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Research Article

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Cholesterol-lowering activity of *Artocarpus ovatus* Blanco (Moraceae) ethanolic leaf extract in animal models

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ABSTRACT

Artocarpus ovatus Blanco is a plant species of the Philippines belonging to the family Moraceae. This study evaluated the cholesterol-lowering activity of the A. ovatus ethanolic leaf extract in Sprague Dawley rats and its acute oral toxicity. It was found to be safe and non-toxic up to 2000 mg/kg BW of test animals based on the guidelines of OECD 425 main test. Post toxicity test gross necropsy results are unremarkable. In the cholesterol-lowering bioassay, the ethanolic extract treated rats at doses of 200, 400 and 600 mg/kg showed time dependent reduction of serum levels of total cholesterol, triglycerides and low density lipoproteins with p-values less than 0.05. High density lipoproteins concentration improved, high catalase enzyme levels and unremarkable degree of lipid peroxidation were measured and observed after 14 days of oral administration of the extract.

Keywords: *Artocarpus ovatus* Blanco, Moraceae, Cholesterol-lowering, Acute oral toxicity, Sprague Dawley

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INTRODUCTION

Scientific researchers worldwide study alternative sources of pharmaceutical remedies one of these possible alternative sources would be from medicinal plants. Plants belonging to the genus *Artocarpus* consist of 50 species belonging to the Moraceae family that contains phytochemical compounds such as flavonoids which have medicinal value [1]. One of the common plant species of the genus *Artocarpus* in the Southeast Asian region is the *Artocarpus heterophyllus*. There are a lot of established biological potentials of *A. heterophyllus* such as hypoglycemic, hypolipidemic, gastroprotective, sedative, antibacterial, antifungal, anti-inflammatory and antineoplastic activities [2]. Its leaves have hypolipidemic effect [3]. An endemic plant species of the *Artocarpus* genus in the Philippines is *Artocarpus ovatus* Blanco locally known as *anubling*, *anobion* or *kanabling*. It is a tree that grows up to 25 meters, its brachlets and petioles are distinctly hirsute with short and less scattered hairs and its leaves are simple, oblong, equilateral. Its latex was studied to be utilized as a chewing gum base and its roasted seeds are usually eaten [4]. Published investigations on its medicinal utilities do not exist. For this reason, this present research study established its pharmacologic potential specifically its cholesterol-lowering activity.

EXPERIMENTAL SECTION

Chemicals and reagents

Analytical grade Ethanol (RCI Labscan, Thailand) and Triton X-100 (Hi-Media, India) were purchased from Bellman Corporation. Atorvastatin Calcium USP (Fluka) AR grade was purchased from Sigma-Aldrich corporation. Clinical chemistry kits Cholesterol liquicolor (HUMAN Diagnostics, Germany), Triglycerides liquicolor mono (HUMAN Diagnostics, Germany) and High Density Lipoproteins Direct homogenous reagents (HUMAN Diagnostics, Germany) were acquired at Biocare Health Resources.

Plant collection and authentication of the sample

Fresh matured leaves of *A. ovatus* were collected from Camarines Sur, Philippines and its sample was authenticated by Dr. Wilfredo F. Vendivil, Curator II at the National Museum of the Philippines with a voucher specimen number 198528.

Plant extraction

A. ovatus leaves were washed and air-dried then ground to fine powder using a Thomas Wiley miller. The powdered leaves were weighed with the aid of a Denver analytical balance instrument and then soaked in 95% ethanol in a percolator for 24 hours and the percolate was collected. This procedure was repeated four to five times and each ethanol percolate was filtered and concentrated using an R-200 rotary evaporator (Buchi, Switzerland) at 45° C until a viscous consistency was obtained. The plant sample to solvent ratio was 1: 10. The concentrated extract was further dried at 35 ° C kept in an amber colored glass at 0 to 8° C and was used for analysis [5].

Acute oral toxicity and *In vivo* cholesterol-lowering bioassay

Prior to the conduct of the acute oral toxicity test and *in vivo* bioassay, animal study protocols were first approved by the Bureau of Animal Industry of the Philippines (animal research permit reference number AR-2014-34) through the University of Santo Tomas Institutional Animal Care and Use Committee (animal study code number RC2014-710120). Sprague Dawley rats (weighing 100 - 250 grams at least 6-8 weeks old) were purchased from the Department of Science and Technology Taguig City, Philippines. The test animals were housed at University of Santo Tomas Research Center for Natural and Applied Sciences animal house. The temperature in the animal room was 22° C (± 3°C) with a 12-hour light and dark cycles. The relative humidity was at 50 ± 5 % [6]. For feeding, conventional rodent chow pellets (Purina Mills) was given with an *ad libitum* supply of distilled water. The test animals were acclimatized for 7 days prior to the experimentations. The doses of the ethanolic extract were prepared and dissolved in a 2% Tween 80 vehicle.

Acute oral toxicity (OECD 425 main test guidelines)

Five female Sprague Dawley rats were used and a control test rat. Three doses were utilized in the testing, 175 mg/kg, 550 mg/kg and 2000 mg/kg as described in the OECD 425 main test guidelines (up-and-down dose procedure). The rats had undergone overnight fasting prior to the oral administration of the extract using a gavage. The first animal is given a dose of 175 mg/kg. When the animal survived after 48 hours; the dose that was given for the next animal was increased by a factor of 3.2 and which is 550 mg/kg. When the second animal survived after 48 hours; the next animal was given a dose of 2000 mg/kg (upper bound dose). The testing was terminated until the last three animals survived at the upper bound dose and all of the animals were observed up to 14 days [7]. Post toxicity test gross necropsy procedures of the test rats were conducted by a licensed veterinarian.

***In vivo* cholesterol-lowering bioassay**

Thirty-six male Sprague Dawley rats were utilized and allocated into 6 different experimental groups (Group I – normal control, Group II – negative control, Group III – 200 mg/kg extract treated group, Group IV – 400 mg/kg extract treated group, Group V – 600 mg/kg extract treated group and Group VI – Atorvastatin 10 mg/kg treated group or the positive control) having 6 test animals per group. The inducing agent for hypercholesterolemia was Triton X-100 with a dose of 100 mg/kg (in physiological saline solution) through intraperitoneal route. Overnight fasting of the test animals was followed prior to the induction of hypercholesterolemia [8]. All of the experimental groups except for Group I was induced with hypercholesterolemia. Oral treatment of the ethanolic extract and atorvastatin lasted for 14 days. Blood extractions through tail clipping and biochemical measurements of the cholesterol parameters were done 4 times (baseline, post induction, week 1 treatment and week 2 treatment periods). Serum samples were obtained after allowing the blood samples to clot for 15 minutes in microtainer tubes with gel separator and centrifugation at 4,000 RPM for 10 minutes [9].

Measurement of cholesterol parameters

Total cholesterol, triglycerides and high density lipoproteins (HDL) serum levels were measured or biochemically estimated using the enzymatic colorimetric methods. Low density lipoproteins (LDL) levels were measured through the use of Friedewald equation.

Measurement of catalase enzyme and lipid peroxidation activity

At the end of the bioassay, the test animals were euthanized using carbon dioxide chamber and their liver organs were extracted and homogenized. The liver homogenate samples were subjected to the measurement of catalase enzyme and lipid peroxidation activity. Procedures of Bogdanska (2003) were followed in determining the catalase enzyme activity [10] and using the Thiobarbituric acid reactive species (TBARS) assay [11] following the methods of Manna *et al*. (2006) was used in evaluating the lipid peroxidation activity of the samples. Catalase enzyme and TBARS levels were computed based from their molar absorption coefficients.

Statistical analysis

Data acquired from the bioassay was statistically analyzed using Two-way Analysis of Variance and Post Hoc LSD test (p = 0.05) through licensed computer package SPSS 20.0.

RESULTS AND DISCUSSION

Plant extraction

Extraction of *A. ovatus* leaves yielded the crude ethanolic extract as a black green viscous mass with a percentage of 19.001% w/w.

Acute oral toxicity test results

Using OECD main test 425 guidelines, it was found that the ethanolic extract was safe and non-toxic at 175 mg/kg, 550 mg/kg up to 2000 mg/kg BW of test animals. There were no variations in normal behavioural pattern and absence of signs and symptoms of toxicity after the administration of the extract. No mortality was observed for 14 days of observation. Post toxicity gross necropsy findings showed that all of the vital organs of the test animals were normal having smooth and firm consistency and also upon opening and examining their abdominal and thoracic areas. This shows that the ethanolic extract is non-toxic up to 2000 mg/kg BW of test animals.

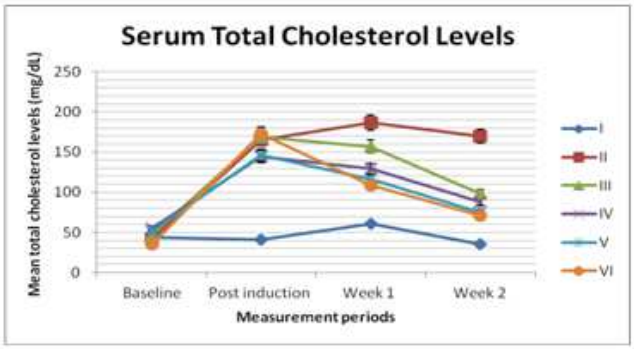

Figure 1. Mean serum total cholesterol levels on baseline to treatment week 2 (Day 14) measurements.

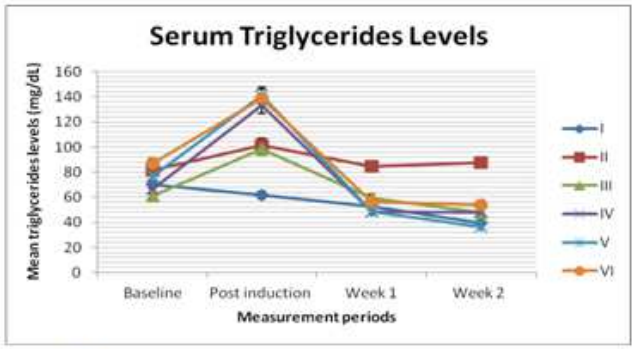

Figure 2. Mean serum triglycerides levels on baseline to treatment week 2 (Day 14) measurements.

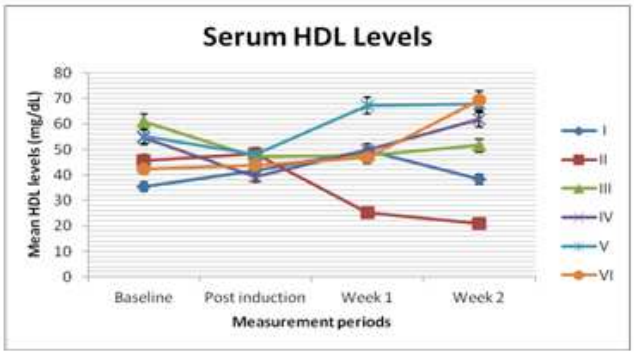

Figure 3. Mean serum HDL levels on baseline to treatment week 2 (Day 14) measurements.

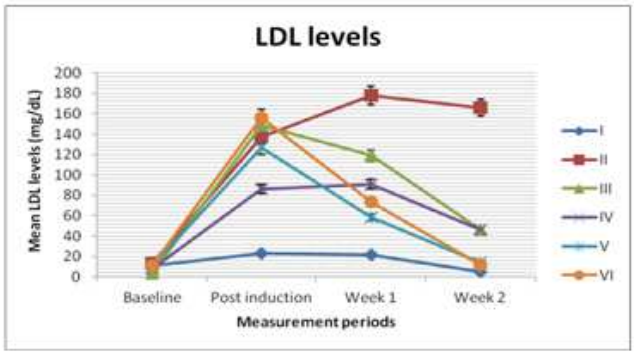

Figure 4. Mean LDL levels on baseline to treatment week 2 (Day 14) measurements.

***In vivo* cholesterol-lowering bioassay results**

Mean serum total cholesterol, triglycerides, HDL and LDL values were significant to all the experimental groups (p values < 0.05). After the 14-day treatment period, groups III, IV, V and VI (200 to 600 mg/kg extract treated groups and positive control group) have decreased total cholesterol, triglycerides and LDL levels. However post hoc analysis showed that groups IV and V (400 and 600 mg/kg extract treated groups) have no significant difference in reducing total cholesterol. The treatment groups (200 to 600 mg/kg extract treated groups) do not statistically differ in improving mean HDL serum levels with the positive control group (p values > 0.05). This demonstrates that the ethanolic extract exhibited cholesterol-lowering effect comparable to the positive control on animal rat models. Presence of excessive levels of cholesterol in the blood indicates a strong risk factor for cardiovascular diseases. Maintenance of low levels of cholesterol in the blood is clinically and importantly considered through the use of pharmaceutical agents such as the statin drugs (HMG-CoA reductase inhibitors) and the fibrates [11]. Medicinal plants can also be utilized as alternative sources of these established remedies but most of them must be clinically studied further. The results of this study suggest that the *A. ovatus* ethanolic leaf extract have a medicinal potential

specifically in lowering cholesterol levels in rat animal models. Figures 1 to 4 present the mean total cholesterol, triglycerides, HDL and LDL levels of the experimental groups.

The lowest levels of catalase enzymes are found at Group II (negative control group). The mean catalase levels of experimental groups III, IV, V (200, 400 and 600 mg/kg extract treated groups) with *p* values greater than 0.05 are not statistically different with group VI (positive control group). Significant levels of the catalase enzymes of the treatment groups demonstrates that at 200 mg/kg up to 600 mg/kg dose of the ethanolic extract was able to scavenge hydrogen peroxide through the action of catalase enzyme present. High levels of TBARS are measured on the negative control group demonstrating a high degree of lipid peroxidation. The 400 and 600 mg/kg extract treated groups and the positive control group have no significant difference in mean TBARS level with the normal control group having low levels of TBARS. This indicates that 400 mg/kg and 600 mg/kg doses of the ethanolic extract were able to prevent lipid peroxidation (degradation of lipids and causes cell damage). In hypercholesterolemic condition, events involving oxidative stress can produce lipoprotein radicals which can interact and accumulate within the cell membrane affecting normal vascular functions [12]. The findings suggest that the *A. ovatus* ethanolic leaf extract can also prevent oxidative reactions such as lipid peroxidation and maintaining high levels of catalase enzymes *in vivo* through rat animal model thus preventing negative interferences of normal vasculature functions and cell damage.

CONCLUSION

Artocarpus ovatus ethanolic leaf extract is safe and non-toxic up to 2000 mg/kg based from the acute oral toxicity OECD 425 main test guidelines. Its doses of 400 mg/kg and 600 mg/kg demonstrated a considerable cholesterol-lowering activity and were able to maintain sufficient levels of catalase enzymes and inhibit lipid peroxidative events *in vivo*.

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