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Effect of Philippine Red *Allium cepa Lin.* (Sibuyas na Pula) on Serum LDL-Cholesterol Level

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Abstract

Philippine red *Allium cepa Lin.* (native red onions), obtained from Pangasinan, Nueva Vizcaya, and Nueva Ecija, were evaluated for its LDL-cholesterol lowering effect in Sprague Dawley rats by measuring the total cholesterol, triglyceride and HDL-cholesterol levels, and by computing LDL-cholesterol using the Friedewald equation. Forty eight (48) rats were used, divided into six groups, a normal control (NC) group and five treatment groups (two control treatment groups: ACC-commercially available atorvastatin calcium tablet and QC-standard quercetin dihydrate; and three experimental treatment groups: RI-Pangasinan, RII-Nueva Vizcaya, and RIII-Nueva Ecija). The LDL-cholesterol lowering effect of the red onions was compared to commercially available lipid lowering drug (atorvastatin calcium). And to prove that the LDL cholesterol lowering effect of the onions is due to flavonoids, standard quercetin dihydrate was used. Results showed that onion extracts exhibited LDL-cholesterol lowering effect like that of the atorvastatin calcium. Likewise, standard quercetin dihydrate showed a significant LDL-cholesterol lowering effect, which proved that flavonoids are effective in lowering LDL-cholesterol. It can be concluded that red onions, obtained from three different sources, are equally effective in lowering LDL-cholesterol, due to the presence of flavonoid in the extracts.

INTRODUCTION

Phytochemicals are substances obtained from plants that provide health benefits. Onions, scientifically named *Allium cepa Lin.*, of the Liliaceae family, have been known to contain phytochemicals (Jaiswal et al., 2013; Gregorio et al., 2010). Numerous health benefits have been identified and have attracted researchers to test the validity of the proposed attributes. Phytochemicals in onions include organosulfur compounds and flavonoids. Organosulfur compounds, like thiosulfates, exhibit anti-inflammatory, anti-allergic, anti-microbial, anti-cancer, anti-diabetes, and anti-thrombotic activity (Albishi et al., 2013; Jaiswal et al., 2013). Flavonoids, on the other hand, are group of polyphenolic compounds that may occur as aglycones, glycosides and methylated derivatives and have several subgroups. Flavonoids are very important in blocking the process of oxidation, which is related to atherosclerosis leading to cardiovascular diseases. They also exhibit anti-inflammatory, anti-hepatotoxic, anti-cancer, anti-bacterial and anti-ulcer actions (Agati et al., 2012).

Cardiovascular diseases like ischemic heart disease and stroke, are results of atherogenesis. Presently, these diseases are the most frequent causes of death throughout the world. Low density lipoprotein cholesterol (LDL-C) has been identified to play a significant role in atherogenesis (Wu et al., 2012).

Epidemiologic studies suggest that consumption of certain fruits and vegetables may protect against stroke and cardiovascular disease using the different mechanisms and properties of either individual and/or combined medicinal substances (Jaiswal et al., 2013; Gregorio et al., 2010). In the Kuopio Ischaemic Heart Disease Risk Factor Study, it was concluded that quercetin, a subclass of flavonoid, promotes good cardiovascular health. Rimm et al., however, did not find a strong inverse association between intake of flavonoids and coronary heart disease (Moline et al., 2000). In another study, it was found out that increased consumption of foods rich in flavonoids decreased cardiovascular risk (Yeganeh et al., 2005; Mennen et al., 2004).

These contradictions on the results of the abovementioned studies regarding the medicinal effects of flavonoids were found to be due to certain factors such as the source of the flavonoid, the food preparation, and processing and the method of flavonoid extraction and administration, whether in vitro or in vivo and/or orally or intravenously (Xu et al., 2007; Katsube et al., 2005). In the case of onions as the source of flavonoids, composition variation, phenotypes like color difference, growing condition, light, seasonal variation and age are considered sources of variations (Lapointe et al., 2006; Xu et al., 2007). Phenotype varieties of onions, whether red, white, yellow or golden, have differences in their flavonoid content (Yamada et al., 2004). On the other hand, growing condition, whether grown hydroponically

or in potting soil, showed no significant difference in the onion composition (Lapointe et al., 2006).

According to the National Onion Association, approximately 87% of the onions produced in the United States are yellow, with about 8% red onions and 5% white onions. While in the Philippines, the most common onion varieties found in the marketplace are the red onions and the white onions (Agribusiness, D.A. 2003). Only the red onions are included in this study. The effect of flavonoids present in the crude ethanolic extract of red *Allium cepa Lin.* was identified.

MATERIALS AND METHODS

Plant Source and Extraction Procedure

Red *Allium cepa Lin. var. ascalonicum* was obtained from three different regions in the Philippines, namely Region I (Pangasinan), Region II (Nueva Vizcaya), and Region III (Nueva Ecija). Onions from the three sources were ground separately, and 80% ethanol was used for extraction. Crude ethanolic extract was then purified through column chromatography using ethyl acetate:formic acid:water (8:1:1) as solvents. Eluates were tested for the presence of flavonoid using Magnesium Turning-Wilstatter Cyanidin test as screening method and Thin Layer Chromatography as confirmatory test (Guevarra *et al.,* 2005). Ferric chloride and potassium ferricyanide were used as spray reagents to visualize the chromatogram.

Approximate Lethal Dose and Median Lethal Dose

Twenty gram-male BALB/c mice were used in the approximate lethal dose (ALD) and median lethal dose (LD_{50}) determinations with an initial dose of 25 mg/kg BW. This dose was logarithmically increased by 6 x 10^{-1} interval and each dose is given to two mice. Concentrations that gave 0% deaths and 100% deaths were noted. For LD_{50} determination, 125.9 mg/20 g BW was used as the starting concentration and increased logarithmically by 2 x10^{-1} interval. This dose was obtained from the result of ALD. Six mice per concentration level were used and the dose that gave 50% deaths was the median lethal dose.

Animals and the Experimental Protocol

The effect of red *Allium cepa Lin.* flavonoid extracts on serum LDL-cholesterol level of hypercholesterol-induced rats was investigated in this study. Forty eight (48) Sprague Dawley rats were used as experimental animals with animal research permit number AR-004-08 issued by the Bureau of Animal Industry (BAI).

Commercially available atorvastatin calcium tablets, which is the standard lipid lowering drug, and quercetin dihydrate powder, a flavonoid standard, were used as treatment controls. The rats were grouped into six (NC-normal control, ACC-atorvastatin calcium control, QC-quercetin control, RI-red onion extract from Region I, RII-red onion extract from Region II, and RIII- red onion extract from Region III) with eight rats per group. Blood extractions were done 6 times to determine LDL-cholesterol level prior to induction (baseline level), after induction (hypercholesterol level), and weekly in one month of treatment. All groups, except NC group, were given 40 mg cholesterol in 0.5 mL canola oil per 100-gram body weight of rat to induce hypercholesterolemia. Afterwhich, the experimentation proper started. Flavonoid extracts were given to groups RI, RII, and RIII together with normal rat diet. ACC group received atorvastatin calcium with normal rat diet. QC group received quercetin dihydrate with normal rat diet. NC group received only the normal rat diet and distilled water. The onion extracts, as well as the control treatments and distilled water, were given via oral gavage based on the weight of each rat at a dose of 10 mg/100 g BW of rat.

Serum Lipid Levels

Blood samples were collected from the tail of the rat before and after induction of hypercholesterolemia and weekly in one month after treatment. All blood samples were placed in a microtainer tube with gel separator, allowed to clot for 15 minutes, and centrifuged for 10 minutes at 4,000 RPM.

LDL cholesterol level was obtained indirectly using Friedewald equation [LDL-C = total cholesterol – high density lipoprotein cholesterol – (triglyceride/2.175)] in mmol/L. Serum total cholesterol (TC), triglyceride (TG), and HDL-C were measured and the results were used in the computation of LDL-C values (Martin *et al.,* 2013). Commercially available TC, TG, and HDL-C kits were used (Human Biocare Company, Europe). Protocol provided by the company was followed in the measurement of TC, TG, and HDL-C. Briefly, 0.01 mL of serum sample was mixed with 1.0 mL of cholesterol reagent containing phosphate buffer at pH of 6.5, aminophenazone, phenol, peroxidase, cholesterol esterase, cholesterol oxidase, and sodium azide or triglyceride reagent containing PIPES buffer at pH of 7.5, chlorophenol, aminoantipyrine, magnesium ions, adenosine triphosphate, lipase, peroxidase, glycerol kinase, and glycerol-3-phosphate oxidase and incubated at room temperature for ten minutes. For HDL-C determination, 0.2 mL of serum sample was mixed with 0.5 mL of phosphotungstic acid and magnesium chloride and centrifuged for 2 minutes 10,000 RPM. Supernatant was collected and 0.1 mL of this was added to 1.0 mL of cholesterol reagent and incubated for five minutes. After incubation, absorbances were read at 500 nm (Spectrophotometer ALS 2000). All serum samples were run together with blank tubes, normal control and

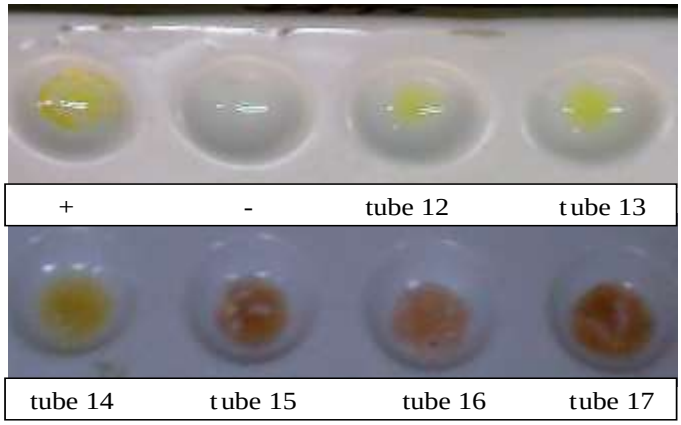

Figure 1: Magnesium Turning-wilstatter Cyanidin Test of column eluates from tubes 12 to 17 on sports plate; Positive and negative controls were run side by side with the elutes. Positive contains standard quercetin dehydrate, formic acid and water in 8:1:1 ratio. Red to orange coloration indicates presence of flavonoid.

Table 1: Approximate lethal dose determination of red *Allium cepa Lin.* Extracts

CONCENTRATION (mg/20 g BW)	LOG INTERVAL (0.6 interval)	RESULT (deaths over number of mice tested)
0.5	-0.3	0/2
2.0	0.3	0/2
7.9	0.9	0/2
31.6	1.5	0/2
125.9	2.1	0/2
501.2	2.7	0/2
1,995.3	3.3	0/2
7,943.3	3.9	0/2

pathologic control sera to monitor accuracy.

Statistical Analysis

Data were assessed using SPSS program. One way Analysis of Variance (ANOVA) was used to determine if flavonoids extracted from the red onions significantly lower the LDL-C. Duncan test was used as the post hoc test to check any significant difference on the LDL-C lowering effect of the flavonoids from red onions from three geographical sources, and as compared to the standard lipid lowering drug and to quercetin dihydrate. P-values of <0.05 between mean values were considered statistically significant.

RESULTS

Flavonoid content of Red *Allium cepa Lin.* extracts

The crude ethanolic extract of red *Allium cepa Lin.* was ran down through a column chromatography to isolate the flavonoid portion of the extracts determined by Magnesium Turning-Wilstatter Cyanidin test and confirmed by Thin Layer Chromatography (TLC). All eluates were collected and placed in 3-mL tared tubes. Only tubes 12 to 30 produced yellow to orange to red coloration in the screening test for flavonoid. Because of the polarity of flavonoids, they are usually eluted first in a column chromatography. Figure 1 below shows the result of Magnesium Turning Test. All eluates with red to orange to red coloration were spotted on TLC plates for confirmation.

Dose Determination

Using male BALB/c mice, the identified approximate lethal dose was 1,999.3 mg/ 20 g BW of mice while the median lethal dose was 794.3 mg/ 20 g BW. Identifying that the onion extract is safe up to this dose, a dose of 10 mg/100 g BW of rat was used. Same dose was used for atorvastatin calcium control and quercetin dihydrate control. Tables 1 and 2 show the approximate lethal dose and median lethal dose results of red *Allium cepa*

Table 2. Median lethal dose determination of red *Allium cepa Lin.* extracts

CONCENTRATION (mg/20 g BW)	LOG INTERVAL (0.6 interval)	RESULT (deaths over number of mice tested)	Rat Dose (mg /100 g BM)
199.5	2.3	0/6	997.5
316.2	2.5	0/6	1,581.0
501.2	2.7	1/6	2,506.0
794.3	2.9	3/6	3,971.5
1,258.9	3.1	6/6	6,294.5
1,995.3	3.3	6/6	9,976.5
3,162.3	3.5	6/6	15,811.5

Table 3: Lipid Profile values of Sprague Dawley rats before inducing hypercholesterolemia (expressed as mean values in mmol/L).

GROUP	TATAL CHOLESTEROL	TRIGYCERIDE	HDL-C	LDL-C
NC	1.65	1.10	1.31	-0.16
ACC	1.69	1.29	1.24	-0.14
QC	1.67	1.24	1.30	-0.20
RI	1.76	1.21	1.48	-0.28
RII	1.78	1.07	1.49	-0.20
RIII	1.63	1.19	1.42	-0.25

Lin. extracts. Approximate lethal dose is the first dose with two out of two mice dead. While the median lethal dose is the first dose where half of the mice is dead and half is alive.

Effect of Red *Allium cepa* extracts on Serum Lipid Levels

To examine the effects of red onion flavonoid extracts on lipid profile, hypercholesterolemia was first induced in Sprague Dawley rats by feeding them a high fat diet for 7 days. All of the rats in this diet developed hypercholesterolemia. Table 3 shows the TC, TG, HDL-C and computed LDL-C of Sprague Dawley rats before giving high fat diet.

From the above values of TC, TG, HDL-C, and LDL-C, the reference values were determined from the mean +/- SD. Normal reference values are: 1.43-1.99 mmol/L for TC; 0.80-1.55 mmol/L for TG; 1.12-1.63 mmol/L for HDL-C; and -0.56-0.14 for LDL-C.

After 7 days of giving high fat diet, lipid profile values were again determined. Table 4. shows the lipid profile values of Sprague Dawley rats after hypercholesterolemic induction and Comparing the lipid profile values before and after induction, TC, TG, and LDL-C values increased after hypercholesterolemic induction while HDL-C values decreased.

Abnormal reference values of the Lipid Profile of Sprague Dawley rats were computed as mean +/-SD in mmol/L: 1.93-2.61 mmol/L for TC; 1.51-1.89 mmol/L for TG; 0.72-1.30 mmol/L for HDL-C; and 0.34-0.54 mmol/L for LDL-C. Levels below these values are considered normal and levels above these are pathologic, except for HDL-C, which has a different pattern of changes. TC, TG, and LDL-C levels increased after hypercholesterolemic induction in groups ACC, QC, RI, RII, and RIII as compared with the NC group, which did not receive high cholesterol diet.

After hypercholesterolemic induction, Sprague Dawley rats received corresponding treatments/extracts depending on the group name for one month and blood samples were collected weekly. NC group received only distilled water. LDL-C values were computed from TC, TG, and HDL-C. Values were compared before and after hypercholesterolemic induction and after giving treatment/extracts. Figure 2 below shows the lipid profile values before and after induction, and after 4 weeks of treatment.

Focusing on the effect of onion extracts on LDL-C levels, it has been observed that immediately after a week of treatment, the LDL-C values declined (not shown). As shown in figure 2-D, LDL-C values decreased after four weeks of treatment or of giving onion extracts as compared with the LDL-C values after hypercholesterolemic induction. The values decreased up to the 'before induction' levels of LDL-C, which indicate that the values normalized after a month of treatment. Using analysis of variance (ANOVA), LDL-C values after 4 weeks of treatment were compared. A p-value of 0.998 was obtained and indicates that there is no significant difference among the compared groups (NC, ACC, QC, RI, RII, and RIII). Using Duncan Test for posthoc analysis, individual groups were compared with

Table 4: Lipid profile values after Hypercholesterolemic induction (expressed as mean values in mmol/L).

GROUP	TOTAL CHOLESTEROL	TRIDYCERIDE	HDL-C	LDL-L	REMARK
NC	1.77	1.53	1.36	-0.33	Not induced
ACC	2.45	1.86	1.26	0.33	*
QC	2.79	1.88	1.37	0.56	*
RI	2.05	1.51	0.82	0.54	*
RII	2.00	1.75	0.80	0.39	*
RIII	2.07	1.51	0.90	0.38	*

*Indicates significant increased as compared with the baseline levels.

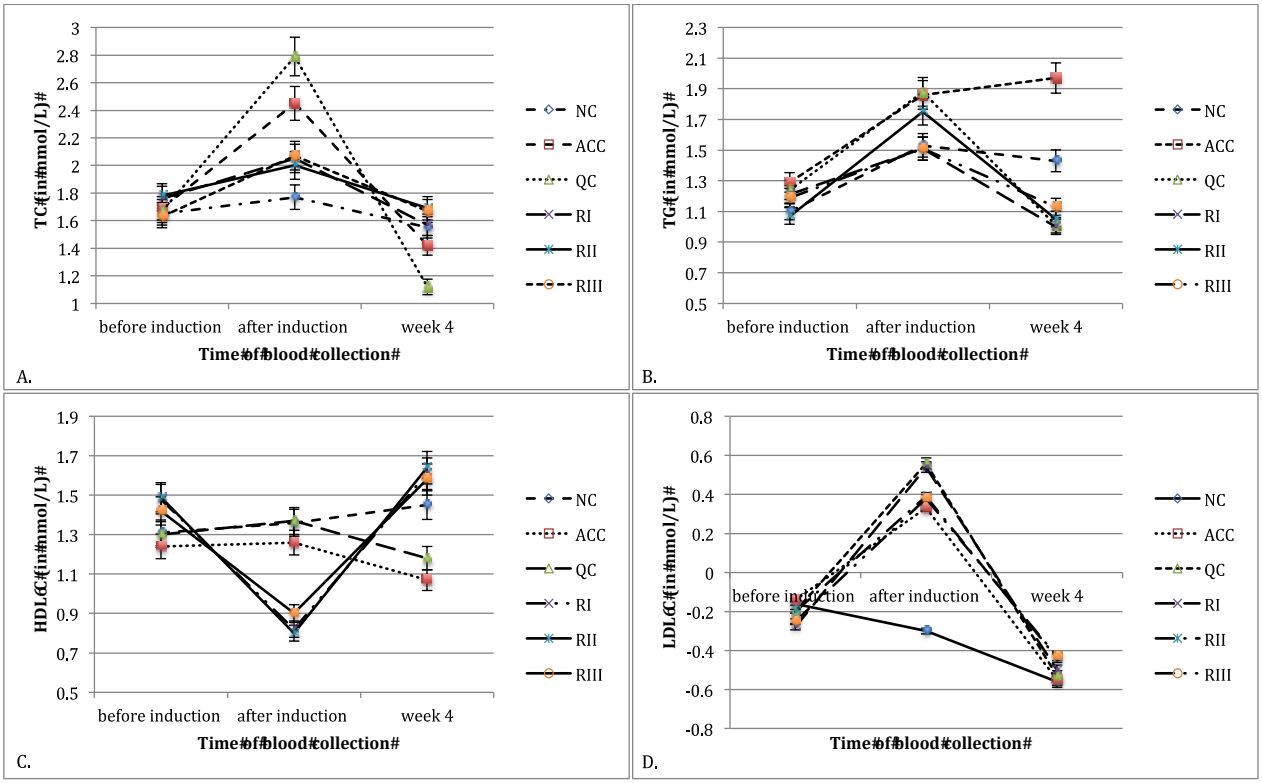

Figure 2: TC, TG, HDL-C and LDL-C values in mmol/L before, and after induction and after 4 weeks of treatement. Blood samples were collected after acclimatization (before giving high fat diet), after hypercholesterolemic induction (after giving high fat diet), and weekly for four weeks afer giving atorvastatin calcium (ACC), quercetin (QC), and onion extracts (RI, RII, and RIII). Results are means+/-SEM**.**

each other (NC vs. RI, NC vs. RII, NC vs. RIII, RI vs. RII, RII vs. RIII, RI vs. RIII, RI vs. ACC, RI vs. QC, RII vs. ACC, RII vs. QC, RII vs. ACC, and RIII vs. QC). A p-value >0.05 was obtained indicating that LDL-C values of all the groups after four weeks of treatment were not different from each other and that the extracts from onions were effective in lowering high levels of LDL-C. It can be concluded that the flavonoid extracts obtained from red *Allium cepa Lin. var. ascalonicum* taken from Region I, Region II, and Region III were equally effective in lowering LDL-C levels of hypercholesterol-induced rats.

DISCUSSION

Here we established that onion extracts from three regions in the Philippines namely, Pangasinan (RI), Nueva Vizcaya (RII), and Nueva Ecija (RIII), contain flavonoids and that the flavonoid content of the onions from three sources are effective in lowering LDL-C in Sprague Dawleys rats and are equally effective as that of the commercially available atorvastatin calcium and of the standard quercetin dihydrate. Flavonoid content of the onions was collected using ethanolic extraction and using column chromatography, and was screened using

Wilstatter Cyanidin-Magnesium Turning test and confirmed using thin layer chromatography.

One of the objectives of this study is to determine if there is significant difference on the onions collected from three difference regions in the Philippines. This study was able to identify that there is no difference in terms of flavonoid content and in terms of its capacity to lower LDL-C levels.

As regards the capacity of the onions extracts to lower LDL-C, this study was the first to identify the effect of onions on LDL-C levels in Sprague Dawley rats. Other studies determined only the antioxidant effect, and anti-inflammatory effects of onions. There is a possibility that the LDL-C lowering effect of onions is actually due to its anti-oxidant property, and the anti-oxidant property is due to its flavonoid content. One study identified the anti-oxidant effect of the phenolic compounds isolated from onion skin and flesh. The ability of the phenolic extracts to inhibit radical-induced DNA scission, human low density lipoprotein cholesterol oxidation, and lipopolysaccharide-stimulated cyclooxygenase-2 expression in J774A.1 mouse macrophage were observed (Albishi *et al.,* 2013). It has been well known that the oxidation of LDL-C by reactive oxygen species (ROS) play a key role in the pathogenesis of atherosclerosis, thus inhibiting the oxidation of LDL-C is a good way to prevent the development of atherosclerosis (Wu *et al.,* 2012). Several studies have identified the potential of flavonoids to inhibit ROS and to reduce the levels of ROS formed. The flavonoid content of many plants has led to study the anti-oxidant effect of each plant. Some of these studies include: the anti-oxidant activity of total flavonoids from persimmon (Sun *et al.,* 2011); flavonoid content and antioxidant activity of *Prunella vulgaris L.* (Zhang *et al.,* 2011)*;* flavonoids in tangelo (*Citrus reticulata x Citrus paradisi)* and their influence on antioxidant properties (Barreca *et al.,* 2013); and a lot more others. These studies can further be pursued by determining the effect of the identified anti-oxidant properties on specific diseases and molecules. In the present study, the lowering effect of flavonoids extracted from onions on the LDL-C level was identified and may indirectly indicate the importance of onions in decreasing the risk of developing atherosclerosis. Further studies on the in vitro anti-oxidant property and the quantitative determination of the flavonoid content of onions may be done to enhance this study.

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