**ORIGINAL ARTICLE**

# HEMATOLOGIC PROFILE AND BIOCHEMICAL VALUES IN ADULT DOGS GIVEN CHOLESTEROL WITH OR WITHOUT NANOLIPOSOME-ENCAPSULATED MALUNGGAY (*Moringa oleifera)* ADMINISTRATION

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## ABSTRACT

**The hematologic profile and biochemical values in adult dogs given nanoliposome-encapsulated malunggay (*Moringa oleifera*) phenolic extract were determined in this study. Six adult dogs were divided into three groups: Group A: cholesterol only; Group B: cholesterol with malunggay phenolic extract; and Group C: cholesterol with nanoliposome-encapsulated malunggay phenolic extract, with two dogs per group. Each dog was subjected to a complete blood count at weeks 0, 1 and 5 and serum biochemistry tests at weeks 0 and 5. The results showed that thrombocytopenia and a decrease in the elevated blood urea nitrogen level were observed after administration of the nanoliposome-encapsulated malunggay phenolic extract. It also showed below normal cholesterol and low density lipoprotein levels and within normal triglyceride and high density lipoprotein levels after the experiment. These preliminary findings suggest that nanoliposome-encapsulated malunggay phenolic extract may have an effect on the platelets, kidneys, and lipid profile. However, further studies should be done to verify this association.**

Keywords: cholesterol, dog, hematology, kidneys, moringa, nanoliposome

## INTRODUCTION

Dyslipidemia is the change in lipid levels (German, 2006). Dogs with chronic kidney disease cause a decrease in the high density lipoprotein-cholesterol (Behling-Kelly, 2014) which leads to higher levels of total WBC and platelets and lower levels of neutrophils, lymphocytes, monocytes and packed cell volume (Emokpae and Kuliya-Gwarzo, 2014). Hyperlipidemia is the increase in the concentration of lipids in the blood more commonly associated with endocrine disorders, obesity and high fat diet that leads to pancreatitis, seizures and ocular and hepatic diseases (Xenoulis and Steiner, 2010). According to Jain *et al*. (2007), a 1% decrease in the total serum cholesterol decreases chronic heart disease risk by 2%. Hyperlipidemia is usually controlled through administration of lipid-lowering drugs (Xenoulis and Steiner, 2010).

Quercetin, a flavanoid component of malunggay (*Moringa oleifera*), can decrease hyperlipidemia (Mbikay, 2012). It was also reported to have antioxidant, hypolipidemic and hepatoprotective properties (Sachan *et al.,* 2011; Tang *et al*., 2012). Quercetin has already been added to commercial dog food; however, quercetin's bioavailability in dogs is only 4% (Reinboth *et al.,* 2010). With this, a way to increase its bioavailability is essential

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to maximize its potential. Nanotechnology, such as nanoliposome-encapsulation, was developed to increase the bioavailability (Fang and Bhandari, 2010) of compounds such as quercetin. It aids in the protection and absorption of the drugs that are being administered. However, quercetin was found to inhibit platelet aggregation (Tzeng *et al.*, 1991) and was also found that high amounts of quercetin can cause kidney damage (Ehrlich, 2011). With the expected increase in quercetin bioavailability because of nanoliposome- encapsulation, it is assumed that these effects will also be increased. McCullagh and Ehrhart (1975) reported that dietary cholesterol can also have an effect on the kidneys of dogs.

However, studies on the hematologic profile and biochemical values in adult dogs given nanoliposome-encapsulated malunggay phenolic extract (which contains quercetin) and cholesterol have not yet been done. Therefore, it was investigated in the current study. The results of this study can be used as a basis for future studies on the use of nanoliposome encapsulation in the production of nutraceuticals for dogs.

## MATERIALS AND METHODS

Six adult male dogs approximately 2-3 years old with body condition score between 4-6/9 (Laflamme, 1997) were used in this study. These dogs were collected from Los Baños, Laguna and were housed in the kennel station at the University of the Philippines' Veterinary Teaching Hospital-Maahas Station. The dogs were randomly assigned to three treatments. Filler capsules containing rice bran and magnesium steate were given to Group A (control) dogs (A1 & A2). Non-encapsulated malunggay phenolic extract capsules were given to Group B dogs (B1 & B2). Group C dogs (C1 & C2) received the nanoliposome-encapsulated malunggay phenolic extract capsules. One hundred milligram cholesterol capsules were also given to all dogs. The capsules were produced and provided by Smart-Functional Biomaterials Laboratory of the College of Arts and Sciences, University of the Philippines Los Baños. The non-encapsulated malunggay phenolic extract was produced using the ethanol extraction process described in the study of Oluduro (2012). The nanoliposome-encapsulated malunggay phenolic extract was produced using thin-film hydration method described by Dua *et al.* (2012).

All dogs underwent initial hematologic profiling and biochemical value testing including blood urea nitrogen (BUN), creatinine, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The blood samples for hematologic profiling and serum samples for the biochemical tests were collected from either the cephalic vein or the jugular vein and were submitted to a laboratory for processing. Hematologic profiling included total white blood cell (TWBC) count, differential WBC count, packed cell volume (PCV) and platelet count.

After the initial testing at Week 0, capsules containing 100 mg cholesterol were given orally to each dog every day for one week. After one week (Week 1) of giving cholesterol, hematologic profiling and lipid profiling were repeated. With the added cholesterol oral administration, it is assumed that dyslipidemia occurred. Then, the assigned treatment capsules were orally given along with the 100 mg cholesterol capsules for four weeks. After four weeks of treatment, all tests were repeated (Week 5). Weekly hematologic profiling and lipid profiling were done for the whole duration of the study. Routine physical examination (obtaining the heart rate, respiratory rate, body temperature and body condition score) was done twice a week. The body weight was measured at the



start and at the end of the study. The hematologic values and lipid levels of the dogs were compared from week 0, week 1, and week 5. Any changes from the initial kidney and liver function values were also compared for each dog.

## RESULTS AND DISCUSSION

### Hematologic profile

Table 1 shows the hematologic profile of dogs given given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay phenolic extract

Table 1.Hematologic values of dogs given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay phenolic extract administration.

| Parameters | | Control | | NonEM.o. Txt | | NLE M.o. Txt | | Normal range |
|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | B1 | B2 | C1 | C2 | |
| Total WBC (×103 cells/µl) | *Wk 0* | 11.5 | 15 | 13.2 | 18.5 | 11.9 | 8.5 | 5.0-14.1 |
| | *Wk 1* | 14.9 | 8.4 | 10.1 | 27.6 | 17.5 | 11.6 | 5.0-14.1 |
| | *Wk 5* | 11.8 | 32 | 13.3 | 35 | 11.9 | 12.6 | 5.0-14.1 |
| Neutrophils (×$10^3$ cells/µl) | *Wk 0* | 6.79 | 9.45 | 10.9 | 12.6 | 7.97 | 6.12 | 2.9-12.0 |
| | *Wk 1* | 11.6 | 5.88 | 7.17 | 20.2 | 14.2 | 7.19 | 2.9-12.0 |
| | *Wk 5* | 6.61 | 14.1 | 9.14 | 24.5 | 7.5 | 7.43 | 2.9-12.0 |
| Lymphocytes (×$10^3$ cells/µl) | *Wk 0* | 3.8 | 4.05 | 1.98 | 3.7 | 2.86 | 2.13 | 0.4-2.9 |
| | *Wk 1* | 2.38 | 1.76 | 1.72 | 4.42 | 2.8 | 1.86 | 0.4-2.9 |
| | *Wk 5* | 3.3 | 8.64 | 2.53 | 7.7 | 3.69 | 3.65 | 0.4-2.9 |
| Monocytes (×$10^3$ cells/µl) | *Wk 0* | 0 | 0 | 0 | 2.04 | 0 | 0 | 0.1-1.4 |
| | *Wk 1* | 0.89 | 0.76 | 1.21 | 3.04 | 0.53 | 2.55 | 0.1-1.4 |
| | *Wk 5* | 1.89 | 9.28 | 1.73 | 0 | 0.6 | 1.51 | 0.1-1.4 |
| Eosinophils (×$10^3$ cells/µl) | *Wk 0* | 0.92 | 1.5 | 0.26 | 0.19 | 0.95 | 0.26 | 0-1.3 |
| | *Wk 1* | 0 | 0 | 0 | 0 | 0 | 0 | 0-1.3 |
| | *Wk 5* | 0 | 0 | 0 | 0.28 | 0.12 | 0 | 0-1.3 |
| Basophils (×$10^3$ cells/µl) | *Wk 0* | 0 | 0 | 0 | 0 | 0.12 | 0 | 0-0.1 |
| | *Wk 1* | 0 | 0 | 0 | 0 | 0 | 0 | 0-0.1 |
| | *Wk 5* | 0 | 0 | 0 | 0 | 0 | 0 | 0-0.1 |
| PCV (%) | *Wk 0* | 41 | 35 | 40 | 35 | 49 | 43 | 35-57 |
| | *Wk 1* | 38 | 35 | 38 | 36 | 50 | 41 | 35-57 |
| | *Wk 5* | 40 | 40 | 37 | 39 | 54 | 46 | 35-57 |
| Platelets (per 100x oil immersion field) | *Wk 0* | 15 | 15.5 | 9 | 13 | 4.5 | 20.5 | 8-29 |
| | *Wk 1* | 22 | 42.5 | 15 | 26.5 | 4.5 | 19.5 | 8-29 |
| | *Wk 5* | 11.5 | 17.5 | 6.5 | 16.5 | 2 | 7 | 8-29 |

administration. Leukocytosis and neutrophilia were observed in dogs A2 and B2. Lymphocytosis was observed in all dogs except dog B1. Monocytosis was observed in dogs A1, A2, B1 and C2. Monocytopenia was observed in dog B2. These results suggest that the dogs may have experienced fear or excitement during blood collection (Schultze, 2010). However, stress and infection can also cause these abnormalities (Weiss and Souza, 2010).

Thrombocytopenia was observed in dogs B1, C1, and C2 which suggests that dogs could have a neoplasia, immune-mediated thrombocytopenia, or platelet loss due to hemorrhage (Russell, 2010). However, this may also suggest that the administration of nanoliposome-encapsulated malunggay phenolic extract had an effect on the platelet count. This result is consistent with the study of Ajibade *et al.* (2012) which shows that administration of methanol extract of *M. oleifera* seeds has a platelet-lowering effect on rats.

**Kidney function tests**

Table 2 showed that the BUN level of dog C1 was elevated.This suggests that the dog could have a gastrointestinal bleeding or fever. However, pre-renal azotemia is also a possible cause of the elevation (Barsanti *et al.*, 2004). It is important to note, however, that the BUN levels of dogs C1 and C2 were higher at the start of the study. Results also showed that the serum creatinine levels of dogs B1 and B2 were elevated. This suggests that there is a decreased glomerular filtration. Myositis or muscle trauma is another possible cause but is very unlikely (Barsanti *et al.*, 2004). Although elevated levels were observed, it was noted that the values on week 5 were lower compared to that of week 0. These results suggest that administration of malunggay phenolic extract containing quercetin, whether nanoliposome-encapsulated or not, may have an effect on the kidneys of dogs. This can be supported by the study of Shoskes (1998) wherein a similar effect was seen in the serum creatinine level of rats administered with quercetin. This is because of the ability of quercetin in preventing reperfusion injury by inhibiting peroxynitrites.

**Lipid profile**

Dogs A1, A2, B1 and B2 had normal total cholesterol levels (125 to 300 mg/dl); however, dogs C1 and C2 both showed hypocholesterolemia (Table 3). Hypertriglyceridemia was also observed in all dogs except dogs C1 and C2 which both had normal triglyceride

Table 2. Blood urea nitrogen (BUN) and serum creatinine (CREA) values of dogs given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay phenolic extract administration.

| Parameters | | Control | | NonEM.o. Txt | | NLE M.o. Txt | | Normal range |
|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | B1 | B2 | C1 | C2 | |
| BUN (mg/dl) | *Wk 0* | 26.4 | 22.3 | 26 | 24.7 | 32.3 | 28.1 | 8-28 |
| | *Wk 5* | 27.1 | 22.8 | 25.2 | 24.2 | 29.6 | 25.2 | 8-28 |
| CREA (mg/dl) | *Wk 0* | 1.2 | 1.1 | 2.6 | 2.3 | 1.3 | 1.2 | 0.5-1.7 |
| | *Wk 5* | 1.3 | 1.1 | 2.4 | 2.2 | 1.2 | 1 | 0.5-1.7 |

Control- filler capsules; NonEM.o. Txt- non-encapsulated malunggay phenolic extract; NLE M.o. Txt- nanoliposome-encapsulated malunggay phenolic extract.



levels (10-150 mg/dl). Normal HDL levels (30.6 to 100.6 mg/dl) were observed in all dogs except dogs A1 and A2 which had below normal HDL levels. Normal LDL levels (43.3 to 179.78 mg/dl) were seen in dogs A1, B1 and B2. Dog A2 had higher than normal levels of LDL while dogs C1 and C2 had lower than normal LDL levels (Nelson *et al.,* 2004). Results suggest that hyperlipidemia in the control group was established due to the presence of hypertriglyceridemia and below normal HDL levels for both dogs A1 and A2 and the presence of above normal LDL levels for dog A2. Results for dogs B1 and B2 suggests that NL-Mo treatment may not be effective in controlling hypertriglyceridemia but may have an effect in controlling other lipid profile parameters. Results for dogs C1 and C2 suggest that NL-E Mo treatment may have an effect in their lipid profiles.

Table 3. Lipid profile results of dogs given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay phenolic extractadministration.

| | Control | | Non-E Mo Txt | | NL-E Mo Txt | |
|---|---|---|---|---|---|---|
| Parameters | A1 | A2 | B1 | B2 | C1 | C2 |
| Cholesterol(mg/dl) | | | | | | |
| Wk 0 | 117.9 | 251.1 | 158.3 | 147.1 | 144.1 | 101.1 |
| Wk 1 | 125.8 | 256.7 | 165.6 | 160.3 | 152.6 | 108.1 |
| Wk 5 | 129.2 | 255.9 | 168.5 | 154.2 | 105.1 | 103.2 |
| Change from Wk 1 | +3.4 | -0.8 | +2.9 | -6.1 | -47.5 | -4.9 |
| Triglyceride (mg/dl) | | | | | | |
| Wk 0 | 95.2 | 230.1 | 134.1 | 165.3 | 120.3 | 80.6 |
| Wk 1 | 193.4 | 241.2 | 172.4 | 192.4 | 184.1 | 147.9 |
| Wk 5 | 200.8 | 255.4 | 167.4 | 187.7 | 115.4 | 98.7 |
| Change from Wk 1 | +7.4 | +14.2 | -5 | -4.7 | -68.7 | -49.2 |
| HDL (mg/dl) | | | | | | |
| Wk 0 | 24 | 51 | 31.8 | 38 | 29.1 | 21.1 |
| Wk 1 | 29.1 | 55.4 | 32.6 | 41.7 | 30.7 | 23.5 |
| Wk 5 | 29.3 | 29.6 | 35.8 | 44.1 | 64.3 | 38.4 |
| Change from Wk 1 | +0.2 | -25.8 | +3.2 | +2.4 | +33.6 | +14.9 |
| LDL (mg/dl) | | | | | | |
| Wk 0 | 74.9 | 159.7 | 99.7 | 76 | 90.9 | 63.9 |
| Wk 1 | 155.2 | 172.1 | 163.2 | 157.2 | 171.2 | 126.2 |
| Wk5 | 167.3 | 195.7 | 105.7 | 97.6 | 31.1 | 37.6 |
| Change from Wk 1 | +12.1 | +23.6 | -57.5 | -59.6 | -140.1 | -88.6 |

Control- filler capsules; NonEM.o. Txt- non-encapsulated malunggay phenolic extract; NLE M.o. Txt- nanoliposome-encapsulated malunggay phenolic extract.
HDL: high density lipoprotein, LDL: low density lipoprotein.

**Liver function tests**

Normal AST (4 – 91 mg/dL) and ALT (<105 mg/dL) levels (Table 4) were observed in all six dogs. Total bilirubin levels (<1 mg/dl) were normal for dogs A1, B2 and C2 (Nelson *et al.,* 2004). Hyperbilirubinemia was observed in dogs A2, B1 and C1 which suggests improper handling of samples, delayed processing of the sample and reagent problems (Thomas, 2004). For dog A2, it may suggest an on-going liver problem due to cholesterol supplementation. Development of fatty liver occurs through excessive lipid deposition in the hepatocytes (Xenoulis, 2008). For dog B1 and C1, results cannot be correlated with the use of the assigned treatment without further research since quercetin has known hepatoprotective effects (Nakagawa *et al.,* 2000).

This study showed that thrombocytopenia was present in both dogs (C1 & C2) administered with the nanoliposome-encapsulated malunggay phenolic extract. In addition, the BUN levels of dogs C1 and C2 decreased after the administration of the same treatment. Moreover, both dogs had normal triglyceride and HDL levels and lower than normal LDL levels. Furthermore, dog C1 had lower than normal cholesterol levels and dog C2 had normal cholesterol levels. The ALT and AST values of these dogs were also within the normal range. Total bilirubin for dog C1 was above the normal range. These results suggest that administration of nanoliposome-encapsulated malunggay phenolic extract may have an effect on platelets, kidneys, and lipid profile. However, further studies are necessary to verify these results. These results can be used as a guide for future researches on the use of nanoliposome encapsulated malunggay (*M. oleifera*) phenolic extract on dogs.

Table 4. Biochemical tests results of dogs given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay phenolic extractadministration.

| | Control | | Non-E Mo Txt | | NL-E Mo Txt | |
|---|---|---|---|---|---|---|
| Parameter | A1 | A2 | B1 | B2 | C1 | **C2** |
| ALT (mg/dL) | | | | | | |
| Wk 0 | 14.1 | 14.2 | 18.9 | 19.3 | 16.2 | 15.4 |
| Wk 5 | 14.2 | 20.8 | 18.7 | 19 | 15.1 | 14.3 |
| | | | | | | |
| AST (mg/dL) | | | | | | |
| Wk 0 | 21.5 | 29.9 | 26.1 | 25.2 | 25.1 | 24.4 |
| Wk 5 | 21.4 | 30.1 | 25.2 | 24.8 | 23.4 | 21.7 |
| | | | | | | |
| Total Bilirubin (mg/dL) | | | | | | |
| Wk 0 | 0.62 | 0.72 | 0.71 | 0.62 | 0.66 | 0.97 |
| Wk 5 | 0.72 | 2.18 | 1.09 | 0.52 | 1.32 | 0.82 |

Control- filler capsules; NonEM.o. Txt- non-encapsulated malunggay phenolic extract; NLE M.o. Txt- nanoliposome-encapsulated malunggay phenolic extract.
ALT: alanine aminotransferase, AST: aspartate aminotransferase.



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The preliminary findings in this study suggest that nanoliposome-encapsulated malunggay phenolic extract may have an effect on the platelets, kidneys, and lipid profile. However, further studies should be done to verify this association. Further studies on nanoliposome-encapsulated malunggay phenolic extract are recommended because of its potential in decreasing an elevated BUN level. However, caution should be taken in giving it as it may cause thrombocytopenia. Also, monitoring of the biochemical values is recommended since results may suggest the development of a liver problem.

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