Lipid lowering and anti-atherosclerotic properties of *Tinospora crispa* aqueous extract on high-cholesterol diet-induced hyperlipidemic rabbits

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This study was aimed to investigate the hypocholesterolemic and anti-atherosclerotic properties of *Tinospora crispa* aqueous extract (TCAE) on rabbits for 10 weeks. The hyperlipidemic rabbits were induced and the rabbit were given different concentration of TCAE (200, 450 and 600 mg/kg). Results from lipid analysis show that the level of total cholesterol (TC), triglyceride (TG) and LDL-C on the hyperlipidemic rabbits were reduced with the treatment of TCAE while HDL level was elevated. Through plasma analysis, the activity of gamma glutamyl transferase (GGT) and alkaline phosphates (ALP) were also reduced with the treatment of TCAE compared to hyperlipidemia group. All group of rabbits tested with TCAE again had significantly higher (p < 0.05) total antioxidant status (TAS), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities. Among the concentrations of TCAE tested, medium dose showed more potent effect in reducing blood serum TC, TG and LDL-C levels and increasing HDL-C level compared to low and high dosages counterparts. No foam cell formation was visible in aorta of rabbits treated with TCAE in dose dependent manner. However, there was visible foam cell formation in the aorta of hyperlipidemia group. In conclusion, this study suggests that supplementation of 450 mg/kg of *T. crispa* extract would be able to reduce or retard the progression of atherosclerotic plaque development induced by dietary cholesterol.

Key words: Hypocholesterolemia, *Tinospora crispa*, anti-atherosclerotic properties, cardiovascular diseases.

INTRODUCTION

Cardiovascular diseases (CVD) is a world’s largest killer, claiming 17.5 million lives a year in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (WHO, 2015). In Malaysia, cardiovascular related diseases contribute to 24.38% of reported death in government hospital (MOH, 2014). Hyperlipidemia is one of contributing factor for cardiovascular diseases (CVD) such as atherosclerosis, stroke and myocardial infarction (Kannel et al., 1979; Xing et al., 2009).
Hyperlipidemia refers to increased level of total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) as well as decreased level of high density lipoprotein (HDL). Reducing blood TC, TG and LDL level, while increasing blood HDL level has become beneficial factor to overcome CVD, thus evoke many investigators to design drugs with the ability to manage blood cholesterol level. Among them, statin has become the most popular kind of drug that has been produced. However, there are many side effects that have been reported on this drug consumption including myotoxicity and liver dysfunction (Sathasivam, 2012). Herbal remedies have become popular in recent decades as an alternative treatment over modern medicines in the screening of hyperlipidemic cure. According to WHO, 80% of the population in some Asian and African countries depend on traditional and herbal medicine for primary health care (WHO, 2002). There are several studies on the therapeutic effect of herbal extracts on various kinds of diseases and ailments including diabetes, hypertension and CVD. There are also many study performed previously which showed that herbal extracts can reduce lipid level on hypercholesterolemia rabbit (Berger et al., 2004; Chen et al., 2011; Khanna et al., 2002).

Tinospora crispa, plant locally known as patawali in Malaysia is a climber that can be found in primary rainforest widely distributed in Malaysia, Indonesia, Thailand and Vietnam. To date, several activity of T. crispa has been recorded including anti-diabetic (Noor and Ashcroft, 1998), hypolipidemia (Praman et al., 2011) and anti-inflammatory (Sulaiman et al., 2008). The aqueous extract of T. crispa stem can be taken to treat diabetes mellitus and improve diabetic conditions (Noor and Ashcroft, 1998). Traditionally, this plant was found to be used to treat diabetes, hypertension and lumbago (Fasihuddin and Hasmah, 1991). The stems of T. crispa were commonly used to treat coughs, asthma, fever and stomach aches (Perry, 1980; Muhammad and Mustafa, 1994). Other than that, it also can be used for purifying blood and as preventions against bacterial and viral infections (Quisumbing, 1978). Besides, the young stems of the plant can be taken raw to reduce high blood pressure, diabetes and relieve abdominal pains (Ministry of Industry and Primary Resources of Brunei Darussalam, 1992).

Present study sought to investigate the lipid lowering properties of T. crispa aqueous extract (TCAE) on the hyperlipidemic rabbit model and the parameters of hyperlipidemia including TC, TG, LDL and HDL were investigated. On the other hand, several biochemical parameters that are related to the CVD occurrence such as SOD and GPx antioxidant enzyme levels and liver function enzymes such as GST and ALP were also measured.

MATERIALS AND METHODS

Preparation of T. crispa extract

Fresh stems of T. crispa were collected from Forest Research Institute Malaysia (FRIM) at Kepong, Selangor after proven by plant taxonomist. The stems were then cut into small pieces of around 2 inches long followed by drying in the oven at 55°C for 72 h. The completely dried stems were then ground into powder by using bench-top blender. T. crispa aqueous extract was prepared by adding 100 g T. crispa powder to 1000 ml distilled water and shaken under shaking water bath at 55°C for 4 h. The solution was then filtered and the supernatant was freeze dried at -80°C. The finished processed of T. crispa aqueous extract in powder form was then store at -20°C until use.

Induction of experimental hyperlipidemic rabbits

Forty two (42) male New Zealand White (NZW) rabbits with initial mean body weight of 2.5 to 3.0 kg were acclimatized under room temperature (28 ± 2°C) with a regular light/dark cycle and free access to food and water for 2 weeks before use. Following acclimatization, the animals were randomly segregated into six groups of seven rabbits each. Normal control (NC) group was given normal chow diet while other 5 groups were given 0.5% cholesterol chow diet for continuous 10 weeks to induce hyperlipidemic rabbit model. Food and water were given ad libitum throughout the experiment. Then, the 5 groups were assigned as hyperlipidemia group (H), simvastatin group (SC), low dosage (200 mg/kg) of TCAE, medium dosage (450 mg/kg) of TCAE and high dosage (600 mg/kg) of TCAE. The TCAE and simvastatin were given via oral gavage while blood sampling was performed 10 weeks of experimental period. The experimental protocol and animal handling throughout the study were in accordance with guidelines approved by the institution ethics committee where the study is conducted.

Biological sample collection

The animal was sacrificed by exsanguinations for blood and organ collection after 12 h of fasting using diethyl ether as anesthetic. Approximately 5 mL of blood was collected by cardiac puncture, segregated into lithium heparin and EDTA tubes. The bloods collected in EDTA tubes were centrifuged at 3000 rpm for 10 min at

Abbreviations: TCAE, Tinospora crispa aqueous extract; GGT, gamma glutamyl transferase; ALP, alkaline phosphates; TAS, total antioxidant status; GPx, glutathione peroxidase; SOD, superoxide dismutase; CVD, cardiovascular diseases; TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; GSH-Px, glutathione peroxidase; VLDL, very low density lipoprotein; IDL, intermediate density cholesterol; ROS, reactive oxygen species.

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4°C to separate the plasma. The serum was then transferred into eppendorf tubes, labeled, covered with aluminium foil and stored at -80°C until further analysis. The blood serum was used for estimation of lipid profiles, liver toxicity enzymes and total antioxidant status (TAS). The bloods collected in heparin tube were centrifuged at 3000 rpm for 10 min at 4°C to separate the plasma from the erythrocyte. The plasma was then removed and erythrocyte was washed with sodium chloride (0.9 w/v NaCl) which was centrifuged at 3000 rpm for 10 min at 4°C. The erythrocyte was washed with sodium chloride and repeated for three times. Before analysis, sodium chloride was added to the erythrocyte.

Lipid analysis

Lipid analysis such as total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) in serum were determined by enzymatic methods using Rosche commercial kit (Germany) in accordance with the manufacturer instruction. All the serums were measured using Hitachi Chemistry Analyzer 902 Machine at the Chemistry Pathology Laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia. The test utilizes the principle of enzymatic colorimetric assay to read the sample.

Antioxidant enzyme test

Antioxidant enzyme activities of glutathione peroxidase G Px, superoxide dismutase (SOD) as well as total antioxidant status (TAS) were determined in the whole blood using a kit of Randox in accordance with manufacturer instruction. All the serums were measured using Cobas Mira Plus analyzer at the Research Laboratory of Faculty of Medicine and Health Sciences, UPM.

Plasma liver function test

The metabolic processes and variety of chemicals that occur in the liver were maintained by the help of liver enzymes including Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGT). In this study, these three enzymes were used to monitor the liver toxicity caused by the treatment effect. The level of ALP and GGT was measured using Roche commercial kit in accordance with the manufacturer instruction. All the serums were measured using Hitachi Chemistry Analyzer at the Chemistry Pathology Laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia. All enzymes of interest were measured using the enzymatic colorimetric test principle.

Histology study

After the rabbits were killed, aorta tissue between its origin and bifurcation into the iliac arteries was taken gently, free of adhering tissues and washed with cold normal saline solution. The large part of origin was cut into 2 mm³, and was put into 10% formalin for haematoxylin and eosin staining, whereas the ascending large part of aorta between its origin and bifurcation into the iliac arteries was opened longitudinally and prepared for plaque assay.

Haematoxylin and eosin staining

The aortic arch were fixed in 10% formalin for a few days and prepared for light microscopy by dehydrating the tissue samples in an ascending series of alcohol dehydration, clearing with xylene and wax impregnation with paraffin wax for 14 h in an automatic tissue processor machine. The tissues were then embedded into block by paraffin wax at 62°C and was cooled at 0°C for 3 h to form solid block. This is followed by the sectioning process, whereas the tissues were trimmed and sectioned with the thickness of 4 to 5 μm using a microtome machine. The tissues were then placed in the water bath, attached on glass slides and then were dried on a hot plate at 50 to 55°C for 30 min and then kept at 37°C. The tissues sections were then stained with Haematoxylin and Eosin (H&E) staining method using Autostainer Machine. The slides underwent processing, colorization and dehydration. After thoroughly dried from xylene, the slides were mounted with cover slips and mounted with DPX. The slides were then dried at room temperature for a few days before being analyzed by an Image Analyzer Machine. The aortic arch aorta was evaluated quantitatively.

Assessment of atherosclerotic plaque lesions

Atherosclerotic plaque areas were assessed by a previously described method (Prasad and Kalra, 1993). Briefly, the aortic strips were dissected from the ascending arch to the iliac bifurcation, and extraneous adipose tissue was removed. The aortas were opened longitudinally, rinsed several times with ice-cold saline and stretched onto a piece of cardboard. Then, it was fixed immersed in neutral 100 g/L buffered formalin solution for 24 h and then rinsed in 70% alcohol. The tissue was then immersed in Herxheimer’s solution containing Sudan IV (5 g), ethyl alcohol (70%, 500 mL) and acetone (500 mL) at room temperature for 15 min and washed in running water for 1 h. Photographs of the intimal surface of the aorta were taken using digital camera (EOS Canon, Japan) and the intimal lipid lesions were determined quantitatively by estimation of the percentage of sudanophilic stained areas in the total aortic area in photographs using Image Analysis Software. The total atherosclerotic area of the intimal surface of the aorta was measured in mm². The extent of atherosclerosis was expressed as a percentage of the luminal surface that was covered by atherosclerotic plaques.

Statistical analysis

Statistical analysis for all assays were performed by one-way ANOVA with Dunnett’s posthoc multiple group comparison using GraphPad Prism software (Version 5). P<0.05 and P<0.01 was considered significant for all tests.

RESULTS

Hypolipidemic effect of TCAE in hyperlipidemic rabbits

Hypolipidemic effect of TCAE and simvastatin are shown in Table 1. When compared to control (normal rabbits), TC, TG and LDL-C and HDL-C level were markedly increased in hyperlipidemic rabbit. After the treatment of TCAE at 200, 450 and 600 mg/kg and simvastatin, there were significant decrease of TC, TG and LDL-C level in serum lipid. TC level of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin decreased by 35.89, 50.38, 33.94 and 95.03%, respectively, compared to untreated hyperlipidemia group while TG level of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin decreased by 73.86, 77.27, 60.23 and 78.98%,
The effect of TCAE on liver enzymes expression is shown in Figure 1. Compared to control (normal rabbits), GGT expression on hyperlipidemic rabbits significantly increased while there was no significant difference in ALP expression between hyperlipidemic rabbit and normal rabbit. Treatment of TCAE at 200, 450 and 600 mg/kg and simvastatin significantly decreased GGT and ALP level, when compared to hyperlipidemic rabbit. GGT level of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin were declined by 66.63, 77.81, 76.00 and 14.42%, respectively, compared to untreated hyperlipidemia group. ALP level of hyperlipidemic rabbit treated with low, medium, high dosages of TCAE and simvastatin decreased by 76.64, 89.38, 83.72 and 71.78%, respectively, compared to untreated hyperlipidemia group.

**Effect of TCAE on antioxidant enzymes activity and total antioxidant status**

Effect of TCAE on antioxidant enzymes activity and total antioxidant status is shown in Figure 2. Compared to those of rabbit in control group (normal rabbits), SOD and GPx activity as well as TAS of hyperlipidemic rabbits was significantly reduced. Treatment of TCAE at 200, 450 and 600 mg/kg and simvastatin significantly increased SOD and GPx activity as well as TAS when compared to hyperlipidemic rabbits. SOD activity of hyperlipidemic

**Effect of TCAE on liver enzymes expression**

Effect of TCAE on liver enzymes expression is shown in

**Effect of TCAE on atherosclerotic plaques coverage**

Atherosclerotic plaques coverage of hyperlipidemic rabbit, treatment group and simvastatin was measured using NC as a baseline. Hyperlipidemia rabbit showed marked increase of plaques coverage with 31.12 ± 2.18% of coverage. TCAE showed marked reduction of atherosclerotic plaques coverage when compared to hyperlipidemic rabbit and the reduction of plaques area was 18.32, 50.87 and 66.25% of reduction for 200, 450 and 600 mg/kg of TCAE, respectively (Table 2). Simvastatin reduced plaques coverage to a greater extent with 92.80% of reduction.

**Table 2.** Percentage of atherosclerotic plaques coverage of all groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TCAE (mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>200</td>
</tr>
<tr>
<td>% of coverage</td>
<td>0.00±0.00*,#</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD. Values with the asterisk (*) are significantly different (p < 0.05) compared to hyperlipidemia model group. Values with * are significantly different (p < 0.05) compared to normal control group. NC, normal control; H, high cholesterol diet group; TCAE, T. crispa aqueous extracts.

respectively, when compared to untreated hyperlipidemia group. LDL-C level of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin decreased by 43.62, 51.12, 39.04 and 94.44%, respectively, while LDL-C level of hyperlipidemic rabbits treated with low, medium, and high dosages of TCAE markedly increased by 4.55, 6.22 and 4.67 fold, respectively, compared to untreated hyperlipidemia group.

**Table 1.** Effect of TCAE and simvastatin on the level of TC, TG, LDL-C and HDL-C in rabbit blood serum following 10 weeks of treatment.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Serum lipid profile (mmol/L) (mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>TC</td>
</tr>
<tr>
<td>NC</td>
<td>0.96±0.02*,#</td>
</tr>
<tr>
<td>H</td>
<td>29.20±1.54</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>1.45±0.74*</td>
</tr>
<tr>
<td>TCAE (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>18.72±0.37*</td>
</tr>
<tr>
<td>450</td>
<td>14.49±1.83*</td>
</tr>
<tr>
<td>600</td>
<td>19.29±1.03*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD. Values with the asterisk (*) are significantly different (p < 0.05) compared to hyperlipidemia model group in respective test group. Values with * are significantly different (p < 0.05) compared to normal control group in respective test group. TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; NC, normal control; H, high cholesterol diet group; TCAE, T. crispa aqueous extracts.

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Figure 1. Effect of TCAE and Simvastatin on the level of A) GGT and B) ALP level of all Groups. Each value represents the mean ± SD. Values with the asterisk (*) are significantly different (p<0.05) compared to hyperlipidemia model group in respective test group. Values with # are significantly different (p<0.05) compared to hyperlipidemia group in respective test group. GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase level; NC, normal control; H, high cholesterol diet group.

Figure 2. Effect of TCAE and Simvastatin on the level of A) SOD level, B) GPx Level and C) TAS of all Groups. Each value represents the mean ± SD. Values with the asterisk (*) are significantly different (p<0.05) compared to hyperlipidemia model group. Values with # are significantly different (p<0.05) compared to normal control group. SOD, superoxide dismutase; GPx, glutathione peroxidase; TAS, total antioxidant status; NC, normal control; H, high cholesterol diet group; TCAE, T. crispa aqueous extracts.

DISCUSSION

Hyperlipidemia is a major pathological basis of CVD such as atherosclerosis, stroke and myocardial infarction. Rabbits treated with low, medium, high dosages of TCAE and simvastatin were increased by 4.22, 7.64, 8.30 and 1.48 fold, respectively, compared to untreated hyperlipidemia group while GPx activity of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin were increased by 1.65, 1.60, 1.85, 1.87 and 4.48 fold, respectively, compared to untreated hyperlipidemia group. TAS of hyperlipidemic rabbits treated with low, medium, high dosages of TCAE and simvastatin were not significantly altered compared to hyperlipidemic rabbits.
Hyperlipidemia, particularly hypercholesterolemia, can increase the risk factor of atherosclerosis by inducing arterial endothelial dysfunction (Chen et al., 2011). LDL also has been diagnosed to be a major determinant to the hypercholesterolemia. Different from LDL, HDL plays a role in reverse transport of cholesterol from artery back to the liver at which it is eliminated. Therefore, managing the elevation of blood LDL, TG, and TC levels are important to prevent HL and atherosclerosis, and are of great significance in reduction of CVD incidence. Result of this study shows that, following a week of acclimatization, there were no significant differences in blood parameters of all rabbits. However, rabbits fed with high cholesterol diet over 10 weeks showed significantly higher level of serum TC, TG and LDL-C compared to normal diet rabbits. This result showed that the hyperlipidemid rabbit model have been established after been introduced to high cholesterol diet over 10 weeks period. The levels of TC, TG and LDL-C on these hyperlipidemid rabbits however, were reduced when it was administered to different dosages of TCAE. Among the concentrations of TCAE tested, medium dose showed more potent effect in reducing blood serum TC and LDL-C levels compared to low and high dosages counterparts. For blood serum TG level, low and medium dosages of TCAE exhibit more potent effect to reduce blood serum TG level, compared to high dosage of TCAE. In addition to that observed result in LDL-C, HDL-C and TC, TCAE also reduced the coverage area of atherosclerotic plaque compared to hyperlipidemic rabbits group in dose dependent manner. It is well known that increased level of TC, TG and LDL-C were the major symptom of hyperlipidemia. Hyperlipidemia, in turn is a major risk factor of atherosclerosis (Nelson, 2013). The decrease of TG, TC and LDL-C level of hyperlipidemia group after treatment of TCAE have showcase the beneficial effect of TCAE consumption in reducing serum cholesterol level and these results have been supported by the reduction of atherosclerotic plaque coverage of hyperlipidemic rabbit that were treated with TCAE. Total cholesterol refers to total amount of cholesterol at a given time and the sum of LDL-C, HDL-C, very low density lipoprotein (VLDL) and intermediate density cholesterol (IDL). In this study, TC was significantly increased in hyperlipidemic rabbit compared to untreated rabbit. However, TCAE at different dosage evidently decrease the TC level and the medium dosage of TCAE has again showed the most evident effect.

Gamma glutamyl transferase (GGT) is an enzyme which plays a role in extracellular catabolism of antioxidant glutathione. It can be found in liver, kidney and cerebrovascular endothelium. GGT acts as pro-oxidant in extra-cellular space. The increase level of GGT may be a reflection of high degree of oxidative stress. GGT is also found to be positively correlated with increase of chronic heart disease events such as congestive heart failure and components of the metabolic syndrome (Niranjan et al., 2012). Results of GGT test in this study shows that TCAE has markedly reduced GGT level on hyperlipidemic rabbit. GGT level in rabbit administrated with medium and high dosages of TCAE decreased by 77.81 and 76.00%, respectively, compared to hyperlipidemic rabbits model. Even though low dosage of TCAE showed lower inhibitory effect than medium and high dosages, it also produced a substantial inhibitory effect with 66.63% of inhibition. Alkaline phosphatase (ALP), a membrane bound isoenzymes that catalyzes the hydrolysis of inorganic pyrophosphate, is expressed in variety of tissues including liver and bone, and in lesser amounts from intestines, placenta, kidneys, and leukocytes (Tonelli et al., 2009). Previous epidemiological studies have associated serum ALP levels to increased coronary calcification and increased risk of CVD (Adeney et al., 2009; Covic et al., 2009; Palmer et al., 2011) and the proposed mechanism in which ALP is related to CVD may be due to inflammation (Tonelli et al., 2009). In this study, medium dosage of TCAE has again showed a strong inhibition activity compared to low dosage counterpart while it is not significantly different compared to high dosage counterpart. The ability of TCAE to inhibit the release of GGT and ALP marker demonstrate its protective effect from oxidative stress and prevent coronary calcification thus decrease the risk of CVD.

Oxidative stress, apart from hyperlipidemia and liver biomarkers, is also an important risk factor for atherosclerosis and CVD. Many reported studies has shown that oxidative stress cause oxidation of LDL to ox-LDL and cause endothelial cell injury which lead to infiltration of monocyte to the arterial intima and its deposition into arterial membrane. Oxidation of LDL to ox-LDL is mainly caused by reactive oxygen species (ROS), particularly superoxide anion and hydrogen peroxide (\(H_2O_2\)) (Liu et al., 2009). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are intracellular enzymes that are involved in cellular defence mechanism from oxidative stress caused by ROS. SOD plays a role in scavenging superoxide anion by forming \(H_2O_2\) while GPx safely decompose \(H_2O_2\) to water and superoxide anion (Yu et al., 2007). In normal circumstances, both enzymes can steadily eliminate oxidative stress by feedback compensatory mechanism. However, under excessive oxidative stress condition caused by superoxide anions, or with limited bioavailability of endogenous antioxidants, both enzymes were depleted and it is reflected by low level of SOD and GPx in plasma. In corroboration with that, result of this study shows that GPx level in hyperlipidemic rabbits was reduced when it was compared to normal rabbit group. Rabbits that were treated with TCAE exhibited increased level of SOD and GPx compared to hyperlipidemic rabbits and even at higher level compared to normal group, demonstrating the protective effect of TCAE in regulating antioxidant enzymes level under oxidative stress circumstance.
In this case, the increased level of SOD and GPx on TCAE-treated rabbits was in dose dependent manner, which contradicts the results of the aforementioned experiments. This result demonstrates the antioxidant capability of TCAE by increasing endogenous antioxidant enzymes on hyperlipidemic rabbits.

Conclusion

These findings suggest that treatment of TAEC were able to positively modulate cholesterol metabolism. Supplementation of 450 mg/kg of T. crispa extract would be the optimum concentration to reduce or retard the progression of atherosclerotic plaque development induced by dietary cholesterol by reducing LDL-C, TC, TG, ALP and GGT and raising HDL-C, TAS, GPx and SOD. T. crispa may, therefore, be beneficial in preventing hypercholesterolemic, atherosclerosis and reducing risk factors for coronary artery disease.

Conflict of interests

The authors did not declare any conflict of interest.

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