

INTRODUCTION

Hyperlipidemia is characterized by an elevated plasma level of triglycerides or cholesterol or both of them. It is associated with diabetes, coronary heart disease (CHD) and atherosclerotic diseases and is considered to be a major cause of mortality and morbidity in developed countries (Kumar et al., 2010, Al-Hiari *et al*., 2011). The overall prevalence of hyperlipidemia in Malaysia was 37.1 % in 2012 (Mohamud et al., 2012), representing the fourth risk factor for metabolic syndrome and occurred most frequently in urban populations. The prevalence is higher among males (43.7 %) than females (33.7 %) and among the Chinese population (47.4 %) as compared to other groups such as Indians (45.3 %), Malay (36.0 %) and others (26.7) (Mohamud *et al.,* 2012).

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Herbs are among the most prominent and useful resources in Malaysian socio-culture owing to their nutritional value as well as their medicinal benefits. In this context, *Cosmos caudatus*, known as *Ulam Raja* in Malaysia, is an important herb and its leaves are often used in salads. It has been proclaimed as a health-promoting vegetable to the extent that it is known as the "King of the Salads." It is consumed as an appetizer because of its rich aroma and taste and its young leaves are consumed raw with chilli or coconut paste. Other culinary uses include its use as a food flavouring agent in the food industry (Rasdi *et al*., 2010) and as one of the ingredients in *kerabu* meal, a famous Malay traditional food (Norhanom *et al.,* 1990). There are several reports of its health benefits due to interesting bioactivities, including antimutagen, antifungal and antioxidant activities (Ragasa *et al.,* 1997, Ikhlas *et al.,* 2011). Traditionally, this plant has been used to improve blood circulation but the pharmacological mechanism of action is still unclear (Shui *et al.,* 2005). A previous study reported antidiabetic properties based on α-amylase and α-glycosidase activity (Loh *et al.,* 2011). However, to date, no studies of its antihyperlipidemic activity using an animal model have been published.

We hypothesized that the effect of *C. caudatus* on the lipid profile of rats could be associated with its traditional use of improving blood circulation. In this study, the effects of C. caudatus on lipid profiles in hyperlipidemic rats was evaluated. Plasma levels of triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, as well as blood glucose were measured, and the atherogenic index was calculated.

MATERIALS AND METHODS

Chemicals: Carboxymethylcellulose (CMC), diethyl ether, deuterated oxide (D_2O) and ketamine HCl were purchased from Sigma-Aldrich (St. Louis, US). Ethanol, NaOD and trimethylsilanepropionic acid sodium salt (TSP) were obtained from Merck (Darmstadt, Germany). While atorvastatin was purchased from Denver Hill Laboratory (Denver, USA).

Plant Preparation: Twelve weeks old, fresh mature leaves of *C. caudatus* were harvested in June 2011 at a local farm in Sepang, Malaysia. A sample was sent to a botanist from the Laboratory of Etnobotany Herbarium, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia, for its botanical identification (voucher specimen number SK 1934/11). The leaves were washed and ground cryogenically in liquid nitrogen and then lyophilized using a freeze dryer (Freezone 6, Labconco, USA). The lyophilized sample was stored at -80ºC until use.

Plant Extraction: The plant material was extracted following a modified version of a method described by Virdi *et al.*(2003). The freeze-dried sample was soaked in 80% ethanol (3:4, w/v) for 24 hrs in room temperature (RT) at 26 $\pm$ 2^{o}C. The extract was then filtered using a Whatman filter paper (15 cm diameter and 8 µm pore size) and the residue was re-extracted twice with fresh solvent (80% ethanol). The filtrates were combined and evaporated at 40 ± 1 ºC using a rotary evaporator (Buchi Rotavor R-200, Flawil, Switzerland). The extract was freeze-dried and then preserved at -80 ºC until feeding it to the rats.

^{1}H-NMR Measurement of *C. caudatus* Leaf Extract: A standard proton analysis method was used in this study following Kim *et al.* (2010) with a few modifications. A sample of 10 mg of dried *C. caudatus* leaf extract was transferred to a 2 mL micro centrifuge tube and mixed with 375 µL of CH_3OH-d_4 and 375 µL of phosphate buffer (pH 6) in D_2O containing 0.1% of TSP. The tube was sonicated for 15 min without heating, vortexed for 1 min and then centrifuged at 10,000 rpm for 10 min. The supernatant (700 µL) was subjected to 500 MHz ^{1}H-NMR spectroscopy (Varian Inc., California, USA) at 26 ^{0}C.

Preparation of Animals: The study was performed on twenty four male *Sprague – Dawley* rats (6 weeks old) obtained from Sapphire Enterprise Sdn Bhd (Kuala Lumpur, Malaysia). Their initial body weight ranged from 100 to 200 g. The approval number for this study was UPM/FPSK/PADS/ BRUUH/00407. Permission to conduct the study was granted by the Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Science, Universiti Putra Malaysia. The rats were kept at RT at 26 $\pm$ 2^{o}C and humidity at 85 $\pm$ 5 % for one week and fed with normal rat pellets (Gold Coin, Kuala Lumpur, Malaysia) and water *ad libitum* with 12 hrs dark/12 hrs light cycle. The rats were placed in polypropylene cage (15cm x 25cm) with 5 rats per cage. After this acclimatization, rats were divided into 4 groups of six rats (*n=6*) per group.

The rats were divided into normal control group and hyperlipidemic group. The latter group was further subdivided into hyperlipidemic control (untreated), atorvastatin-treated group, and *C. caudatus*-treated group. The rats were placed randomly into the cage with 2 rats per cage.

Hyperlipidemia Induction of Rat Model using a High Fat Diet: During the first week of the acclimatization period, rats were fed with normal rat pellets (Gold Coin, Selangor, Malaysia), composed of 14% fat, 25% protein and 61% carbohydrate of the total energy. After that, a high fat diet (HFD) was used to develop hyperlipidemia and obesity in the rats. HFD was made following the formulation developed by Jalil *et al.* (2009). This HFD consisted of 19% protein, 49% fat and 32% carbohydrate (calculated from total energy); the fat content was provided by pure ghee (QBB, Selangor, Malaysia) and corn oil (Vecorn, Selangor, Malaysia). The detail of HFD ingredients is shown in Table 1. This HFD biscuits were prepared according to ingredients given in Table 1 with baking condition of 40^0C for 15 hrs. This HFD was administered to the rats during 3 months (12 weeks) starting from week 2 to 13 of the experiment, after which normal rat pellets (Gold Coin, Selangor, Malaysia) feeding was continued from week 14 to 17. The body weight of each rat was recorded weekly. The rats from the normal group were fed with normal rat pellets from week 1 to 17.

Table 1. Ingredients of the high fat diet (HFD) per kg

Material	g/kg
Casein	236.00
Corn starch	182.62
Maltodextrin	120.00
Corn oil	90.00
Pure ghee	100.00
Sucrose	160.00
Cellulose	40.00
Cholesterol	10.00
DL-Methionine	3.54
Calcium phosphate dibasic	4.72
Mineral mix	41.30
Vitamin mix	11.80
Ethoxyquin	0.02

Administration of the *Cosmos caudatus* Leaf Extract: The *C. caudatus* dried extract was diluted in 0.03% (w/v) carboxymethylcellulose (CMC) and administered every day (200 mg/kg BW) by oral gavage using a force-feeding needle to rats of *C. caudatus*-treated group for 4 weeks (week 14 to week 17). Rats in groups normal control and hyperlipidemic control received 0.03% (w/v) CMC starts from week 14 to week 17, while the rats in atorvastatin-treated group were given atorvastatin (35 mg/kg BW) suspended in 0.03% (w/v) of CMC starting from week 14 to week 17, according to Manish *et al*. (2011).

Determination of Glucose and the Lipid Profile: Samples of 2.5 mL of blood were extracted from the retro-orbital of each fasted rat at the beginning (baseline) (week 13), middle (week 15), and end of the treatment (week 17). The rats were given 100 mg/kg BW of ketamine (Merck, New Jersey, U.S.A) via intraperitoneal for anesthetic purpose. The collected blood was transferred to an EDTA tube and centrifuged (Hettich EBA 20, Buckinghamshire, England) at 3500 rpm for 15 min at RT. The lipid profile and plasma glucose levels were analyzed using an automatic biochemical analyzer (Automatic Analyzer 902, Hitachi, Tokyo, Japan). The Atherogenic index (AI) was calculated as the ratio of LDL-cholesterol to HDL-cholesterol (Fki *et al*., 2005).

Statistical Analysis: Data were interpreted using one-way analysis of variance (ANOVA; Minitab version 16; Coventry, London). Tukey's test was applied to determine the difference within groups. The results were displayed as mean ± standard deviation. The results with a P-value below 0.05 were considered significantly different.

RESULTS

Constituents of Crude Ethanolic *C. caudatus* Leaf Extract: Figure 1 shows the ^{1}H-NMR spectrum of ethanolic *C. caudatus* leaf extract. The assigned compounds were alanine (δ 1.45), choline (δ 3.24), sugars (δ 3.0- 5.5), β-glucose (δ 4.60), α-glucose (δ 5.20), sucrose (δ 5.40), rutin (δ 4.55, 4.95,6.20, 6.40, 6.90, 7.58), quercetin (δ 6.20, 6.40, 6.83, 7.60,), and catechin (δ 2.80, 3.90, 4.58, 6.46). Other metabolites could not be assigned due to the overlapping peak signals and their low concentration.

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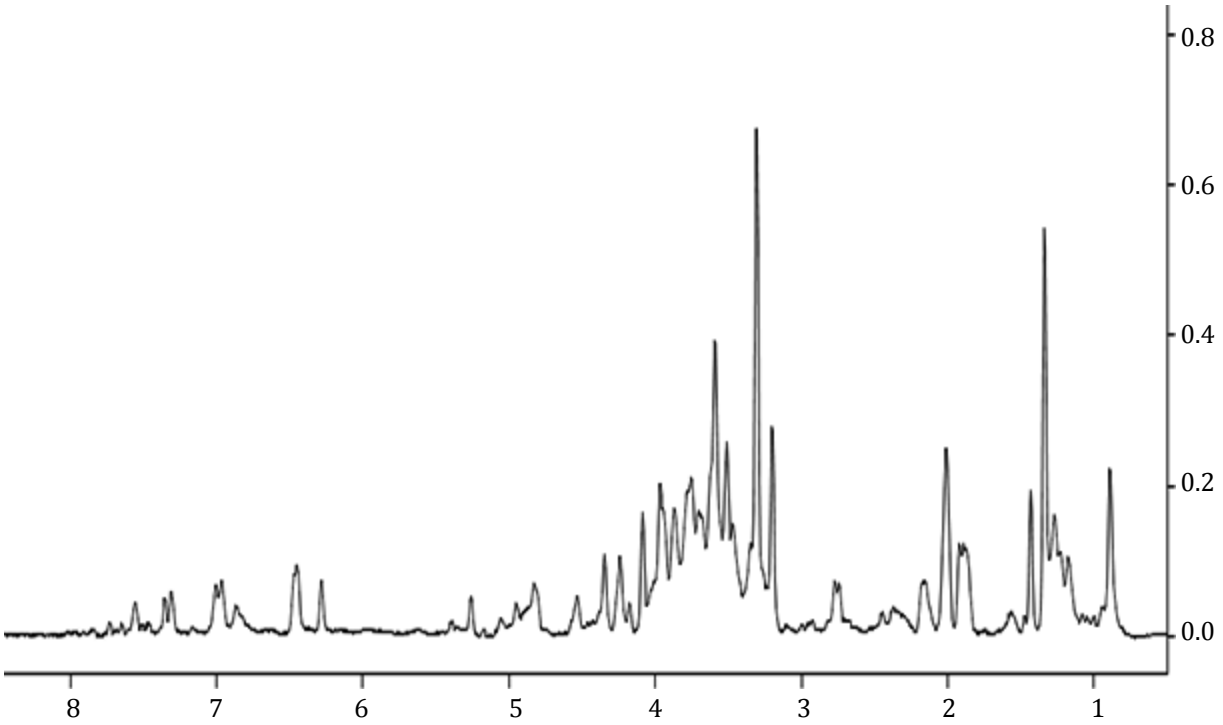

Figure 1. The ^{1}H-NMR spectrum of the ethanolic *C. caudatus* leaf extract.

Antihyperlipidemic Activity of *C. caudatus*: The initial body weight of rats ranged from 150 – 218 g. After 3 months of feeding with the HFD, the body weight of the obese rats increased to a range of 499 - 570 g, while the body weight of the normal-fed rats increased to the range of 398- 441 g. The total plasma cholesterol (TC) levels in the baseline, middle, and final period of treatment are shown in Table 2. The administration of the HFD for 12 weeks significantly ($P < 0.05$) increased the TC in the obese, *C. caudatus*- and atorvastatin-treated groups as compared to the normal rats to TC values of 78.97, 80.12, 81.75, and 53.97 respectively at the baseline. In the middle of the treatment, the TC values were significantly ($P < 0.05$) reduced from 80.12 to 68.09 mg/dL in the group treated with the *C. caudatus* extract while the group treated with atorvastatin decreased from 81.75 to 75.64 mg/dL. During the final period, there was a further marked reduction of the TC value in the *C. caudatus*- and atorvastatin-treated group to 57.70 and 71.25 mg/dL, respectively. Interestingly, while the TC value in the *C.caudatus*-treated group returned to the normal range of 33-65 mg/dL this did not happen in the atorvastatin-treated group. It is possible that a longer period of treatment with atorvastatin is required to normalize the TC value in hyperlipidemic rats.

Table 2. Total cholesterol plasma levels (mg/dL) in rats at baseline (week 13), middle (week 15) and final period (week 17) of the treatments.

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	53.97 ± 4.81 ^{Ac}	78.97 ± 3.17 ^{Aa}	80.12 ± 7.48 ^{Aab}	81.75 ± 3.91 ^{Aa}
Midddle	55.13 ± 4.59 ^{Ac}	78.81 ± 3.72 ^{Aa}	68.09 ± 8.42 ^{Bb}	75.64 ± 3.85 ^{Bab}
Final	55.18 ± 4.77 ^{Ac}	79.55 ± 3.38 ^{Aa}	57.70 ± 6.99 ^{Cbc}	71.25 ± 3.85 ^{Bb}

The normal range is between 35 – 65 mg/dL according to Matsuzawa et al. (1997). Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time intervals (n=6).

Compared to normal rats, HFD significantly ($P < 0.05$) increased plasma triglycerides at the baseline period of the obese, *C. caudatus*- and atorvastatin-treated rats to 120.06, 124.84, and 128.23, respectively. This is clearly shown by the results presented in Table 3. In the middle of the treatment, plasma triglyceride levels were significantly ($P < 0.05$) reduced to 106.99 and 119.81 mg/dL in *C. caudatus*- and atorvastatin-treated rats

respectively, and these were further significantly ($P < 0.05$) reduced in the final period to 96.70 mg/dL and 113.82 mg/dL, respectively. The *C. caudatus* extract proved to reduce plasma triglyceride levels in the final period of treatment more efficiently than atorvastatin, producing a 22.54% reduction from the baseline period, while that of atorvastation was lower (11.24%). However, both *C. caudatus* and atorvastatin failed to reduce triglyceride levels to the normal range of 42-82 mg/dL. This failure by atorvastatin to normalize plasma triglyceride of hyperlipidemic rats was also observed by Stringer (2011).

Table 3. Plasma levels of triglycerides (mg/dl) in rats at baseline (week 13), middle (week 15) and final period of the treatments (week 17).

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	73.66 ± 5.64 ^{Ab}	120.06 ± 9.23 ^{Aa}	124.84 ± 12.16 ^{Aa}	128.23 ± 3.03 ^{Aa}
Midddle	74.21 ± 5.04 ^{Ab}	121.04 ± 8.85 ^{Aa}	106.99 ± 14.57 ^{Ba}	119.81 ± 1.10 ^{Ba}
Final	74.92 ± 5.41 ^{Ac}	121.11 ± 9.02 ^{Aa}	96.70 ± 4.37 ^{Bb}	113.82 ± 3.12 ^{Ca}

The values are expressed as mean ± SD. The normal range is between 42 - 82 mg/dL according to Matsuzawa *et al.* (1997). Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time interval (n=6).

Table 4. Plasma HDL-cholesterol (mg/dL) of rats at baseline (week 13), middle (week 15) and final period of the treatments (week 17).

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	21.72 ± 0.17 ^{Aa}	22.01 ± 0.47 ^{Aa}	21.39 ± 0.73 ^{Ba}	21.97 ± 0.57 ^{Aa}
Midddle	21.75 ± 0.13 ^{Aa}	22.14 ± 0.59 ^{Aa}	22.14 ± 0.59 ^{ABa}	21.97 ± 0.55 ^{Aa}
Final	21.77 ± 0.14 ^{Ab}	22.17 ± 0.60 ^{Ab}	22.66 ± 0.21 ^{Aa}	22.00 ± 0.55 ^{Ab}

The values are expressed as mean ± SD. Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time interval (n=6).

The levels of high-density lipoprotein (HDL)-cholesterol in plasma at the baseline, middle, and final period of the treatments are shown in Table 4. A significant ($P < 0.05$) increment of HDL-cholesterol was observed at the final period compared to the baseline in *C. caudatus*-treated rats (from 21.39 to 22.66 mg/dL), while the HDL-cholesterol level remained unchanged in the normal, obese and atorvastatin treated rats. The plasma LDL-cholesterol levels in obese, *C. caudatus*- and atorvastatin-treated rats were significantly increased as compared to the normal rats at the baseline period (Table 5). At the middle of the treatment, *C. caudatus*- and atorvastatin-treated rats showed significantly ($P < 0.05$) reduced levels of LDL-cholesterol (16.74 and 17.65 mg/dL, respectively) and in the final period these levels were significantly ($P < 0.05$) further reduced to 14.63 mg/dL for *C.caudatus*-treated rats as well as for those treated with atorvastatin ($P > 0.05$).

Table 5. Plasma LDL-cholesterol of rats at baseline (week 13), middle (week 15) and final period of the treatments (week 17).

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	13.07 ± 0.47 ^{Ab}	18.33 ± 0.63 ^{Aa}	18.51 ± 0.63 ^{Aa}	18.54 ± 0.26 ^{Aa}
Midddle	13.11 ± 0.48 ^{Ac}	18.33 ± 0.63 ^{Aa}	16.74 ± 0.88 ^{Bb}	17.65 ± 0.52 ^{Bab}
Final	13.26 ± 0.48 ^{Ad}	18.38 ± 0.61 ^{Aa}	14.63 ± 1.28 ^{Cc}	16.96 ± 0.78 ^{Bb}

The values are expressed as mean ± SD. Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time interval (n=6).

A significant ($P < 0.05$) increase of the AI was observed in the obese, atorvastatin- and *C. caudatus*-treated rats compared to the normal rats at the baseline period with an AI value of 0.83, 0.84, 0.87 and 0.60 respectively (Table 6). In the middle of the treatment, both *C. caudatus*- and atorvastatin-treated rats showed significant ($P < 0.05$) reduction of the AI value as compared to that of the baseline. In the final period of

treatment, the AI values of *C. caudatus*- and atorvastatin-treated rats were significantly ($P < 0.05$) reduced to 0.74 and 0.77, respectively. Interestingly, *C. caudatus* reduced the AI value more effectively than atorvastatin: *C. caudatus* reduced AI values by 14.94%, while atorvastatin only produced a 8.33% decrease from baseline levels.

Table 6. Atherogenic index (AI) of rats at baseline (week 13), middle (week 15) and final period of the treatments (week 17).

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	0.60 ± 0.02 ^{Ab}	0.83 ± 0.04 ^{Aa}	0.87 ± 0.03 ^{Aa}	0.84 ± 0.03 ^{Aa}
Midddle	0.60 ± 0.02 ^{Ab}	0.83 ± 0.04 ^{Aa}	0.81 ± 0.04 ^{ABa}	0.80 ± 0.04 ^{ABa}
Final	0.61 ± 0.02 ^{Ab}	0.83 ± 0.04 ^{Aa}	0.74 ± 0.05 ^{Ba}	0.77 ± 0.04 ^{Ba}

The values are expressed as mean ± SD. Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time interval (n=6).

Effect of *C. caudatus* Leaf Extract on Fasting Plasma Glucose Level: The plasma glucose level for the baseline, middle and final period of the treatments are shown in Table 7. In the baseline period, glucose levels were not significantly ($P > 0.05$) different between groups fed with the HFD and normal rats.

In the middle of the treatment, *C. caudatus* significantly ($P < 0.05$) reduced the plasma glucose level to 5.57 mmol/L. Atorvastatin did not significantly ($P < 0.05$) increase the plasma glucose level. In the final period of the treatment, *C.caudatus* significantly ($P < 0.05$) reduced the plasma glucose level to 4.48 mmol/L compared to the middle and baseline period. It normalized the fasting plasma glucose to its normal range which is 2.75 – 7.49 mmol/L according to Johnson (1996). Conversely, atorvastatin significantly ($P < 0.05$) increased the plasma glucose level compared to the baseline.

Table 7. Plasma glucose of rats at baseline (week 13), middle (week 15) and final period of the treatments (week 17).

Periode	Normal	Obese	*C. caudatus* treated	Atorvastatin treated
Baseline	6.35 ± 0.32 ^{Aab}	7.37 ± 0.33 ^{Aa}	7.08 ± 0.58 ^{Aa}	7.43 ± 0.78 ^{Ba}
Midddle	6.53 ± 0.31 ^{Ab}	7.20 ± 0.26 ^{Aab}	5.57 ± 0.50 ^{Bc}	7.83 ± 0.85 ^{ABa}
Final	6.75 ± 0.39 ^{Ab}	7.17 ± 0.21 ^{Ab}	4.48 ± 0.52 ^{Cc}	8.78 ± 0.35 ^{Aa}

The values are expressed as mean ± SD. The normal range is between 2.75 – 7.49 mmol/L according to Johnson (1996). Block letters indicate the significant difference within the same group at different time intervals, while lower case letters indicate the significant difference between different groups regarding the corresponding time interval (n=6).

DISCUSSION

The ^{1}H-NMR spectrum of ethanolic *C. caudatus* leaf extract (Figure 1) shows the presence of quercetin and catechin which were similar to those reported in detail by Mediani *et al*. (2012). There are several reports of the effect of polyphenols and flavonoids in reducing cholesterol levels. Quercetin has been reported to decrease total cholesterol and LDL-cholesterol plasma levels in humans (Arai *et al.,* 2000). A study conducted by Erlund *et al.* (2008) showed the HDL-cholesterol increasing effects of catechin-containing berries in hypercholesterolemic patients. A catechin- and polyphenolic-rich cocoa powder was able to decrease plasma LDL-cholesterol levels and increase plasma HDL-cholesterol levels in mildly hypercholesterolemic humans (Baba *et al.,* 2007).

Numerous studies reported the use of HFD as a means of developing a hyperlipidemic rat model (Akiyama *et al.*, 1996, Yan *et al.,* 2006, Jalil *et al.,* 2009, Ji *et al.,* 2011). In this study, we used a HFD composition following Jalil *et al.* (2009) because this diet prepared from pure ghee containing high saturated animal fat, requires a short period of time to develop the hyperlipidemic condition.

Various plasma parameters were monitored throughout the study in order to evaluate the antihyperlipidemic effect of *C. caudatus*, e.g. total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, AI and glucose level. Hyperlipidemia is characterized by elevated levels of triglycerides, total cholesterol and LDL-cholesterol, and

decreased level of HDL-cholesterol, all of which can lead to diabetes and cardiovascular disease (Rose *et al.,* 2012). High LDL-cholesterol levels are known to be the triggering factor for hypercholesterolemia, while HDL-cholesterol is known as protective cholesterol and is responsible for the transportation of cholesterol (Fernandes *et al.,* 2007).

Cholesterol is an essential component in the synthesis of vitamin D, steroid hormones and bile acids. However, elevated levels of total serum cholesterol and LDL-cholesterol increase the risk of hyperlipidemia. A decreased level of HDL-cholesterol increases hyperlipidemia since HDL aids in translocation of cholesterol from arterial walls and other peripheral tissue to the liver for catabolism (Koh *et al.,* 2010). In the present study, rats treated with 200 mg/kg BW of *C. caudatus* showed a significant increase in the serum HDL-cholesterol levels and a significant decrease in AI, LDL-cholesterol, triglyceride and total cholesterol. Considering the overall effects on the lipid profile of obese rats, it may be concluded that *C. caudatus* possesses antilipidemic properties. These findings suggest that it may not only be potentially beneficial because of its antihyperlipidemic properties, but consequently may also be effective as a therapeutic agent for hyperlipidemic associated disorders such as diabetes and atherosclerosis.

In the present study atorvastatin was used as a positive control, as it is approved as a cholesterol-lowering drug alongside other statins such as lovastatin, simvastatin and pravastatin. Statins block the endogenous synthesis of cholesterol and reduce LDL and IDL (intermediate density lipoprotein) levels due to the up-regulation of the LDL receptor in the liver (Kanda *et al.,* 2003). Atorvastatin is classified as a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, which inhibits the conversion of HMG-CoA to mevalonate. The overall consequence of this is an increase in the receptor- mediated uptake and catabolism of IDL and LDL in the liver and decrease in cholesterol synthesis. As a consequence, levels of LDL-cholesterol and triglycerides are reduced while HDL-cholesterol plasma levels increase slightly (Tripathi, 2003).

Our results showed that atorvastatin significantly reduced the cholesterol, triglyceride and LDL-cholesterol level but did not increase the HDL-cholesterol level in plasma. The HDL-cholesterol result of this study was not in agreement with Tripathi (2003) who observed that atorvastatin caused a mild increase of in HDL-cholesterol levels. Thus it is probably necessary to administer atorvastatin for a longer period for a future study to better evaluate its effect. There was a significant increase in plasma glucose levels in the group of rats treated with 35 mg/kg BW of atorvastatin. This could be explained by the fact that atorvastatin is a lipophilic statin and can have a deleterious effect on glucose metabolism.. This is in agreement with Koh *et al.* (2010), who reported that despite being effective in reducing LDL-cholesterol, total cholesterol and apolipoprotein B, atorvastatin treatment increased plasma glucose levels in hypercholesterolemic patients.

The presence of polyphenols (Mustafa *et al.,* 2010), anthocyanins (Mustafa *et al.,* 2010, Sukrasno *et al.,* 2011) and quercetin (Hoek-van *et al.,* 2013) in *C. caudatus* has been reported by several researchers. Polyphenols are reported to be responsible for lipid lowering properties by slowing down triacylglycerol absorption through the inhibition of pancreatic lipase (Nakai *et al*., 2005), enhancement of cholesterol excretion in feces (Hsu *et al*., 2006), attenuation of hepatic lipid accumulation through activation of adenosine monophosphate (AMP-) activated protein kinase (Zang *et al*., 2006), enhancement of the expression of LDL receptors in liver (Dávalos *et al*., 2006), and inhibition of the hepatic secretion of apolipoprotein B100 (Jalil *et al.,* 2009). On the other hand, quercetin is able to suppress hyperlipidemic activity by increasing the ω-oxidation pathway in hepatic lipid metabolism (Sukrasno *et al.,* 2011), whereas anthocyanins work by stimulating the cholesterol transport (Xia *et al*., 2005). They have been reported to promote the efflux of excess cholesterol from peripheral tissues to the liver for biliary excretion. Although these compounds may be related to the antihyperlipidemic activity of *C. caudatus*, the exact mechanism for the lipid lowering effect of *C. caudatus* still remains unclear and further investigation is required to determine this.

CONCLUSION

This study suggests that feeding 200 mg/kg BW of an ethanolic extract of *C. caudatus* leaves to hyperlipidemic rats during 4 weeks is effective in improving their lipid profile and reducing their glucose level. The total cholesterol and plasma triglyceride level were normalized while plasma LDL-cholesterol levels and the atherogenic index were reduced. The exact mechanism

for this activity remains unknown and has yet to be investigated. To further understand and validate this, the metabolomic profile of the *C. caudatus* extract used in this study should be determined. This aspect of the study is currently being pursued using metabolomics.

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