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Antihyperlipidemic Effect of *Sarabat* (*Diplazium polypodiodes* Blume.) Aqueous Extract in Diet-Induced Hyperlipidemic Rats

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Abstract:

Hyperlipidemia remains an important modifiable risk factor in cardiovascular diseases. The aim of the present study was to investigate the potential role of the aqueous decoction of *Sarabat (Diplazium polypodiodes* Blume) in lowering plasma lipid profile in albino rats fed a high fat diet (HFD). Thirty Wistar albino rats were randomly divided into five groups of six rats and for 42 days were administered plain water and standard pellets (negative controls), lard and cholesterol (hypercholesterolemic animals), low and high dose *Sarabat* decoction (1and 2 g/100ml water respectively and Simvastatin as positive control. The effects of D. *polypodiodes* Bl. (*Sarabat*) decoction on rat lipid profiles was assessed by measuring the plasma Total cholesterol (TC),triglyceride (TG),Low density Liporprotein (LDL), High Density Lipoprotein (HDL) and Very Low Density Lipoprotein (VLDL) .Administration of lard and cholesterol showed a significant elevation ($p<0.006$) of cholesterol during the third data gathering (week 4) and similarly for the other lipid parameters with an increasing lipid profile after a two-week metabolic adjustment period. Concurrent administration of *Sarabat* (D.*polypodiodes* Bl.) decoction showed a promising decrease in cholesterol serum concentration($p<0.006$) , an increase in High Density Lipoprotein (HDL) was notable although not statistically significant. These findings suggest the cholesterol and lipid buffering effects of saponin rich *Sarabat* as potentially useful in the management of hyperlipidemia as part of diet therapy.

Key Words: hyperlipidemia; anticholesterol; diet therapy, saponins; antioxidants

1. INTRODUCTION

Hypercholesterolemia and hypertriglyceridemia are major risk factors either, alone or together that accelerate the development of coronary artery disease and the progression of atherosclerosis(Lusis,2000). Atherosclerosis is thought to involve lipid deposition, oxidative modification and cellular uptake followed by release of inflammatory and growth factors resulting in smooth muscle cell proliferation and collagen matrix production.(Singh et al., 2002). Although treatment of hyperlipidemia can cause slow physical regression of plaques, the well-documented reduction in acute coronary events that follows vigorous lipid-lowering treatment is attributable chiefly to mitigation of the inflammatory activity of macrophages(Malloy et al 2012) Pharmacologic treatment of hyperlipidemia include Lipid lowering drugs like fibrates, statins and bile acid binding resins, niacin, ezetimibe, propprotein convertase inhibitors are used to treat hyperlipidemia (Baron, 2018) and are known to possess some side effects (Chattopadhyaya et al.,1996). The imperative to use treatments with lesser side effects is necessary which includes diet

therapy. Because oxidation of LDL cholesterol is a potential initiating event in atherogenesis, diets rich in antioxidants, found primarily in fruits and vegetables, may be helpful. Studies have suggested that when all of these elements are combined into a single dietary prescription, the impact of diet on LDL cholesterol may approach that of statin medications, lowering LDL cholesterol by close to 30%(Baron, 2018). World ethnobotanical information reports a number of herbal medicines from plants and vegetables that can be used to control hyperlipidemia and related complications in patients (Dahanukar et al., 2000; Scartezzini and Speroni, 2000).In our efforts to find sources from natural products with cholesterol lowering effects, we focus our present investigation to a fern medicinal vegetable commonly called *Sarabat (Diplazium polypodiodes* Bl.).It has been shown that some fern vegetables possess antidiabetic effects due to their anti-alpha glucosidase activity as well as powerful antioxidant activity (Chai et al., 2015; Chai, Panirchellvum, Ong, & Wong, 2012; Chai et al., 2013).Atherosclerosis prevention potential of the fern *Pteris ensiformisis* supposedly attributed to its glycosylated phenolic acid constituent (7-O-caffeoylhydroxymaltol-3-b-dglucopyranoside)(Wei et al., 2007).The present study examines the antihyperlipidemic activity of *Sarabat* which is a species of the pako group of fern vegetables. The young large croziers are gathered in the wild and consumed as vegetables in parts of Northern and Northeastern Luzon. Pako vegetable (*Diplazium esculentum* Retz.)has been demonstrated to possess antioxidant properties due to their contents like phenolics, flavonoids, saponins, terpenoids like triterpenes, diterpenes, and phytosterols and glycosides phytocontituents(Tongco et al., 2014).Saponins, phytosterols, phenolics and the rest of the phytoconstituents in particular have been shown to possess anticholesterol or antihiperlipidemic activity(Akdogan et al., 2012;Rupasinghe et al., 2003;Leontowicz et al., 2002). The presence of the abovementioned constituents in *Diplazium polypodiodes* Bl. as well particularly saponins and flavonoids prompted us to evaluate its antihyperlipidemic properties .

Specifically the study:

1.Evaluated the antihyperlipidemic and antihypercholesterolemic activity of the aqueous decoction extract of *Sarabat (Diplazium polypodiodes* Bl.) in hyperlipidemia induced rats through lipid profile parameters.

2.Evaluated high dose(HD)2gm/100ml and low dose(LD)1gm/100ml boiled decoction with antihyperlipidemic activity

3. Determined the acute toxicity manifestations in rats fed with the extracts following OECD guidelines.

2. METHODOLOGY

The experimental protocol for this study followed the methods as adopted by Sudha R. Pendurkar and Sushma A. Mengi in their study titled "Antihyperlipidemic effect of aqueous extractof *Plumbago zeylanica* roots in diet-induced hyperlipidemic rats" with substantial modifications as well as the study by Kaup et al. (2011) titled Antihyperlipidemic activity of *Cynodon dactylon* extract in high-cholesterol diet fed Wistar rats.

Plant Material

Fiddleheads of the vegetable fern locally called *Sarabat (Diplazium polipoidiodes* Blume.) were purchased from the public markets of Solano and Bayombong Nueva Vizcaya Region 2, Philippines and samples were brought to the Biology department of De La Salle University for identification and subsequent authentication under DLSUH voucher sp no.5602.

Preparation of Extract

The 5kg fern croziers were washed then air dried in shade for one week then pulverized to fine particles using mechanical grinder yielding 320gms powdered fern. About 10gms of the powdered fiddleheads was prepared and added 90 ml distilled water and continuously stirred.Prepared filtered extracts were then brought to the Chemistry Dept. of DLSU and lyophilized to yield a solid to semisolid consistency of crude extract and stored in sealed plastic opaque containers for future use. Another set of 5 kg *sarabat* was procured, air dried, grinded following same procedure, 10gms of dried powdered fern was boiled in 500 ml water with continuous stirring for 30 min until the volume was reduced to 200 ml which made up the 100% *Sarabat* decoction. The same was filtered and stored for acute toxicity study. The decoction utilized in the study made use of 2g/100 ml as high dose and 1g/100m as low dose.

Animals

Healthy albino rats of both sexes were obtained from in house bred and raised experimental albino rats at the Phil. Institute of Traditional and Alternative Healthcare in Region 2. The animals were housed under controlled conditions of light (12h) and temperature 25° ± 1°C in the animal house and allowed to acclimatize for 1 week. All had free

access to water and standard laboratory animal diet. The experimental protocol for this study was reviewed and approved by the IACUC of the same Institute and permit to conduct research using animal models was obtained from the Bureau of Animal Industry under AR-2016-145 .

Acute toxicity study

The acute toxicity was performed according to the OECD 423 guidelines (Ecobichon, 1997). The rats were divided into groups of four each containing six animals (n = 6). The lyophilized aqueous extract at the dose of 10, 20, 50, 100,200,and 400,2000, 5000 mg kg/ body weight was administered by oral gavage to rats after overnight fasting. Another set of rats of the same grouping was prepared for acute toxicity testing of the concentrated boiled *Sarabat* decoction .For the boiled decoction, the extract was well tolerated as no death was found up to the maximum dose of 5gm/kg however in the unboiled lyophilized concentrated crude extract one death was immediately noted at the maximum dose of 5gm/kg The rats were subsequently observed closely for the first 3 h for any untoward symptoms such as tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy followed by observation for a further 24 hrs. A mortality of 1 out of 6 in the 5gm/kg group was noted after 24 hrs in the lyophilized concentrated crude extract of sarabat. No abnormal behavioral patterns was noted.

Induction of hyperlipidemia in rats

The hyperlipidemic condition was induced in rats by feeding a high fat diet (HFD) comprising of 1% cholesterol powder,20% olive oil along with 30% lard oil(pork fat),1 ml honey admixed with 20gm of standard pellet food for an induction period of 15 days. The fatty food was ensured to be consumed daily by the rats.

Experimental design

The rats were divided into five treatments of six rats each. The T1 (normal control) animals was maintained on standard laboratory animal diet. However, the remaining animals in groups T2 to T5 were fed with a high fat diet for a period of 15 days.The induction period was followed by 20 days of drug intervention period where the animals continued with the high fat diet along with drug and decoction experimental treatments. T2 animals (high fat diet control) receive high fat diet for 15 days and onwards. T3 received high fat diet plus simvastatin 8mg/kg po on the 16 day onwards T4 received high fat diet plus boiled *Sarabat* low dose decoction 50%(LD)1gm/100ml water on the 16 day onwards T5 received high fat diet plus boiled *Sarabat* high dose decoction 100%(HD) 2gm/100ml water on the 16th day onwards to day 42.

Determination of serum lipid profile

Blood was withdrawn through tail tipping of rats on day 0 (before the induction of hyperlipidemia), day 14 and day 42 (end of the drug intervention period) of the study. The animals were fasted for 18 h prior blood withdrawal. The blood was collected using red marked tubes and centrifuged at 3000 rpm for 10 min to separate serum for estimation of lipid profile. The serum total cholesterol, triglyceride and HDL-C levels were analyzed using a blood chemistry analyzer machine and by enzymatic colorimetric methods.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukeys t-test.Values are expressed as means ± SEM and $p < 0.05$ was considered to be significant.

3. RESULTS AND DISCUSSION

3.1. Results

Table 1 shows the Total cholesterol (TC) data where first data gathering show significant mean differences between and among treatments particularly T1 and T5.Cholesterol elevations are noted beginning the third week and 4th weeks as shown by elevated mean data during the 3rd data gathering. The table likewise shows that during this period of concurrent administration of the low and high dose *Sarabat* decoction a buffering effect of *Sarabat* particularly high dose against elevation of cholesterol is beginning to take effect as shown by the significant p-value of 0.006 between T1 control and T5(High dose) during the 3rd data gathering and the trend towards lower serum cholesterol is seen between the mean values of the second and third data gathering High dose *Sarabat* T5. This seem to show the possible cholesterol lowering effect of Sarabat which is consistent with its rich saponin content along with other lipid lowering phytoconstituents like plant sterols which reduces the absorption of cholesterol increasing its fecal excretion. Other lipid lowering constituents are polyphenols, flavonoids and tannins. On the one hand for triglyceride (TG), The second data gathering was undertaken during the end of the second week of hyperlipidemic induction where the data seem to show that the tests rats are undergoing metabolic adjustments including induction of lipoprotein

lipases to the high fat diet. The gradual onset of chronic triglyceride elevations can be noted only during the 3rd data gathering period which is the 4th week while not statistically significant it seem to show that elevations are not abrupt and possibly modified and affected by the concurrent administration of the *Sarabat* decoctions which are rich in saponins with detergent like activity along with plant sterols.

Table1.Total cholesterol (TC) and Triglyceride (TG) at varying periods of data gathering in mmol/l

Treatments	Total cholesterol		Triglyceride	
	mean±sd	sig	mean± sd	sig
1st Data Gathering		0.020		0.421
T_1- Pellet control	1.72^{a}±0.09		1.717±0.09	
T_2- HFD (high fat diet)	1.68^{ab}±0.09		1.681±0.09	
T_3- Simvastatin	1.69^{ab}±0.09		1.689±0.09	
T_4- LD (low dose Sarabat)	1.59^{ab}±0.09		1.593±0.09	
T_5– HD (high dose Sarabat)	1.64^{b}±0.09		1.640±0.09	
2nd Data Gathering		0.608	0.649	0.649
T_1- Pellet control	1.55±0.19		0.69±0.17	
T_2- HFD (high fat diet)	1.63±0.22		0.87±0.56	
T_3- Simvastatin	1.62±0.27		0.82±0.21	
T_4- LD (low dose Sarabat)	1.38±0.73		0.72±0.44	
T_5– HD (high dose Sarabat)	1.74±0.21		1.001±0.35	
3RD Data Gathering		0.006	0.421	0.421
T_1- Pellet control	2.11^{a}±0.31		1.19±1.13	
T_2- HFD (high fat diet)	2.04^{ab}±0.25		1.55±0.33	
T_3- Simvastatin	2.03^{ab}±0.35		0.97±0.32	
T_4- LD (low dose Sarabat)	1.87^{bc}±0.15		0.76±0.24	
T_5– HD (high dose Sarabat)	1.57^{c}±0.13		1.08±0.74	

**superscript with different letters indicates significant mean difference at 0.05 level*

Table 2 shows the serum HDL data which indicates that after induction of hyperlipidemia show a nonsignificant but decreasing trend of HDL from mean values obtained initially with p-value of 0.664 to decreased mean values with p value of 0.086. Concurrent administration of the simvastatin(T3) and low and high dose *Sarabat* (T4 and T5 respectively) elevated back the HDL serum levels as shown in the 3rd data gathering mean values and reversed back the p value to 0.664. Although not statistically significant, the complete reversal back to its normal initial values and possibly increasing values with continuous administration of the *Sarabat* decoctions points to its cardioprotective effects. Increased HDL to LDL ratio is beneficial to maintain the continuous translocation of cholesterol from peripheral tissues to the liver for catabolism. The *Sarabat* administered seemed to have influenced the elevation of HDL and restored it to its former prehyperlipidemic levels. Table2 also shows the low density lipoprotein(LDL) and VLDL serum mean values estimated via Friedwalds formula: LDL-c=Total cholesterol-(HDL-c)-(VLDL-c). Data seem to indicate that it is only towards the end of the 4th week that elevations of LDL begins to increase as the first two weeks of hyperlipidemic induction are periods of metabolic adjustments as shown by mean values obtained in the second data gathering. A longer period of treatment and observation is therefore warranted to observe any significant effect as shown by the non-statistically significant differences between the hyperlipidemic control(T2) and treatments 3,4 and 5 (simvastatin and Sarabat groups, respectively).The derived mean values of Very Low Density Lipoprotein is consistent with the LDL,TG and HDL . The various periods of data gathering show similar trends with the exception of total cholesterol(TG).Like HDL there is restoration of the initial mean values (p-value of 0.409) obtained initially going back to the same values obtained during the third data gathering(p-value 0.409) where concurrent administration of Sarabat with the hyperlipidemia induction was undertaken.

Table 2. HDL, LDL and VLDL at varying period of data gathering(mmol/l)

Treatments	HDL		LDL		VLDL	
	mean±sd	sig	mean± sd	sig	mean± sd	sig
1st Data Gathering		0.664		0.421		0.409
T_1- Pellet control	2.25±0.76		-0.68±0.92		0.54±0.51	
T_2- HFD (high fat diet)	1.78±0.20		-0.45±0.21		0.70±0.15	
T_3- Simvastatin	1.98±0.72		-0.39±0.53		0.44±0.14	
T_4- LD (low dose Sarabat)	2.02±0.18		-0.49±0.20		0.34±0.11	
T_5– HD (high dose Sarabat)	2.08±0.13		-1.00±0.38		0.49±0.34	
2nd Data Gathering		0.086	0.649	0.649		0.644
T_1- Pellet control	1.49±0.42		-0.24^{b}±0.35		0.31±0.08	
T_2- HFD (high fat diet)	1.45±0.16		-0.21^{b}±0.25		0.39±0.25	
T_3- Simvastatin	0.87±0.33		0.28^{a}±0.30		0.37±0.20	
T_4- LD (low dose Sarabat)	1.30±0.69		$-.24^{ab}$±0.23		0.33±0.20	
T_5– HD (high dose Sarabat)	1.45±0.22		-0.17^{c}±0.26		0.46±0.16	
3RD Data Gathering		0.664	0.421	0.421		0.409
T_1- Pellet control	2.25±0.76		-0.68±0.92		0.54±0.51	
T_2- HFD (high fat diet)	1.78±0.12		-0.45±0.21		0.70±0.15	
T_3- Simvastatin	1.97±0.72		-0.39±0.53		0.44±0.14	
T_4- LD (low dose Sarabat)	2.01±0.18		-0.49±0.20		0.34±0.11	
T_5– HD (high dose Sarabat)	2.08±0.13		-1.00±0.38		0.49±0.34	

Triglyceride and cholesterol lowering effect in this plant is due to the predominance of saponins. Previous studies have shown that saponin lowers serum cholesterol levels in animals including humans (Kuppusamy et al 2015,Matsui et al.2009,Ghule et al.2006).Sterols also present in this plant reduces the absorption of cholesterol and thus increase the fecal excretion of steroids that results in decrease of body lipids(Ghule et al.2006,Guimaraes et al 2000)

4. CONCLUSIONS

In the present study,we tested the lipid lowering effect of a wild fern vegetable locally called *Sarabat* (*Diplazium polypodiodes* Blume) in rats fed with high fat and cholesterol diet for 42 days. It will be noted that there is a gradual elevation in the serum lipids of the high fat diet fed rats after 4

weeks of feeding with increase in cholesterol(p-value 0.006) and total triglycerides(p value 0.421). Likewise the aqueous extracts high dose(HD) effected a decrease in blood cholesterol in the hyperlipidemic rats when compared with the control and a comparatively similar effect with the simvastatin group. HDL was likewise noted to be elevated with the administration of high and low dose *Sarabat* treatments which seem to show beneficial effects of this fern vegetable being cardioprotective. Boiled *Sarabat* of up to 5gm/kg did not cause any mortality in the acute toxicity test done. A longer period of administration and assessment is however warranted for better results

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