ORIGINAL ARTICLE

BODY FAT STATUS AND HEPATIC AND RENAL ULTRASONOGRAMS OF ADULT DOGS GIVEN CHOLESTEROL WITH OR WITHOUT NANOLIPOSOME ENCAPSULATED MALUNGGAY (*Moringa oleifera*) ADMINISTRATION

Nelson B. Malabanan[1], Cherissa A. Garcia[1], Marianne Leila S. Flores[1], Jezie A. Acorda[1] and Evelyn B. Rodriguez[2]

ABSTRACT

The effect of administration of nanoliposome encapsulated malunggay (*Moringa oleifera*) phenolic extract in dogs given cholesterol on body fat and hepatic and renal ultrasonograms was investigated in this study. Six adult male dogs were given cholesterol without (T1) or with non-encapsulated (T2) and nanoliposome encapsulated (T3) malunggay (*M. oleifera*) phenolic extract. Data on the back fat thickness, body condition score and hepatic and renal ultrasonograms were collected at weeks 0, 1 and 5. Dogs in T3 showed inconsistent changes in the back fat thickness and body condition score. Moreover, T3 dogs exhibited decrease in both hepatic and renal echo mean values. The initial findings suggest that monitoring of the liver and kidney in cases of dyslipidemia or hyperlipidemia should be considered since ultrasonogram results indicate probable development of liver and kidney problems. Further studies are recommended to verify these results.

Keywords: back fat, dog, kidney, liver, moringa, nanoliposome, ultrasonography

INTRODUCTION

Dyslipidemia is the change in lipid levels (German, 2006) and hyperlipidemia is the increase in the concentration of lipids in the blood. Both are commonly associated with endocrine disorders, obesity and high fat diet that may cause diseases such as pancreatitis, seizures and ocular and hepatic diseases (Xenoilis and Steiner, 2010). Behling-Kelly (2014) reported that dyslipidemia is consistently seen in dogs with chronic kidney disease and nephrotic syndrome.

To prevent dyslipidemia and hyperlipidemia, studies on nutraceuticals are being done (Choudhary and Grover, 2012; Aggarwal, 2010). According to Kalra (2003), "nutraceutical is a functional food that aids in the prevention and/or treatment of diseases and/or disorders". One of the most common ingredients of nutraceuticals is malunggay (*Moringa oleifera)*. One of its components is the flavonoid quercetin which can decrease hyperlipidemia (Mbikay, 2012). But for its benefits to take effect, large amounts of these substances must be consumed.

Nanotechnology, such as nanoliposome-encapsulation, was developed to increase the bioavailability (Fang and Bhandari, 2010) of compounds such as quercetin. However, quercetin was found to inhibit platelet aggregation (Tzeng *et al.*, 1991) and was also

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine (email: leiladvm@yahoo.com), [2]Institute of Chemistry, College of Arts and Science, University of the Philippines Los Baños, Laguna, Philippines.

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 increase. McCullagh and Ehrhart (1975) reported that dietary cholesterol can also have an effect on the kidneys of dogs.

Studies on the body fat status and hepatic and renal ultrasonogram features 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 (BCS) 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 Los Baños Veterinary Teaching Hospital Maahas Station. The dogs were randomly assigned to three treatments. Filler capsules containing rice bran and magnesium steate were given to the dogs (A1 & A2) in the control group (T1). Non-encapsulated malunggay (NEM) phenolic extract capsules (T2) were given to the next two dogs (B1 & B2). And the third pair of dogs (C1 & C2) was assigned to T3, receiving the nanoliposome-encapsulated malunggay (NLEM) 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 (*Moringa oleifera*) phenolic extract was produced using thin-film hydration method described by Ramana *et al.* (2007).

One week acclimatization was done where the dogs were fed commercially prepared dog food two times a day and water *ad libitum*. Baseline data were collected and accomplished by physical examination (heart rate, respiratory rate, body weight, temperature, body condition score) and ultrasonographic examination of the back fat at the level of the 2nd to the 4th lumbar vertebra using a 7.5 MHz linear scanner and of the liver and the left kidney using a 5.0 MHz microconvex scanner and ultrasound machine (WellD® WED-3100V, Well.D Electronics Co., Szechuan, China) was conducted.

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, all tests were repeated. With the added oral administration of cholesterol, it was assumed that dyslipidemia occurred. Then the assigned treatment capsules were given orally along with the 100 mg cholesterol capsules for four weeks. After four weeks of treatment, all tests were repeated (Week 5). Routine physical examination (obtaining the heart rate, respiratory rate, body temperature and body condition score) was done twice a week.

Histogram analyses (1 cm x 1 cm of the ultrasonogram) of three sites for the liver parenchyma, renal cortex and pelvis were done using the computer software Adobe® Photoshop CS5 (Adobe Systems Inc., San Jose, CA). The echo mean values for each parameter were computed from these analyses to quantify any changes, if any, in the liver and kidney. The hepatic and renal architecture were also described.

RESULTS AND DISCUSSION

Backfat thickness and body condition score

Since lumbar subcutaneous tissues are one of the major sites of fat deposition, any change in back fat thickness reflects the change in body fat status. Back fat thickness and BCS are used together to assess the fat status of the animal especially in swine practice. However, many studies have shown that there is poor association between back fat thickness and BCS due to the wide range of back fat thickness measurement that is present in a certain body condition score (Young and Aherne, 2005). In addition, though BCS is a criterion for evaluating an animal, it is still a subjective assessment and not all subjectivity can be eliminated (Toll *et al.,* 2010). Back fat thickness (Table 1 and Figure 1) decreased for dogs A1, A2, B2 and C1, increased for dog B1 and had no

Table 1. Backfat thickness (mm) and body condition score of dogs given cholesterol with or without nanoliposome encapsulated or non-encapsulated malunggay (*M. oleifera*) administration.

	T1		T2		T3	
Parameter	A1	A2	B1	B2	C1	C2
Back fat thickness (mm)						
Wk 0	2.2	1.4	1.7	2	2.4	1.2
Wk 1	2.2	1.9	1.5	2	2.2	1.2
Wk 5	1.9	1.5	1.7	1.5	1.7	1.2
Body condition score						
Wk 0	4/9	4/9	4/9	5/9	6/9	3/9
Wk 1	4/9	5/9	4/9	5/9	6/9	3/9
Wk 5	5/9	4/9	4/9	4/9	5/9	3/9

T1: Control, filler capsules; T2: non-encapsulated malunggay phenolic extract; T3: nanoliposome-encapsulated malunggay phenolic extract.

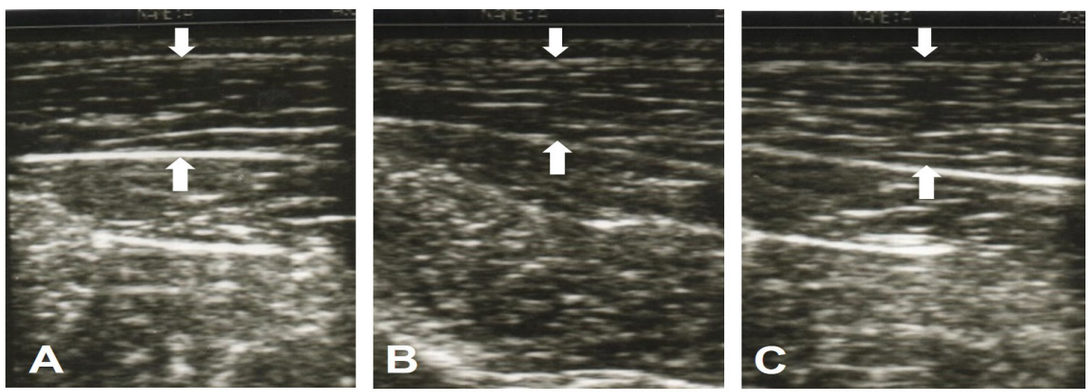

Figure 1. Back fat ultrasonograms of dogs at week 0 (A), week 1 (B) and week 5 (C). Dorso-ventral borders of the back fat are indicated by arrows.

change for dog C2. Decreased BCS results were observed in dogs A2, B2 and C1 while increased BCS result was seen in dog A1. On the other hand, there were no changes in the BCS results of dogs B1 and C2. Since there were differences in the results, back fat thickness was analyzed to have an objective assessment. Results for dogs A1 and A2 suggest fatty liver development. According to Staufenbiel *et al.,* (1993), fatty liver is correlated with a decrease in back fat thickness due to the presence of lipolysis. The increase in back fat thickness in dog B1 can be due to the supplementation of cholesterol. This result contradicts the findings of Dong *et al.,* (2014), wherein quercetin treated mice had decreased fat deposition. Results for dogs B2, C1 and C2 may be due to assigned treatments since quercetin has been known to decrease fat deposition (Dong *et al.,* 2004). Further studies have shown that quercetin can decrease high-fat diet induced body fat gain (Henagan *et al.,* 2014).

Hepatic ultrasonographic findings

Hepatic ultrasonograms for all dogs revealed homogenous hypoechoic parenchyma with evenly distributed coarse granules and visible circular gall bladder and hepatic and portal veins (Figure 2). Decreased echo mean values (Table 2) were observed in dogs A1, A2, C1 and C2 which can be due to lymphosarcoma, amyloidosis or acute hepatitis (Morris, 2008). According to Yasutake *et al*. (2012), hepatitis may develop in individuals with high levels of dietary cholesterol due to the development of hepatocyte triglyceride accumulation. This may also be true for dogs A1 and A2 due to cholesterol supplementation. Results for dogs C1 and C2 cannot be concluded as an effect of the NLEM treatment. The mechanism on how NLEM affects or acts on the liver has not yet been explored. However, the hepatoprotective effects of quercetin have already been established and must be considered (Nakagawa *et al.,* 2000). An increase in the echo mean value of dog B1 was observed, which may suggest the presence of fatty infiltration, chronic hepatitis and cirrhosis (Morris, 2008). The increase in liver mean echogenicity could be due to fatty infiltration or an effect of NLEM treatment. Echo mean value for dog B2 did not change drastically which may suggest that the liver is normal throughout the experiment. These findings also support the known hepatoprotective effects of quercetin (Nakagawa *et al.,* 2000).

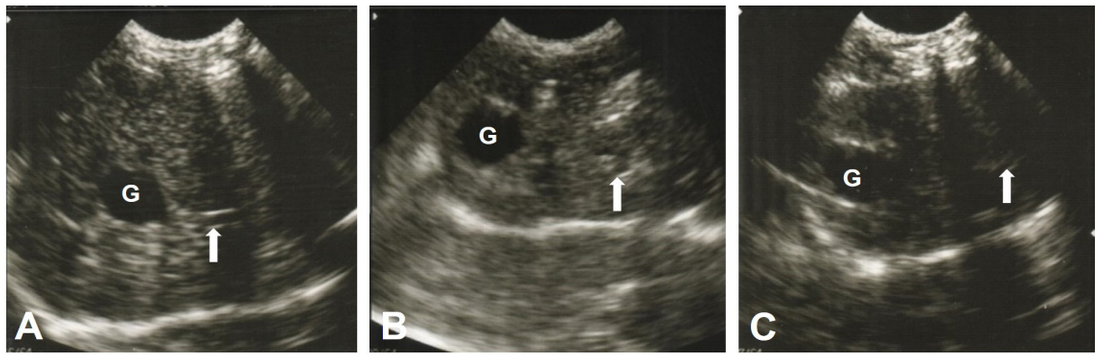

Figure 2. Hepatic ultrasonograms of the dogs showing the gall bladder (G) and portal vein (arrow) at week 0 (A), week 1 (B) and week 5 (C).

Table 2. Hepatic echo mean values in dogs given cholesterol with or without nanoliposome encapsulated or non-encapsulated malunggay (*M. oleifera*).

	T1		T2		T3	
Parameter	A1	A2	B1	B2	C1	C2
Wk 0						
High	144.98	123.36	107.02	114.82	144.6	123.72
Low	44.55	47.83	42.89	41.73	57.45	43.36
Intermediate	79.76	87.42	87.53	81.63	73.08	85.09
Echo mean value	89.76	86.20	79.15	79.39	91.71	84.06
Wk 1						
High	106.33	104.54	129.09	120.94	116.68	110.71
Low	39.43	31.75	35.75	42.64	35.49	44.69
Intermediate	64.72	72.7	73.75	67.43	66.95	89.6
Echo mean value	70.16	69.66	79.53	77.00	73.04	81.67
Wk 5						
High	111.93	87.02	131.31	111.83	103.67	110.71
Low	38.95	38.96	46.67	52.82	30.69	50.78
Intermediate	72.11	63.35	75.02	69.94	71.12	68.63
Echo mean value	74.33	63.11	84.33	78.20	68.49	76.71

T1: Control, filler capsules; T2: non-encapsulated malunggay phenolic extract; T3: nanoliposome-encapsulated malunggay phenolic extract.

Renal ultrasonographic findings

The echo mean values (Table 3) of the cortex and pelvis of dogs A1, B1, B2 increased at week 5 suggesting glomerulosclerosis or tubular lipidosis because of the administration of cholesterol (McCullagh and Ehrhart, 1975). In addition, it may also indicate a chronic inflammatory disease or renal dysplasia (Anderson, 2011). Moreover, acute glomerulonephritis, amyloidosis, nephrosis, squamous cell carcinoma or mast cell tumors can cause an increase in the cortical echogenicity (Lang, 2006). For dogs A2 and C1, all echo mean values decreased at week 5. This is suggestive of necrosis (Lang, 2006) or lymphoma (Anderson, 2011). A decrease in the echo mean value of the cortex and an increase in the echo mean value of the pelvis were observed on dog C2 at week 5 which may indicate necrosis or lymphoma and acute pyelonephritis, respectively (Lang, 2006). This may suggest different stages of a disease. The renal cortex, medulla, pelvis and capsule were visible and identifiable (Figure 3).

Table 3. Renal echo mean values in dogs given cholesterol with or without nanoliposome-encapsulated or non-encapsulated malunggay (*M. oleifera*) administration.

Parameter		T1		T2		T3	
		A1	A2	B1	B2	C1	C2
Cortex	Wk 0	97.87	127.86	79.04	94.98	96.33	98.16
	Wk 1	56.52	73.82	74.02	61.04	84.86	75.79
	Wk 5	70.01	64.95	85.57	72.3	59.32	67.43
Pelvis	Wk 0	168.01	214.82	178.34	165.41	197.09	214.92
	Wk 1	152.35	173.13	194.4	166.54	185.91	205.4
	Wk 5	173.32	84.83	233.87	182.34	142.6	234.34

T1: Control, filler capsules; T2: non-encapsulated malunggay phenolic extract; T3: nanoliposome-encapsulated malunggay phenolic extract.

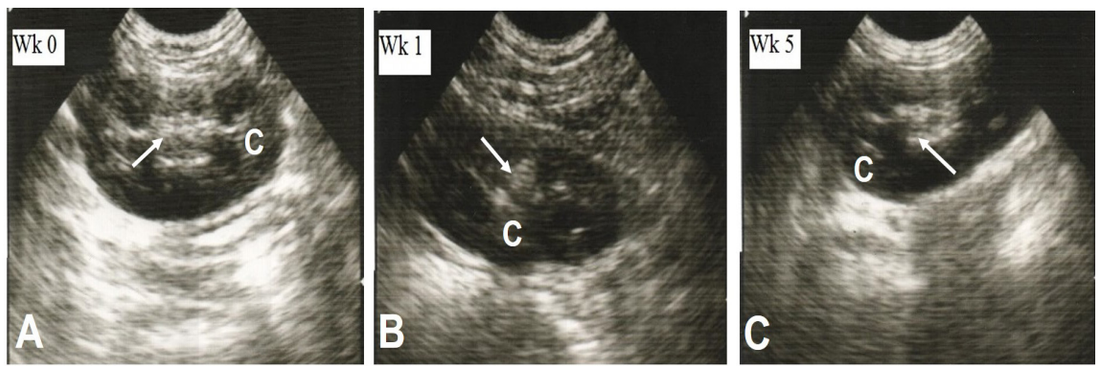

Figure 3. Renal ultrasonograms of dogs showing the renal cortex (C) and renal pelvis (arrow) at week 0 (A), week 1 (B) and week 5 (C).

CONCLUSIONS

In this study, dogs treated with nanoliposome-encapsulated malunggay phenolic extract showed different results in the back fat thickness and body condition score, athough similar hepatic and renal ultrasonogram findings were observed. Decrease in the hepatic echo mean values was seen but the study cannot correlate this to the use of the nanoliposome-encapsulated malunggay treatment. Decrease in renal echo mean values may suggest an effect of nanoliposome-encapsulated malunggay on the kidneys. However, further studies should be done to verify these associations. These results can be used as a basis for future studies on the use of nanoliposome-encapsulated malunggay phenolic extract in dogs for treatment of dyslipidemia or hyperlipidemia.

REFERENCES

Aggarwal BB. 2010. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. *Ann Rev Nutr* 30: 173-199.

Anderson KL. 2011. *Ultrasonography of Upper Urinary Tract*. Accessed 27 April 2015. http://goo.gl/PflS0l

Behling-Kelly E. 2014. Serum lipoprotein changes in dogs with renal disease. *J Vet Intern Med* 28: 1692-1698.

Choudhary M and Grover K. 2012. Development of functional food products in relation to obesity. *Func Foods Hlth Dis* 2(6): 188-197.

Dong J, Zhang X, Zhang L, Bian H, Xu N, Bao B and Liu J. 2014. Quercetin reduces obesity-associated adipose tissue macrophages infiltration and inflammation in mice: a mechanism including AMPKα1/SIRT1. *J Lipid Res* 55: 363-374.

Dua JS, Rana AC and Bhandari AK. 2012. Liposome: methods of preparation and applications. *Int J Pharmaceut Stud Res* 3(2): 14-20.

Ehrlich SD. 2011. *Quercetin*. http://goo.gl/vo3Kfy. Accessed 11 October 2013.

Fang Z and Bhandari B. 2010. Encapsulation of polyphenols- a review. *Trends Food Sci Tech* 21: 510-523.

German AJ. 2006. The growing problem of obesity in dogs and cats. *J Nutr* 136 (Suppl): 1940S-946S.

Henagan TM, Lenard NR, Gettys TW and Stewart LK. 2014. Dietary quercetin supplementation in mice increases skeletal muscle PGC1α expression, improves mitochondrial function and attenuates insulin resistance in a time-specific manner. *Public Libr Sci ONE* 9(2): e89365.

Kalra EK. 2003. Nutraceutical- definition and introduction. *Am Assoc Pharm Scientists* 5 (3): 27-28.

Laflamme DP. 1997. Development and validation of a body condition score system for dogs. *Canine Pract* 22: 10-15.

Lang J. 2006. Urinary tract In: Paddy Mannion (ed.). *Diagnostic Ultrasound in Small Animal practice.* Ames, Iowa: Blackwell Science Ltd.

Mbikay M. 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Front Pharm* 3 (24): 1-12.

McCullagh KG and Ehrhart LA. 1975. Non-arteriosclerotic lesions in the kidneys of dogs fed an atherogenic diet. *Exp Mol Pathol* 22: 400-416.

Morris D. 2008. Diagnostic testing for liver disease. *Bandfield* 4(3): 34-44.

Nakagawa K, Kawagoe M, Yoshimura M, Arata H, Minamikawa T, Nakamura M and Matsumoto A. 2000.Differential effects of flavonoid quercetin on oxidative damages induced by hydrophilic and lipophilic radical generators in hepatic lysosomal fractions of mice. *J Health Sci* 46(6): 509-512.

Oluduro AO. 2012. Evaluation of antimicrobial properties and nutritional potentials of *Moringaoleifera* Lam. leaf in South-Western Nigeria. *Malaysian J Microbiol* 8 (2): 59-67.

Ramana LN, Sethuraman S, Ranga U and Krishnan U. 2010.Development of liposomal nanodelivery system for nevirapine. *J Biomedical Sci* 17:57.

Staufenbiel R, Lachmann I, Bauer J and Staufenbiel B. 1993. Energie- und Fettstoffwechsel des Rindes - Beziehungen der RückenfettdickezurEnergieaufnahme und zurEnergiebilanz. *MonatsheftefuerVeterinaermedizin* 48:58-66.

Toll PW, Yamka RM, Schoenher WD and Hand MS. 2010. Obesity. In: Hand MS, Thatcher

CD, Remillard RL, Roudebush P and Novotny BJ. (eds.) *Small Animal Clinical Nutrition*, 5th ed. Topeka, KS: Mark Morris Institute.

Tzeng SH, Ko WC, Ko FN, and Teng CM. 1991. Inhibition of platelet aggregation by some flavonoids. *Thromb Res* 64 (1): 91-100.

Xenoulis PG and Steiner JM. 2010. Lipid metabolism and hyperlipidemia in dogs. *Vet J* 183: 12-21.

Yasutake K, Kohjima M, Nakashima M, Kotoh K, Nakamuta M and Enjoji M. 2012. Nutrition therapy for liver diseases based on the status of nutritional intake. *Gastroenterology Research and Practice.* Article ID 859697: 1-8.

Young M and Aherne F. 2005.Monitoring and Maintaining Swine Condition. *Adv Pork Prod* 16: 299-313.