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EFFECTS OF GYNURA PROCUMBENS EXTRACT ON LIVER FUNCTION TEST OF HYPERCHOLESTEROLEMIA INDUCED RABBITS

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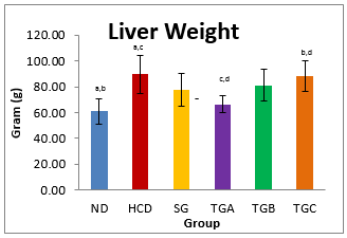
Graphical abstract



Abstract

Antioxidant works against oxidant and free radicals is by donating electrons it encompasses to the radical molecules and therefore neutralising the oxidation process in the biological system. Hypercholesterolemia has been identified as one the primary predisposing factor for chronic health diseases in most industrial and developed countries.Hypercholesterolemia contributes to nonalcoholic fatty liver diseases (NAFLD). Gynura procumbens is not toxic and exhibit anti-diabetic, anti-inflammatory, anti-hypertensive and wound healing properties where the extract of this plant contains active chemical constituents such as tannins, terpenoids, sterol glycoside, saponins and flavonoids. New Zealand White Rabbits were fed with high cholesterol diet for ten weeks. Liver weight and serum liver function test (LFT) including ALP, ALT, AST and GGT were determined and analyzed. Rabbits that were given high cholesterol diet show the symptom of liver injury. ALP, ALT, AST and GGT levels indicate the liver injury. Liver injury of rabbits that were supplemented with Gynura procumbens extracts were reduced compared to rabbits that were given high cholesterol diet but not given any supplement of extract. ALP, ALT, AST and GGT level on rabbits were given the extract were improved compared to HCD. Supplementation of the extract lowered the levels of liver enzymes compared to the HCD.

Keywords: Antioxidant, atherosclerosis, liver function test, gynura procumbens, fatty liver

1.0 INTRODUCTION

Antioxidant is a compound that can postpone or restrain the oxidation process occur in lipids or others by inhibit the initiation or propagation of oxidative chain reactions caused by free radicals and able to protect damage of the biological molecules such as lipids and DNA from free radical-oxidation interactions [1]. Antioxidant works against oxidant and free radicals is by donating electrons it encompasses to the radical molecules and therefore neutralising the oxidation process in the biological system. According to [2] because of the nucleophilic and reductant properties of antioxidants that able to react with oxidants, it allow the donation of one or two electrons.

Hypercholesterolemia has been identified as one the primary predisposing factor for chronic health

diseases in most industrial and developed countries. High levels of cholesterols in the plasma promotes cardiovascular diseases (CVD), peripheral vascular diseases (PVD) and coronary artery diseases (CAD) [3]. Besides, statistics shows that about 6.91% or 147,843 admissions from total admission to the government hospitals are CVD-related cases and has recorded as 24.5% death of overall admissions [4]. Hypercholesterolemia contributes to nonalcoholic fatty liver diseases (NAFLD) [5]. Fatty liver is the state where there is deposition of lipid in the liver that disturb the function and physiology of the liver. According to [6], NAFLD is a symptom of abnormal liver function development. They also added, establishment of NAFLD in the body causes liver inflammation, cirrhosis, fibrosis and liver failure because of the deposition of fat. The amount of cholesterols produced will not be determined well at this stage leading to deterioration of circulating cholesterol in the body.

Gynura procumbes is a local herb that widely distributed throughout Southeast Asia. The herbs have been practiced as an alternative medicine by old folk as they believe the herb is able to heal different types of diseases. Gynura procumbens belongs to Asteracea family and is an annual evergreen shrub with fleshy stems that have been traditionally used among old folk in Southeast Asia especially Malaysia, Thailand and Indonesia as medicinal plants to treat certain diseases such as cancer, migraines, hypertension, kidney problem and diabetes [7]. Previous studies has documented that Gynura procumbens is not toxic and exhibit anti-diabetic, anti-inflammatory, anti-hypertensive and wound healing properties where the extract of this plant contains active chemical constituents such as tannins, terpenoids, sterol glycoside, saponins and flavonoids [8]. The objective of this study is to determine the effects of Gynura procumbens leaves aqueous extract on liver function test of hypercholesterolemic-induced New Zealand White rabbits.

2.0 EXPERIMENTAL DESIGN

2.1 Preparation of *Gynura procumbens* Leaves Aqueous Extract

Gynura procumbens were purchased from local supplier from Kg. Ayer Hitam, Alor Gajah, Malacca and been identified by botanist from Forest Research Institute Malaysia (FRIM). Only leaves part were collected and washed to remove dust and foreign substance. Then, the leaves were oven dried at 50˚C for 72 hours [9]. The dried leaves were pulverized into powder and kept in air-tight container for future use. 10% leaves extraction was performed by soaking 100g of *Gynura procumbens* dried leaves powder into 1L of distilled water and mix thoroughly. Then, the mixture was heated at 60˚C for 6 hours in water bath. The heated mixture was filtered and the filtrate was subjected to freeze dried procedure to obtain freeze dried powder for in vivo experimental study.

2.2 Experimental Design

Thirty six (36) male New Zealand White Rabbits weight about 2kg to 2.5kg were purchased from local supplier. Upon arrival, the animals were acclimatized for a week in the Animal House of Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA Puncak Alam Campus before the experiment started. During acclimatize process; the animals were kept at room temperature with 12 hour light-dark cycle whereas water and food were given *ad libitum*. Experimental procedures were approved by research Committee on the Ethical Use in Research (UiTM Care), Universiti Teknologi MARA (65/2014).

2.3 Induction of Hypercholesterol Diet

Thirty six male New Zealand White Rabbits were randomly divided into six groups shows in Table 1. Each group consist of six male rabbits and labelled as Normal Diet (ND), High Cholesterol Diet (HCD), Simvastatin (SG) and treatment groups (TGA, TGB and TGC). Except to the ND group that was given normal rabbit chow and acted as a negative control, each groups were given 100g per day of 0.5% high cholesterol diet [10]. Treatment groups were supplemented with *Gynura procumbens* leaves extract at a ransom of 100mg/kg/day, 200mg/kg/day and 400mg/kg/day respectively [11]. Water was given ad libitum. Experimental period was set for 10 weeks. Body weights were monitored at week 0, 2, 4, 6, 8 and 10.

Table 1 Description of each groups and its treatment

Groups	Description
ND	Normal diet + distilled water
HCD	0.5% high cholesterol diet + distilled water
SG	0.5% high cholesterol diet + 20mg/kg simvastatin
TGA	0.5% high cholesterol diet + 100mg/kg *Gynura procumbens* extract
TGB	0.5% high cholesterol diet + 200mg/kg *Gynura procumbens* extract
TGC	0.5% high cholesterol diet + 400mg/kg *Gynura procumbens* extract

2.4 High Cholesterol Diet Preparation

Normal chow pallets (CARGILL) were purchased from One Stop Rabbit Farm & Trading, Kuala Selangor. 0.5% high cholesterol diet were prepared based on [12] with slightly modification. To do this, 0.5 g of pure cholesterol powder were dissolved in 45 mL of chloroform. Then, the liquid were sprayed thoroughly

to 200 g of chow pallets before oven dried for 24 hours at 50˚C.

2.5 Blood Sampling

Blood sampling were taken before (week zero) and after the experimental period (week 10). Approximately twelve (12) millilitres of whole bloods were collected through marginal ear vein. Blood were collected in plain and EDTA blood tubes. The blood was centrifuged at 3000 rpm for 10 minutes at 4˚C and the serum/plasma obtained was aliquoted accordingly for biochemistry analysis.

2.6 Liver Weight

At week 10, the animals were sacrificed, the liver were carefully removed out from the body, cleaned and weight. The tissue was kept at -80^{0}C until analyzed.

2.7 Liver Function Test (LFT)

Serum liver enzymes that have been analyzed were alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyltransferase (GGT). Serum samples were sent to CPDRL, UiTM Sungai Buloh Campus.

2.8 Statistical Analysis

All data were expressed as mean ± standard deviation (SD). One way ANOVA was used to compare the statistical differences between the treatment groups and the control group using SPSS version 21. Turkey post-hoc test was used for multiple group comparison analysis. Significant different was set at $p<0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Liver Weight

Figure 1 show the liver weight for each group at the end of the experimental period. Prolong consumption of high cholesterol diet was found to cause liver tissue deterioration as shown in the study. HCD group was recorded to have the highest liver weight (89.533±14.661g) compared to other group, meanwhile ND was the lowest (60.895±9.800g). Supplementation of *Gynura procumbens* leaves aqueous extracts was found to be able in reducing liver weight towards normal level, comparable to the ND group. Liver weight of each treatment groups were 73.048±20.204g, 66.582±6.605g, 82.357±24.167g and 82.982±16.217g for SG, TGA, TGB and TGC respectively.

Continuous intake of high cholesterol diets affects the physical structure and the physiological of liver. In the present study demonstrated that long term of high cholesterol intake will changed the weight of the liver and that reflected as an increased liver function towards metabolising the increased concentration of circulating cholesterol. Liver is important in maintaining homeostasis [13]. Based on the result, liver of animals that have been given 0.5% high cholesterol diet were higher compare to animals that have been given only the normal diet. In contrary, animals that have been given high cholesterol diet supplemented with *Gynura procumbens* leaves aqueous extracts showed a reduction of liver weight compared to the HCD group. This can be postulated that *Gynura procumbens* extract are able to reduce the risk of liver damaged. The presence of active compound in the extract was presumably able to scavenge the free radicals that were produced from excessive levels of circulating cholesterol. Plant extract works well for chemical-induced hepatic damage [14].

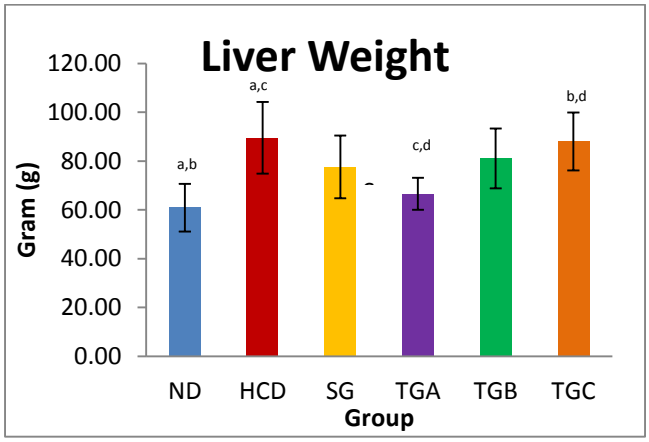


Figure 1 Each value represents the mean ± SD; the same alphabet represents significantly different ($p<0.05$) between groups

3.2 Alkaline Phosphatase (ALP)

At the end of week 10, serum was collected and determined. The level of ALP in the groups was 59.183±5.926 U/L, 76.425±8.957 U/L, 71.800±13.628 U/L, 77.075±1.769 U/L, 95.533±10.350 U/L and 85.675±9.207 U/L for ND, HCD, SG, TGA, TGB and TGC respectively (Figure 2). HCD and TGB groups showed a significant difference ($p<0.05$) between week 0 and week 10. The rest were not significant changed between week 0 and week 10. Surprisingly, TGB showed the highest level of ALP on week 10 followed TGC and TGA. Group that was not given high cholesterol diet and plant extract recorded the lowest ALP levels. ALP is used to determine the membrane permeability and produce instability in the transport of metabolites if the ALP level is altered [15]. This is because ALP is membrane bound. ALT is specific for liver and is appropriate as indicator for liver injury because it facilitates the conversion of alanine to pyruvate and glutamate meanwhile AST indicator for hepatic

injuries that similar to viral hepatitis, infarction and muscular damages.

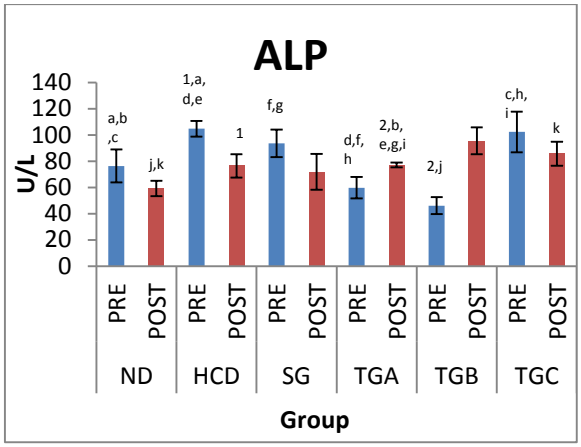


Figure 2 Each value represents the mean ± SD; same number represent significantly different (p<0.05) within groups at specific time interval; the same alphabet represents significantly different (p<0.05) between groups

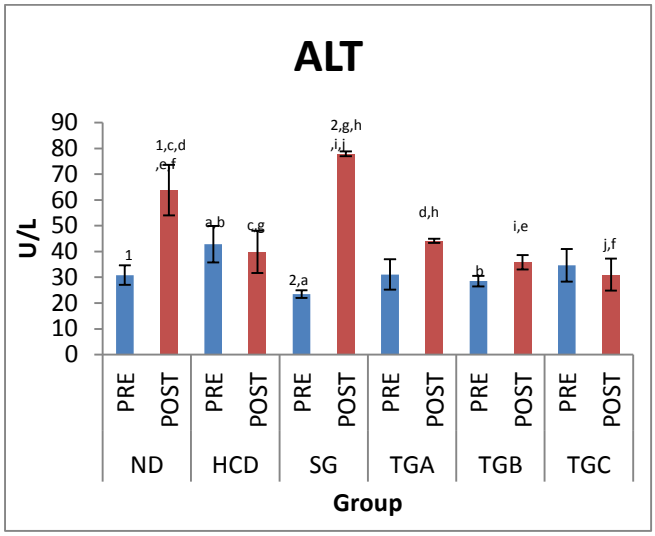


Figure 3 Each value represents the mean ± SD; same number represent significantly different (p<0.05) within groups at specific time interval; the same alphabet represents significantly different (p<0.05) between groups

3.3 Alanine Transaminase (ALT)

Figure 3 show the levels of ALT for each group at week 0 and week 10 of the experiment. The level of ALT at week 0 was ND=(30.833±3.811 U/L), HCD=(42.867±7.094 U/L), SG=(23.475±1.461 U/L), TGA=(31.100±5.892 U/L), TGB=(28.525±2.071 U/L) and TGC=(34.6406.328 U/L). At the end of experiment, the levels of ALT for most of the groups were increased which was 63.833±9.767 U/L for the ND group, HCD was 39.825±8.213 U/L, SG (77.933±0.907 U/L) and all the three *Gynura procumbens* leaves aqueous extract TGA, TGB and TGC treatment groups (44.100±0.819 U/L, 35.833±2.767 U/L and 31.017±6.197 U/L) respectively compared to week 0.

Serum ALT levels for the *Gynura procumbens* leaves aqueous extract treatment groups were lower than the HCD and SG groups. This might be due to the ability of the extract to lower the damage effects of cholesterol in the mitochondrial sites of the liver. Improved mitochondrial activity causes less ALT secretion. This is supported by [16] where *Moringa oleifera* Lam extract were able to reduce the level of ALT in the bloodstream. They also explained that serum ALT levels were decreased because the liver parenchymal and mitochondrial parts were less damaged due to the protective effect by the extract given. Therefore, nontoxic and tissue protective nature against many types of toxic metabolites was significantly reduced the liver toxicity in herbal treated groups.

3.4 Aspartate Transaminase (AST)

Figure 4 illustrates the levels of aspartate transaminase (AST) of each group for week 0 and week 10 of the experiment. There were no significant difference (p<0.05 between every groups) was observed in week zero where the level of AST was as follows: ND = (19.733±3.846 U/L), HCD=(18.260±3.593 U/L), SG=(12.783±2.194 U/L), TGA=(14.175±1.477 U/L), TGB=(17.133±3.191 U/L) and TGC=(18.567±2.980 U/L).

At the end of experiment (week 10), the level of AST were increased compared to week zero. The level of AST at week 10 in ND, HCD, SG, TGA, TGB and TGC were 57.457±1.084 U/L, 43.950±3.366 U/L, 42.450±7.019 U/L, 49.750±8.655 U/L, 42.467±1.801 U/L and 31.780±4.739 U/L respectively and were significantly higher (p<0.05) in comparison to the level of the same group at week 0.

According to [17], serum AST was located in cytoplasm and mitochondria that comprised of two isozymes. AST activities in the liver were 60-80% origin from the mitochondrial and liver injury causes a robust increased of serum AST levels. The present study also found out that the serum AST levels were significantly increased compared to serum AST before the treatment. Findings from the data demonstrated that high cholesterol diet causes detrimental effect that lead to liver injury hence increase AST production. It was found that *Gynura procumbens* extract possess protective effect against oxidative stress from excessive circulating cholesterol and prevents liver injury.

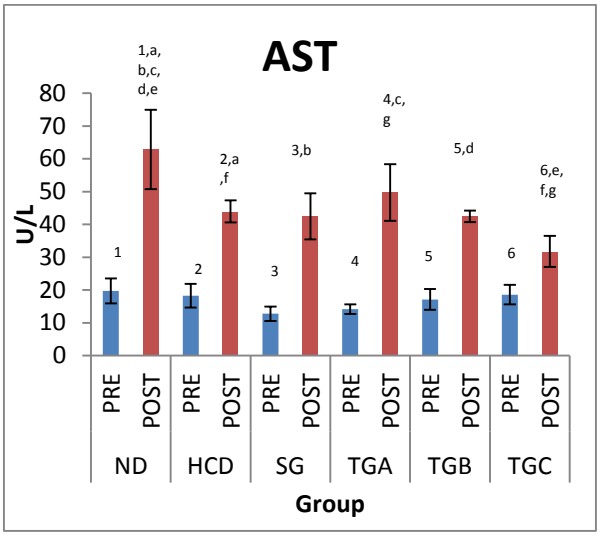


Figure 4 Each value represents the mean ± SD; same number represent significantly different ($p<0.05$) within groups at specific time interval; the same alphabet represents significantly different ($p<0.05$) between groups

3.5 Gamma-Glutamyltransferase (GGT)

Figure 5 show the levels of serum gamma-glutamyltransferase (GGT) for each group at the beginning and at the end of the treatment. At week zero, serum GGT level were observed to be not significantly difference ($p<0.05$) between all groups, which was 4.00±0.779 for ND group, HCD = (6.300±0.925 U/L), SG=(6.425±0.350 U/L) and treatment groups TGA =(4.550±0.839 U/L), TGB=(5.000±0.927 U/L)and TGC=(5.867±0.902 U/L).

At the end of treatment (week 10), the level of serum GGT was increased except for TGA compared to week 0. ND, HCD and SG showed a significance increased ($p<0.05$) on the serum GGT level compared to week 0. Treatment groups were not significantly changed in comparison between week 0 and week 10. ND was 7.975±1.576, HCD was 11.950±2.079, SG was 9.567±1.316 meanwhile the treatment groups (TGA, TGB and TGC) were 3.650±0.714 U/L, 7.680±1.337 U/L and 5.980±1.066 U/L respectively.

Gamma-glutamyltransferase (GGT) is also use as a marker for liver injury where it is an enzyme that located in the cell with high secretory activity. According to [18], GGT has been related in various other diseases such as diabetes, metabolic syndrome, malignancies, cardiovascular disease and also related to the increment of all-cause mortality. They also added that serum GGT can be used as a marker of liver dysfunction and excessive alcohol consumption. In the present study, it was found out that most of the groups show that the level of GGT were increased on the week 10 compared to the week 0. Daily intake of high cholesterol diet show the highest increment of GGT levels compared to others. It shows that daily intake of high cholesterol diets will cause in liver injury that might leads to various types of diseases. This is supported by [19], stated that increasing of serum GGT levels indicate incidence of hypertension, diabetes and CVD. This is also supported by Kozakova *et al.,* (2012), which they have concluded that GGT levels are related to the pathophysiologic link between early atherosclerosis process and hepatic steatosis and they suggested that the increase serum GGT levels can be used as a biomarker of atherosclerosis.

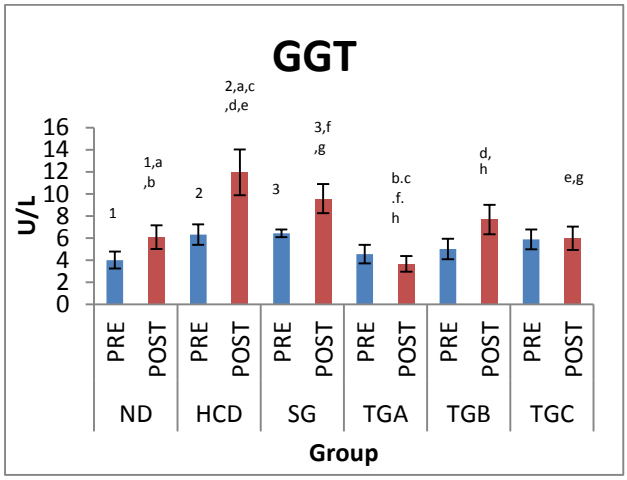


Figure 5 Each value represents the mean ± SD; same number represent significantly different ($p<0.05$) within groups at specific time interval; the same alphabet represents significantly different ($p<0.05$) between groups

4.0 CONCLUSION

Gynura procumbens leaves aqueous extract showed good potential effects and lower the risk of liver injury. In contrary, high cholesterol diet causes oxidative stress and lead to liver injury characterise by the increment of serum ALP, AST, ALT and GGT levels. Supplementation of the extract lowered the levels of liver enzymes compared to the HCD.

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