International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 11 (6): 542-551, 2015
ISSN 1811-7775

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.542.551

Antihyperglycemic Effects of *Nephelium lappaceum* Rind Extract in High Fat-Induced Diabetic Rats

[1]Shonia Subramaniam, [1]Ammu Radhakrishnan, [2]Srikumar Chakravarthi, [3]Uma Devi Palanisamy and [4]Nagaraja Haleagrahara

[1]School of Medicine and Health Sciences, International Medical University, Bukit Jalil, 57000, Kuala Lumpur, Malaysia

[2]Department of Pathology, Faculty of Medicine, Perdana University Graduate School of Medicine, Selangor, Malaysia

[3]School of Medicine and Health Sciences, Monash University, Sunway Campus, 46150, Bandar Sunway, Malaysia

[4]Discipline of Biomedicine, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia

ARTICLE INFO

Article History:
Received: March 04, 2015
Accepted: July 01, 2015

Corresponding Author:
Nagaraja Haleagrahara
Discipline of Biomedicine,
College of Public Health,
Medical and Veterinary Sciences,
James Cook University,
Townsville, Australia

ABSTRACT

This study investigated the antidiabetic effects of the rind of *Nephelium lappaceum* extract in a high fat-induced diabetic rat model. Ethanolic *N. lappaceum* rind extract was prepared and standardised with geraniin using high performance liquid chromatography. Male Sprague Dawley rats were fed on a high fat diet followed by 210 mg kg^{-1} nicotinamide and 55 mg kg^{-1} streptozotocin injection to induce type 2 diabetes. The diabetic rats were treated with *N. lappaceum* rind at concentrations of 500 and 2000 mg for 28 days. Positive control rats were treated with 200 mg metformin. A 41.1% yield of ethanolic extract was obtained from powdered *N. lappaceum* rind while geraniin present in the extract was quantified to be 33.0±0.2 mg geraniin/g extract. Our study also showed that the diabetic rats treated with 2000 mg *N. lappaceum* had reduction in blood glucose level and improved insulin levels which were similar to the metformin-treated group. Pancreas histology showed that the group treated with 2000 mg of *N. lappaceum* had healthy pancreas morphology and the treatment was comparable to the effects observed in the metformin-treated group. In conclusion, *N. lappaceum* rind extract showed anti-hyperglycaemic activity at a dose of 2000 mg kg^{-1} without any major toxic effects in high-fat diet induced diabetic rats.

Key words: *Nephelium lappaceum*, diabetes mellitus, insulin, pancreas, alpha amylase

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder and characterized by hyperglycemia with impairment in fat, carbohydrates and protein metabolism resulting from defects in insulin production, action or both (Zheng *et al*., 2012). Currently, diabetes affects 366 million people worldwide and this is likely to increase to 522 million by the year 2030 (Whiting *et al*., 2011). Type 2 diabetes mellitus (T2DM) accounts for 90-95% of diabetic cases and is strongly associated with insulin resistance (Perez and Medina-Gomez, 2011). Obesity is found to be the major risk factor for insulin resistance due to high fat diet and sedentary lifestyle.

Although, oral hypoglycaemic agents such as sulphonylureas, biguanides and metformin (Pepato *et al*., 2005) were widely used to control hyperglycemia in diabetic

patients, they do produce side effects such as obesity, osteoporosis and sodium retention (De Souza *et al*., 2001). Hence, efficacy of these anti-diabetic drugs is questionable and hence there is need to look for newer compounds to treat diabetes. There is an increase in demand in developing drugs or alternative therapy with the least side effects using natural products as potential anti-diabetic drug in managing hyperglycemia. Rambutan (*Nephelium lappaceum* L) is a subtropical fruit from the Sapindaceae family and is found abundantly in Malaysia. It is a commercial fruit appreciated for its refreshing taste and is consumed fresh, processed as well as in canned form (Palanisamy *et al*., 2008). The peel of *N. lappaceum* possesses antioxidant and antiglycemic properties. Methanolic and aqueous extracts of lyophilized rambutan peels and seeds were evaluated for phenolic contents, antioxidant and antibacterial activities (Palanisamy *et al*., 2011a; Thitilertdecha and Rakariyatham, 2011). Geraniin, which is an ellagitannin is reported to be the main active compound in the *N. lappaceum* rind extracts. Geraniin is also found in other species, including *Phyllantus urinaria* L. (Yang *et al*., 2007), *Phyllantus amarus* (Notka *et al*., 2003) and *Phyllatus muellerianus* (Agyare *et al*., 2011). Studies have shown that geraniin exhibits high antioxidant activity (Palanisamy *et al*., 2008; Thitilertdecha *et al*., 2010), hepatoprotective action (Ambrose *et al*., 2012), antihypertensive activity (Lin *et al*., 2008), anti-inflammatory activity (Agyare *et al*., 2011) and antiviral activity (Notka *et al*., 2003; Li *et al*., 2008; Yang *et al*., 2012). Previously, *in vitro* assays revealed the ability of *N. lappaceum* rind extract as well as geraniin to play important role in the management of hyperglycemia (Palanisamy *et al*., 2011a, b). In this study, the rambutan rind was evaluated for its potential as an anti-glycaemic agent using a rat model of T2D induced by high fat diet.

MATERIALS AND METHODS

Materials: The biochemical kits used in this experiment were obtained as follows: Streptozotocin (STZ) and nicotinamide (Sigma Aldrich, USA); Metformin (500 mg) tablets (Ranbaxy laboratories Limited, USA); Rat/Mouse Insulin ELISA kit from Merck Millipore (Darmstadt, Germany); Rabbit GLUT-4 polyclonal antibody and rabbit PPAR-gamma polyclonal primary antibody from Bita Lifesciences (Abcam, UK). Normal rat chow (ND, 5% minimum crude fat content) from Specialty Feed (Kuala Lumpur, Peninsular Malaysia). One-Touch Ultra Glucometer from Roche (LifeScan, USA). All other chemicals and reagents from Becton, Dickinson and Company (New Jersey, USA), Millipore (Massachusetts, USA), Sigma-Aldrich (Missouri, USA) and Terumo (Tokyo, Japan).

Preparation of ethanolic extract from the rind of the *Nephelium lappaceum* fruit: The peel of rambutan (*Nephelium lappaceum* L) fruit was obtained from Kuala Lumpur, Malaysia and authenticated by the Herbarium of the Forest Research Institute of Malaysia (FRIM). The crude ethanolic extract from the rind of the *N. lappaceum* fruit was prepared as previously described (Palanisamy *et al*., 2008). Briefly, the rind was washed thoroughly with clean water and dried at room temperature for 2-3 h before being placed in a circulating oven set at 30°C until completely dry. The dried rind was milled using a Fritsch dry miller. Ethanol extraction was carried out by adding ethanol to the powdered rind at 1:10 (w:v) in the orbital shaker for 24 h at room temperature. The suspension was filtered using Whattman filter paper and concentrated using a rotary evaporator.

Standardization of ethanolic extract from the rind of rambutan fruits: Geraniin, the main bioactive compound in the ethanolic extract of *N. lappaceum* rind (Palanisamy *et al*., 2011b), was used to prepare the standardised extract based on its composition following HPLC method. The extract (1 mg) was dissolved in distilled water with a minimum amount of acetonitrile and the presence of geraniin was determined by analytical HPLC using a Merck Chromolith Performance RP-18 column (100×4.6 mm). This was performed on a Shidmazu Prominence Liquid Chromatography system equipped with LC-20AD/T liquid pump, SPD-m20A diode array detector, SIL-20A auto-injector, DGU-20A system controller and CTO-20AC column oven. The mobile phase used was mobile A: 0.1% formic acid in acetonitrile and mobile B: 0.1% formic acid in water at a flow rate of 0.5 mL min^{-1}. The elution was performed on a solvent gradient from 0-100% acetonitrile over 30 min and the peaks were monitored at 254 nm (Perera *et al*., 2012).

Alpha-glucosidase inhibition assay: The alpha-glucosidase (α-glucosidase) inhibition assay was carried out according to method described by Palanisamy *et al*. (2011a). The assay was carried out in 96 well plates. The α-glucosidase (0.4 U mL^{-1}) from *Saccharomyces cerevisiae* was dissolved in 0.1 M sodium phosphate buffer (pH 6.8) and supplemented with 0.2% BSA. Then, 20 µL of DTT (1 mM), 20 µL of substrate PNPG (para-nitrophenyl glucopyranoside) were added and the mixture was incubated for 15 min at 37°C. Then, 80 µL of 0.2 M sodium carbonate solution was added to this mixture to stop the reaction and the intensity of the yellow colour which corresponds to the amount of para-nitrophenol (PNP) present was determined using a UV-spectrophotometer (Cary 50 Bio UV-spectrophotometer, Varian, Inc., Palo Alto, CA) with absorbance set at 400 nm. All measurements were performed in triplicates. Acarbose was used as positive control while mixtures without the *N. lappaceum* rind extract served as the negative control. Samples without the enzyme served sample blank ($A_{sample\ blank}$) and samples with enzyme but without sample acted as blank (A_{blank}). The A_{blank} absorbance was used to exclude back ground absorbance. The percentage inhibition was determined using the formulae shown below:

$$\text{Inhibition (\%)} = \frac{(A_{\text{neg control}} - A_{\text{blank}}) - A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{neg control}} - A_{\text{blank}}} \times 100$$

The activity of the ethanolic extract of *N. lappaceum* was assessed by plotting percentage inhibition against the various concentrations of the rind extract. Effective concentration of 50% inhibition (EC_{50}) was determined from this plot and data was expressed as Mean±SD of triplicate measurements.

Alpha-amylase inhibition assay: The alpha-amylase (α-amylase) inhibition assay was carried out as described by Palanisamy *et al*. (2011b). The assay was conducted in 96 well plates. Porcine pancreatic α-amylase (2 U mL^{-1}) (Sigma Type IV-B) was dissolved in ice-cold distilled water. A solution of potato soluble starch (1%) was prepared in 20 mM phosphate buffer (pH 6.9). Then the 3,5-Dinitrosalicyclic acid (DNS) solution was prepared by dissolving DNS (1 g) with 30 g sodium potassium tartrate dehydrate in 2 M NaOH to prepare 100 mL DNA solution. In the assay, 40 µL of the α-amylase enzyme was incubated with 80 µL of various dilutions of rind extract for 10 min at room temperature. Following this, 40 µL of starch was added to the mixture and incubated for another 10 min at room temperature. Then, 80 µL of the DNS solution was added to the tubes and incubated for another 10 min at 95°C. The reducing sugar will change the DNS from orange to brick red colour. Absorbance reading was noted using a UV-spectrophotometer (Cary 50 Bio UV-spectrophotometer, Varian, Inc., Palo Alto, CA) with absorbance set at 540 nm. Acarbose was used as positive control while absence of *N. lappaceum* rind extract was the negative control. Sample without enzyme as sample blank and without sample and enzyme as blank were used to exclude background absorbance. The percentage inhibition and EC_{50} value were determined as described above for the α-glucosidase assay.

Animals and experimental design: Male Sprague-Dawley rats (4 weeks old) were obtained from Cheneur Animal Supplier, Selangor, Malaysia. The animals were housed in polypropylene cages at 25±2°C, with 12:12 h dark/light cycle. All rats were acclimatised for a week before the start of the experiment and had free access to standard rat pellet and water. The experimental procedures carried out were in accordance to the guidelines approved by the IMU Joint Research and Ethics committee.

Induction of Type 2 Diabetes Mellitus (T2DM): The T2DM was induced by high fat diet in combination with streptozotocin and nicotinamide injection (Arya *et al*., 2012; Tahara *et al*., 2011). A total of 45 rats were fed with high fat diet (containing 58% fat, 23% carbohydrate, 9% protein and 10% sugar as a percentage of total kilocalorie) for 12 weeks. The composition (Table 1) and preparation of HFD was formulated based on methods modified for the literature review (Tahara *et al*., 2011; Zheng *et al*., 2012; Srinivasan *et al*., 2005). After 12 weeks, the overnight fasted rats were injected intraperitoneally with 210 mg kg^{-1} NTM followed by 55 mg kg^{-1} of STZ. Blood glucose levels were determined after 3 and 7 days of the NTM-STZ injections. Rats with blood glucose levels ≥11.0 mmol L^{-1} were randomly placed into four experimental groups consisting of five rats per group. These rats were fed with high fat diet throughout the whole study.

Table 1: Composition of high fat diet

Ingredients	Composition per 100 g of feed (g)
Normal pellet	50
Ghee	30
Casein	15
Sugar	5

Rats in group A were non-diabetic rats which served as normal control. The rats in group B were diabetic rats fed with saline and these were the untreated group. The rats in groups C received 200 mg kg^{-1} metformin daily and served as positive control. This dose of metformin was chosen based on the previous studies (Zheng *et al*., 2012). The rats in group D and E were given doses of 500 or 2000 mg kg^{-1}, respectively, of the standardised ethanolic extract of *N. lappaceum* rind via oral gavage for of 28 days.

Blood glucose measurement: Fasting blood glucose was determined in overnight fasted rats using a blood glucose meter (ONETOUCH, Ultra, Lifescan, USA) (Veerapur *et al*., 2012). Briefly, blood was obtained from the rat's tail vein using a lancet. Blood glucose level was then determined using the commercial glucose strips (ONETOUCH, Ultra Test Strips, Lifescan, USA) recommended for the glucometer. Rats exhibiting fasting blood glucose levels more than >11.0 mmol L^{-1} were considered diabetic (Islam and Choi, 2007).

Oral glucose tolerance test: The Oral Glucose Tolerance Test (OGTT) was performed at the beginning and the end of treatment as described in the literature (Pushparaj *et al*., 2000). The overnight-fasted rats were given 2 g kg^{-1} glucose via oral gavage (Sharma *et al*., 2009). Tail vein blood was obtained at 30 min intervals (0, 30, 60, 120 min) and blood glucose levels were estimated as using a blood glucose meter (ONETOUCH, Ultra, Lifescan, USA).

Biochemical analysis: At the end of the treatment period, the animals were sacrificed. The blood samples were collected by cardiac puncture. The plasma was stored at -20°C until assayed. The plasma insulin level was measured using a commercial ELISA kit using the protocol recommended by the manufacturer (Rat/Mouse Insulin Elisa kit, Merck Millipore, Darmstadt, Germany) (Bera *et al*., 2012). The estimation of alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, total protein, triglycerides (TG), Total Cholesterol

(TC), Low Density Lipoprotein cholesterol (LDL) and High Density Lipoprotein cholesterol (HDL) in the plasma were analyzed using commercial kits in a semi-automated biochemical analyser (Hitachi 902 Boehringer Mannheim).

Histopathology: At autopsy, various organs (liver, kidney, heart and pancreas) from the experimental animals were harvested and weighed to determine relative organ weight. All the organs were fixed in 10% buffered-formalin and processed for paraffin embedment. A 4 µm thick sections were cut and stained with haematoxylin and eosin for histopathological examination.

Statistical analysis: Analysis was carried out using One-way ANOVA followed by post-hoc Dunnett's test (SPSS version 16), where the data was compared with control. All data points were expressed as Mean±Standard Deviation (SD). The p-value ≤ 0.05 was considered to be statistically significant (Rasekh *et al*., 2008).

RESULTS

Isolation and standardization of *N. lappaceum* rind extract: A standardised preparation of *N. lappaceum* extract was identified, having a yield of 41.6±0.9% (Table 2). This yield was seen to be higher than that obtained in our previous studies 33.2% (Palanisamy *et al*., 2011a) and 24.06% from large scale purification study (Perera *et al*., 2012). Thitilertdecha *et al*. (2010), as well showed 25.1% yield using methanol as a extraction solvent to extract 120 g of *N. lappaceum* rind. The possible reason for the higher yield obtained in this study will be due to the smaller extraction scale and selection of ethanol as extraction solvent.

In order to ensure consistency, the ethanolic extract obtained from the rind of *N. lappaceum* fruit was standardised against geraniin using HPLC (Fig. 1). Geraniin was identified as the major bioactive compound in *N. lappaceum* rind. Palanisamy and co-workers (Palanisamy *et al*., 2011b) reported a geraniin peak at 13 min of retention time and an approximately 37.9 mg g^{-1} geraniin in the crude extract of *N. lappaceum* rind. In our studies, geraniin was eluted at 10.74 min in the acetonitrile:water solvent system. The amount of geraniin in the standardised extract was found to be 33.0±2.1 mg g^{-1}, which was comparable with the previous study.

Alpha glucosidase and alpha amylase inhibition assay: The *N. lappaceum* rind extract inhibited α-glucosidase (EC_{50} = 6.44±5.4 µg mL^{-1}) at significant levels compared to the control drug acarbose. However, inhibition of α-amylase (EC_{50} = 93.35±18.2 µg mL^{-1}) was lower compared to α-glucosidase and acarbose (Table 3).

Body weight: As shown in Fig. 2, the control group showed constant increase in body weight throughout the study. All the treatment groups were compared with diabetic control group.

Table 2: Yield from ethanolic extract of *Nephelium lappaceum* rind

Extraction cycle	Sample	Yield (%)*
1	*Nephelium lappaceum* rind	41.6±0.9
2	*Nephelium lappaceum* rind*	33.2±0.1

*Yield (%) is a percentage of the weight of the extract in relation to the weight of the raw material used p values are Mean±SD (n = 3)

Table 3: Inhibitory effects of *Nephelium lappaceum* extract on the activities of α-glucosidase and α-amylase

	*EC_{50} (µg mL^{-1})	
Test compounds	α-glucosidase	α-amylase
Ethanolic extract of *N. lappaceum* rind	6.44±5.44	93.35±18.19
Acarbose	6578.42±364.42	15.52±11.13

*Data is expressed as Mean±SD (n = 3)

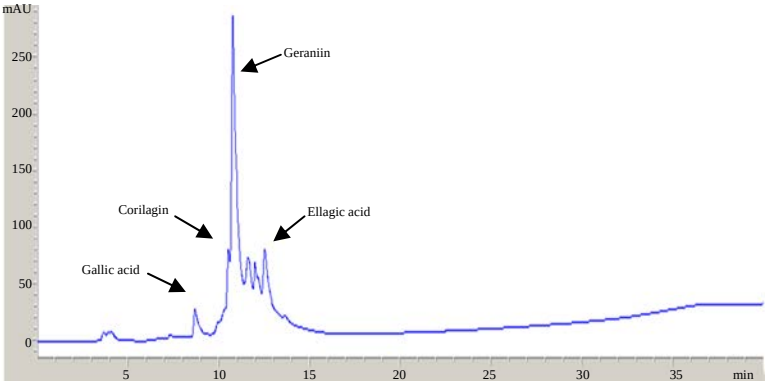

Fig. 1: HPLC profile of *Nephelium lappaceum* rind extract showing the separation of various compounds in the developing systems and conditions used separation on a Chromolith Performance RP-18 column using analytical HPLC with a solvent gradient of acetonitrile and water, flow rate was 0.5 mL min^{-1}. Geraniin was detected at 254 nm with retention time of 10.74 min

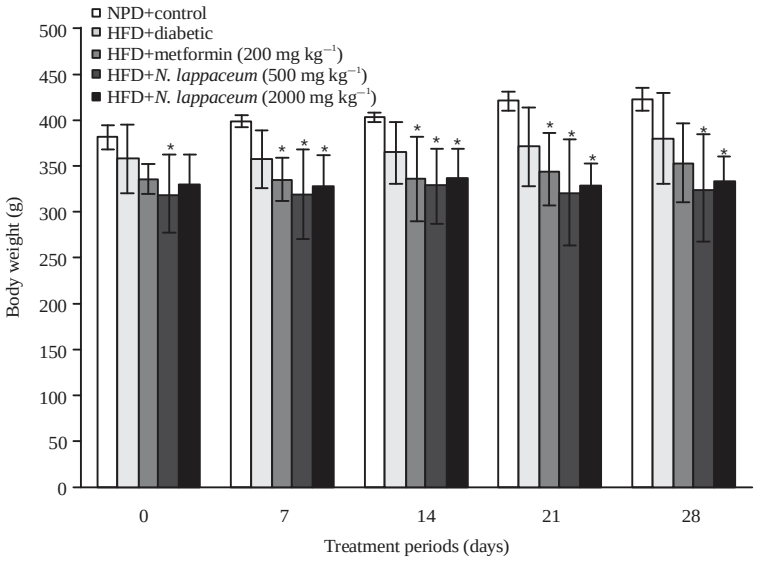

Fig. 2: Body weight changes of rats during 28 days treatment. Control group fed with normal pellet diet and the remaining groups fed with high-fat diet. Data expressed in Mean±SD, for five rats in each group. *Significant difference observed compared with diabetic control group ($p<0.05$)

Table 4: Fasting blood glucose level of type 2 diabetic rats treated with *Nephelium lappaceum* rind for 28 days

	Blood glucose mmol L^{-1})				
Treatments	0 days	7 days	14 days	21 days	28 days
Control	4.3±0.2*	4.5±0.2	4.3±0.2	4.3±0.2	4.0±0.9
Diabetic	12.2±8.2	15.3±10.1	10.6±8.7	11.3±9.2	14.9±8.9
Metformin (200 mg kg^{-1})	11.3±6.5	8.1±2.9	9.8±8.1	9.3±7.7	9.5±8.7
Nephelium lappaceum (500 mg kg^{-1})	11.0±4.1	12.3±7.9	14.0±6.0	10.6±7.1	10.6±6.4
Nephelium lappaceum (2000 mg kg^{-1})	11.0±5.1	8.5±4.1	10.9±5.2	10.9±5.6	9.6±6.3

Data is in Mean±SD for five rats in each group. *Significant difference observed compared with diabetic control group $p<0.05$

Rats received 500 mg kg^{-1} *N. lappaceum* and *N. lappaceum* (2000 mg kg^{-1}) showed significant ($p<0.05$) decrease in body weight throughout the treatment period. Besides that, at day 7, 14 and 21, rats in the treatment groups that received metformin (200 mg kg^{-1}) showed significant ($p<0.05$) decrease in body weight compared to the diabetic control group.

Blood glucose: The reduction in fasting blood glucose level was observed in the treatment groups at the end of 28 days treatment (Table 4). Groups treated with metformin and *N. lappaceum* extracts showed insignificant reduction in the blood glucose level compared to diabetic control group. In addition, rats treated with higher dose of *N. lappaceum* extract (2000 mg kg^{-1}) exhibited similar effects observed with the metformin-treated rats in terms of decreasing blood glucose levels.

Oral Glucose Tolerance Test (OGTT): Diabetic control group showed lower glucose intolerance compared to other treatment groups. Groups treated with metformin and *N. lappaceum* rind extract (2000 mg kg^{-1}) showed better improvement in glucose tolerance at 120 min compared to diabetic control group. There was no significance difference observed in the treatment groups compared to diabetic control group (Fig. 3).

Organ weights: Table 5 depicts the effects of *N. lappaceum* extract on relative organ weight of rats. There was significance difference observed in the relative weight of liver in the treatment groups of metformin (200 mg kg^{-1}), *N. lappaceum* rind extracts (500 or 2000 mg kg^{-1}) against the diabetic control group. Meanwhile, relative weight of kidney, heart, pancreas and adipose tissue of all treated groups did not show any difference compared to the diabetic control group (Table 5).

Biochemical analysis: As shown in Table 6, the TG level of rats fed with HFD was higher than control group fed with the NPD. Groups treated with *N. lappaceum* (2000 or 500 mg kg^{-1}) had the highest levels of triglyceride (TG) compared to the control diabetic group. On the other hand, there were no significant differences in the total cholesterol, HDL-C and LDL-C between the treatment groups compared to the diabetic control group. There were no significant

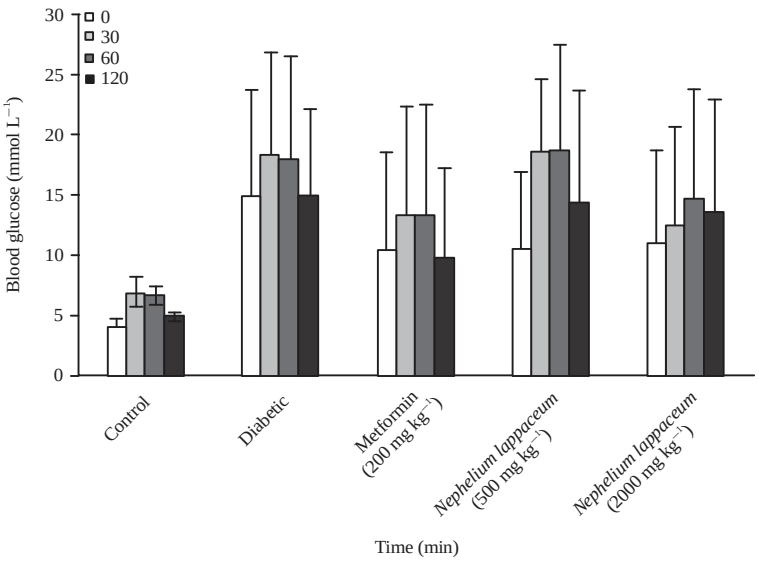

Fig. 3: Fasting blood glucose level at 0, 30, 60 and 120 min in overnight fasted rats, which was orally loaded with glucose solution. Data values are in Mean±SD, for five rats in each group

Table 5: Relative organ weight of type 2 diabetic rats treated with *Nephelium lappaceum* rind extract

Groups (%)	Liver	Adipose	Kidney	Heart	Pancreas
Control	3.0±0.8	1.4±0.2	0.3±0.0	0.3±0.1	0.1±0.0
Diabetic	3.2±0.9	2.2±3.9	0.3±0.0	0.4±0.1	0.1±0.0
Metformin (200 mg kg^{-1})	2.9±1.4*	1.3±3.7	0.3±0.0	0.3±0.1	0.1±0.0
Nephelium lappaceum (500 mg kg^{-1})	2.9±1.1*	1.6±3.8	0.3±0.1	0.4±0.1	0.1±0.0
Nephelium lappaceum (2000 mg kg^{-1})	2.9±1.1*	1.3±3.7	0.3±0.2	0.3±0.1	0.1±0.0

Data represented in Mean±SD for five rats in each group. *Significant difference observed compared with diabetic control group $p<0.05$

Table 6: Plasma lipid profile, kidney and liver function profile of rats treated with *Nephelium lappaceum* rind extract

Groups	Control	Diabetic	Metformin (200 mg kg^{-1})	*Nephelium lappaceum* (500 mg kg^{-1})	*Nephelium lappaceum* (2000 mg kg^{-1})
TG (mg dL^{-1})	62.80±7.7	178.00±73.2	109.10±83.8	194.70±101.1	243.10±222.5
TC (mg dL^{-1})	52.50±9.7	72.40±22.8	78.10±28.5	74.30±20.3	77.20±33.8
HDL-C (mg dL^{-1})	8.80±2.3	14.00±7.3	17.60±9.0	14.60±5.7	20.00±17.5
LDL-C (mg dL^{-1})	41.00±6.4	45.20±5.0	46.50±17.4	48.40±9.9	47.30±5.8
ALT (IU L^{-1})	78.60±19.0	82.40±19.2	72.90±27.9	64.50±15.5	72.30±34.3
ALP (IU L^{-1})	378.00±291.7*	605.00±286.6	364.80±237.8	329.80±155.7	319.80±281.5
Creatinine (mg dL^{-1})	0.54±7.5	0.58±7.4	0.570±6.8	0.57±6.9	0.60±7.5
Total protein (g dL^{-1})	7.50±0.2	7.40±0.5	6.800±0.3	6.90±0.4	7.50±0.3
PI (ng mL^{-1})	2.31±0.1	0.38±0.1	1.080±0.1	0.49±0.0	0.80±0.0

TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, ALT: Alanine transaminase ALP: Alkaline phosphatase, PI: Plasma insulin. Data expressed in Mean±SD for five rats in a group. *Significant difference observed compared with diabetic control group $p<0.05$

differences observed in ALT, creatinine and total protein level of *N. lappaceum* treated rats compared to control diabetic rats. Control rats showed significant decrease in ALP level compared to diabetic control group. In the present study, a marked increase in lipid profile was observed in diabetic rats. However, the diabetic rats treated with either metformin or the extract of *N. lappaceum* rind did not appear to alter the blood cholesterol, Low-Density Lipoprotein (LDL) or High-Density Lipoprotein (HDL) levels. The TG level in treated diabetic rats was not altered in this study.

There was a significant reduction in liver weight in diabetic rats treated with metformin or 2000 mg kg^{-1} *N. lappaceum* rind extract. In addition, the ALT and AST levels were unaltered in these rats which suggest that there was no liver damage in these rats. Urea and creatinine used as marker for kidney function test. In this study, the diabetic rats treated with the ethanolic extract of *N. lappaceum* rind or metformin treated rats did not show any significant changes in their blood urea, creatinine and total protein levels.

Effects of *Nephelium lappaceum* rind on histology findings: In the diabetic group (Fig. 4b), pancreas showed diffused areas of damage to both the endocrine and exocrine parts. There was loss of architecture and acini appeared necrotic in focal areas.

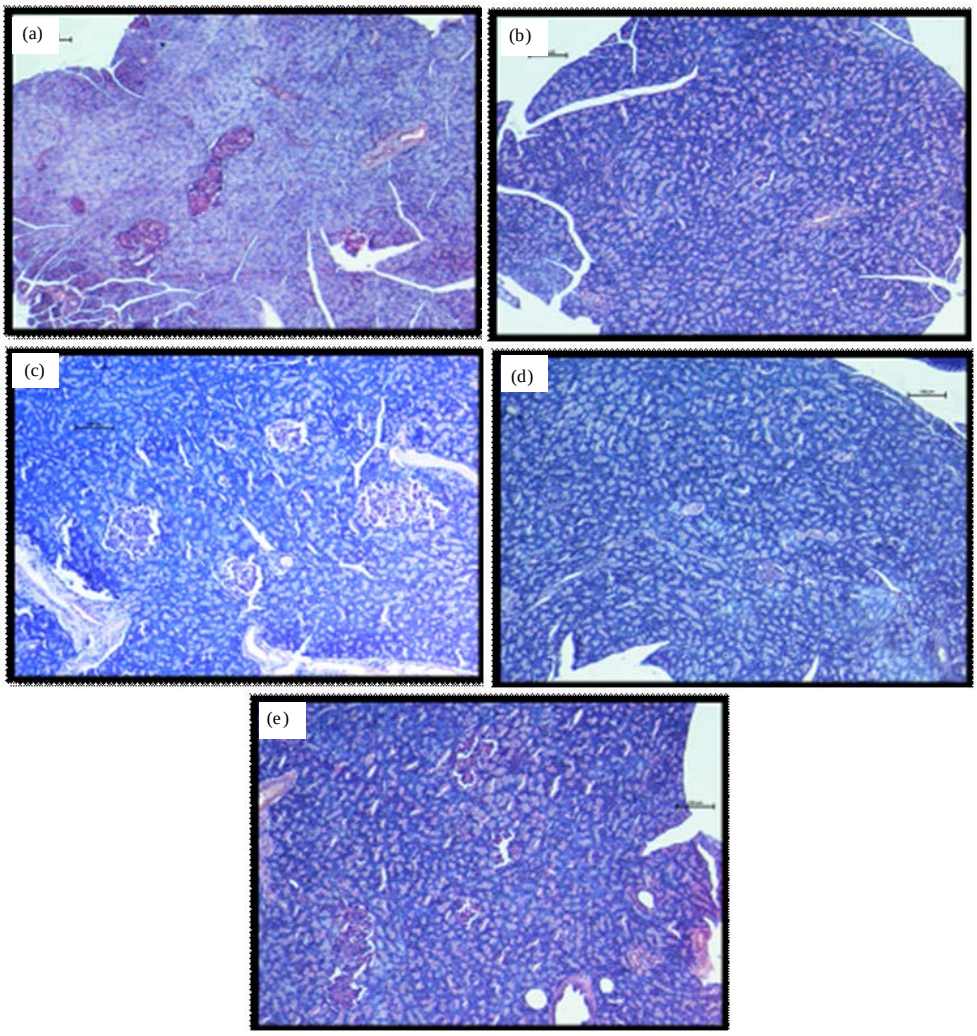

Fig. 4(a-e): Photomicrograph of H and E stain at 100x showing anti-hyperglycemic effects of *Nephelium lappaceum* in type-2 diabetic rats on pancreas (a) Control group healthy pancreatic islets, (b) Diabetic group showing absence of islets normal morphology, (c) Metformin 200 mg kg^{-1} and (d) *Nephelium lappaceum* showing few clusters of regenerating islets and (e) *Nephelium lappaceum* 2000 mg kg^{-1}

The endocrine islets showed reduction in size, number and distribution. Each islet itself had reduced number of cells. Some of the rats showed total absence of islets in the pancreas. In the metformin group, pancreas showed normal architecture of the endocrine islets (Fig. 4c). The distribution of islets was adequately spaced. The cellularity in the islet was good with some of the islets larger than seen in the normal group. In the 500 mg kg^{-1} *N. lappaceum* treatment group (Fig. 4d), focal areas of recovery were seen in the exocrine acini with normal acini seen in the periphery of the exocrine areas. However there were not much significant proliferation of the islets in terms of distribution, number and cellularity. The 2000 mg kg^{-1} *N. lappaceum* group showed pancreas with few residual areas of damage admixed with abundant areas of regenerating acini and good distribution of islets with healthy cellularity. The 2000 mg kg^{-1} *N. lappaceum* group was comparable to the effect of the metformin group and the islets were almost normal and in some cases also showed hyperproliferation (Fig. 4e).

DISCUSSION

The earliest metabolic abnormality in type 2 diabetic patients was reported to be postprandial hyperglycaemia which can lead to serious complications and mortality. The inhibition of the α-glucosidase and α-amylase produce promising solutions to control postprandial plasma glucose levels (Thilagam *et al*., 2013). We demonstrated that, the ethanolic extract of *N. lappaceum* rind exhibited anti-hyperglycaemic activity *in vitro*, whereby the extract inhibited the activities of two carbohydrate-hydrolyzing enzymes, namely, α-glucosidase and α-amylase. The *N. lappaceum* rind extract

inhibited α-glucosidase at significant levels compared to the drug acarbose. However, inhibition of α-amylase was lower compared to α-glucosidase and acarbose. These findings are similar to the findings by Palanisamy *et al*. (2011a), who reported that the ethanolic extract of *N. lappaceum* rind inhibited α-glucosidase (EC_{50} = 2.7±2.3 μg mL^{-1}) greater than α-amylase (EC_{50} = 70.8±49 μg mL^{-1}). In another study, Palanisamy *et al*. (2011b) reported that geraniin, the bioactive compound in *N. lappaceum* rind extract had the more profound inhibitory effects on both enzymes [α-glucosidase and α-amylase (IC_{50} = 0.92±0.1 μg mL^{-1})] compared to ethanolic extract of *N. lappaceum* rind and acarbose. Therefore, the inhibitory activity of carbohydrates enzymes by the ethanolic extract of *N. lappaceum* rind in our study could be due to geraniin present in the extract. In this study, there was a significant reduction in the body weights of the rats treated with *N. lappaceum*. The decrease in body weight could be due to anti-obesity potential of *N. lappaceum* rind in rats. A study showed that, high fat diet fed rats treated with 50 mg kg^{-1} geraniin showed significant reduction in body weight and displayed anti-obese activity (Chung *et al*., 2014). Another study also revealed, presence of geraniin in rambutan rind extracts alter the carbohydrate metabolism by delay in glucose absorption and further resulting in body weight reduction (Thinkratok *et al*., 2014).

The rats treated with higher dose of *N. lappaceum* extract (2000 mg kg^{-1}) exhibited similar effects to the metformin-treated rats in terms of decreasing blood glucose levels. Hyperglycaemia can result in elevated ROS which can lead to oxidative DNA damage in STZ-induced diabetic rats (Bansal *et al*., 2012; Robertson, 2006). Therefore, antioxidants may have role in reducing some of the effects of diabetes. The ethanolic extract of rambutan rind has been reported to possess high antioxidant activities (Palanisamy *et al*., 2008). It was found that geraniin which is the main bioactive compound found in the ethanolic extract of rambutan rind (Palanisamy *et al*., 2008) also has high antioxidant activity as the *N. lappaceum* rind extract (Palanisamy *et al*., 2011a). The free radical scavenging activities observed in the *N. lappaceum* rind extract was largely contributed by the presence of geraniin (Thitilertdecha *et al*., 2010). Therefore, geraniin which is a potent antioxidant present in *N. lappaceum* rind extract may have some beneficial effects in lowering hyperglycemia in diabetic rats. In the present study, we observed severe glucose tolerance in diabetic rats which was partially improved with a high dose (2000 mg kg^{-1}) of *N. lappaceum* rind extract or metformin. These findings suggest that the ethanolic extracts (2000 mg kg^{-1}) of *N. lappaceum* rind, may act by increasing peripheral utilization of glucose. The observed biochemical changes were supported by histological findings in this study. Diabetic rats showed reduced islet size and islet cell number. But treatment with metformin and *N. lappaceum* showed an improvement in islet cell size and numbers. There were areas of recovery observed with 500 and 2000 mg groups and a significant improvement in islet cell morphology was observed with 2000 mg *N. lappaceum* treatment.

Diabetes is strongly associated with hyperlipidaemia. Elevation in TG and total cholesterol are important markers of hyperlipidaemia in HFD/STZ-induced diabetic rats (Maiti *et al*., 2005). In the present study, a marked increase in lipid profile was observed in diabetic rats. However, the diabetic rats treated with either metformin or the extract of *N. lappaceum* rind did not appear to alter the blood cholesterol, Low-Density Lipoprotein (LDL) or High-Density Lipoprotein (HDL) levels. Under normal conditions, the lipoprotein lipase enzyme will be activated by insulin and can hydrolyse TG (Nikkila and Kekki, 1973). However, when there is insulin deficiency, the lipoprotein lipase enzyme cannot be activated and this results in the hypertriglyceridemia condition (Shirwaikar *et al*., 2004). The TG level in treated diabetic rats was not altered in this study. This may be due to the wide variations in the TG levels in the experimental rats which in turn affected the value of the data; i.e. the high SD in the hypertriglyceride measurements failed to provide a clear conclusion on the effect of treating with *N. lappaceum* rind.

The ALT enzyme is a widely used marker for hepatocellular injury whereas ALP used as marker for hepato-billiary injury (Chin *et al*., 2008). Liver damage due to accumulation of fat can cause an increase in liver weight (Sallie *et al*., 1991). However, in our study, we observed a significant reduction in liver weight in diabetic rats treated with metformin or 2000 mg kg^{-1} *N. lappaceum* rind extract. In addition, the ALP and AST levels were unaltered in these rats which suggest that there was no liver damage. Urea and creatinine used as markers for kidney function test. Elevation of these two parameters indicates injury in kidneys (Satyanarayana *et al*., 2001). In this study, the diabetic rats treated with the ethanolic extract of *N. lappaceum* rind or metformin rats did not show any changes in their blood urea, creatinine and total protein levels. We had previously reported that, supplementation of 2000 mg kg^{-1} of *N. lappaceum* rind extract for 28 days did not induce liver or kidney injury in rats (Subramaniam *et al*., 2012).

CONCLUSION

The standardised ethanolic extract of *N. lappaceum* rind was found to be effective in inhibiting carbohydrate hydrolyzing enzymes. Geraniin, the active compound in *N. lappaceum* rind also possesses high antioxidant activity mediating its anti-hyperglycemic effects in type 2 diabetic rats through inhibition of oxidative stress and scavenging free radicals. The data from the current study revealed that oral administration of the standardised extract of *N. lappaceum* rind in the HFD-induced diabetic rats exhibited moderate

anti-hyperglycaemic effects. The higher dose of *N. lappaceum* (2000 mg kg^{-1}) rind was found to be more effective than the lower dose (500 mg kg^{-1}) used. Therefore, both *in vitro* and *in vivo* studies of ethanolic extract of *N. lappaceum* rind shown to have good potential in managing hyperglycemia in diabetes. The molecular mechanism of *N. lappaceum* induced anti-diabetic effect is currently being investigated.

ACKNOWLEDGMENTS

This research work was supported in part by Research Grants from the Ministry of Sciences, Technology and Innovation Malaysia.

REFERENCES

Agyare, C., M. Lechtenberg, A. Deters, F. Petereit and A. Hensel, 2011. Ellagitannins from *Phyllanthus muellerianus* (Kuntze) Exell.: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. Phytomedicine, 18: 617-624.

Ambrose, S.S., P. Solairaj and A. Subramoniam, 2012. Hepatoprotective activity of geraniin isolated from *Thespesia lampas* Dalz. and Gibson. Int. Res. J. Pharm. Pharmacol., 2: 92-96.

Arya, A., S.C. Cheah, C.Y. Looi, H. Taha, M.R. Mustafa and M.A. Mohd, 2012. The methanolic fraction of *Centratherum anthelminticum* seed downregulates pro-inflammatory cytokines, oxidative stress and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats. Food Chem. Toxicol., 50: 4209-4220.

Bansal, P., P. Paul, J. Mudgal, P.G. Nayak, S.T. Pannakal, K.I. Priyadarsini and M.K. Unnikrishnan, 2012. Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of *Pilea microphylla* (L.) in high fat diet/streptozotocin-induced diabetes in mice. Exp. Toxicol. Pathol., 64: 651-658.

Bera, T.K., K. Chatterjee, K. Jana, K.M. Ali, D. De, S. Maiti and D. Ghosh, 2012. Antihyperglycemic and antioxidative effect of hydro - methanolic (2:3) extract of the seed of *Swietenia mahagoni* (L.) Jacq. in streptozotocin-induced diabetic male albino rat: An approach through pancreas. Genomic Med. Biomarkers Health Sci., 4: 107-117.

Chin, J.H., H.H. Abas and I. Sabariah, 2008. Toxicity study of orthosiphon stamineus benth (Misai Kucing) on sprague dawley rats. Trop. Biomed., 25: 9-16.

Chung, A.P.Y., S.H. Ton, S. Gurtu and U.D. Palanisamy, 2014. Ellagitannin geraniin supplementation ameliorates metabolic risks in high-fat diet-induced obese Sprague Dawley rats. J. Funct. Foods, 9: 173-182.

De Souza, C.J., M. Eckhardt, K. Gagen, M. Dong, W. Chen, D. Laurent and B.F. Burkey, 2001. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes, 50: 1863-1871.

Islam, M.S. and H. Choi, 2007. Nongenetic model of type 2 diabetes: A comparative study. Pharmacology, 79: 243-249.

Li, J., H. Huang, M. Feng, W. Zhou, X. Shi and P. Zhou, 2008. *In vitro* and *in vivo* anti-hepatitis B virus activities of a plant extract from *Geranium carolinianum* L. Antiviral Res., 79: 114-120.

Lin, S.Y., C.C. Wang, Y.L. Lu, W.C. Wu and W.C. Hou, 2008. Antioxidant, anti-semicarbazide-sensitive amine oxidase and anti-hypertensive activities of geraniin isolated from *Phyllanthus urinaria*. Food Chem. Toxicol., 46: 2485-2492.

Maiti, R., U.K. Das and D. Ghosh, 2005. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biol. Pharmacol., 28: 1172-1176.

Nikkila, E.A. and M. Kekki, 1973. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism, 22: 1-22.

Notka, F., G.R. Meier and R. Wagner, 2003. Inhibition of wild-type human immunodeficiency virus and reverse transcriptase inhibitor-resistant variants by *Phyllanthus amarus*. Antiviral Res., 58: 175-186.

Palanisamy, U., H.M. Cheng, T. Masilamani, T. Subramaniam, L.T. Ling and A.K. Radhakrishnan, 2008. Rind of the rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants. Food Chem., 109: 54-63.

Palanisamy, U., T. Manaharan, L.L. Teng, A.K.C. Radhakrishnan, T. Subramaniam and M. Masilamani, 2011a. Rambutan rind in the management of hyperglycemia. Food Res. Int., 44: 2278-2282.

Palanisamy, U.D., L.T. Ling, T. Manaharan and D. Appleton, 2011b. Rapid isolation of geraniin from Nephelium lappaceum rind waste and its anti-hyperglycemic activity. Food Chem., 127: 21-27.

Pepato, M.T., D.M. Mori, A.M. Baviera, J.B. Harami, R.C. Vendramini and I.L. Brunetti, 2005. Fruit of the Jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. J. Ethnopharmacol., 96: 43-48.

Perera, A., D. Appleton, L.H. Ying, S. Elendran and U.D. Palanisamy, 2012. Large scale purification of geraniin from *Nephelium lappaceum* rind waste using reverse-phase chromatography. Separat. Purif. Technol., 98: 145-149.

Perez, M.R. and G. Medina-Gomez, 2011. Obesity, adipogenesis and insulin resistance. Endocrinol. Nutr., 58: 360-369.

Pushparaj, P., C.H. Tan and B.K.H. Tan, 2000. Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J. Ethnopharmacol., 72: 69-76.

Rasekh, H.R., P. Nazari, M. Kamli-Nejad and L. Hosseinzadeh, 2008. Acute and subchronic oral toxicity of *Galega officinalis* in rats. J. Ethnopharmacol., 116: 21-26.

Robertson, R.P., 2006. Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr. Opin. Pharmacol., 6: 615-619.

Sallie, R., J.M. Tredger and R. Williams, 1991. Drugs and the liver Part 1: Testing liver function. Biopharm. Drug Dispos., 12: 251-259.

Satyanarayana, P.S.V., D. Singh and K. Chopra, 2001. Quercetin, a bioflavonoid, protects against oxidative stress-related renal dysfunction by cyclosporine in rats. Methods Findings Exp. Clin. Pharmacol., 34: 175-181.

Sharma, B., R. Salunke, S. Srivastava, C. Majumder and P. Roy, 2009. Effects of guggulsterone isolated from *Commiphora mukul* in high fat diet induced diabetic rats. Food Chem. Toxicol., 47: 2631-2639.

Shirwaikar, A., K. Rajendran, C.D. Kumar and R. Bodla, 2004. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. J. Ethnopharmacol., 91: 171-175.

Srinivasan, K., B. Viswanad, L. Asrat, C.L. Kaul and P. Ramarao, 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res., 52: 313-320.

Subramaniam, S., S. Chakravarthi, U.D. Palanisamy, A. Radhakrishnan and N. Haleagrahara, 2012. Acute and sub chronic oral toxicity assessment of the Ethanolic extract from the rind of *Nephelium lappaceum* in rats. J. Pharmacol. Toxicol., 7: 378-385.

Tahara, A., A. Matsuyama-Yokono and M. Shibasaki, 2011. Effects of antidiabetic drugs in high-fat diet and streptozotocin-nicotinamide-induced type 2 diabetic mice. Eur. J. Pharmacol., 655: 108-116.

Thilagam, E., B. Parimaladevi, C. Kumarappan and S.C. Mandal, 2013. α-Glucosidase and α-amylase inhibitory activity of *Senna surattensis*. J. Acupunct. Meridian Stud., 6: 24-30.

Thinkratok, A., P. Suwannaprapha and R. Srisawat, 2014. Safety assessment of hydroethanolic rambutan rind extract: Acute and sub-chronic toxicity studies. Indian J. Exp. Biol., 52: 989-995.

Thitilertdecha, N., A. Teerawutgulrag, J.D. Kilburn and N. Rakariyatham, 2010. Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. Molecules, 15: 1453-1465.

Thitilertdecha, N. and N. Rakariyatham, 2011. Phenolic content and free radical scavenging activities in rambutan during fruit maturation. Sci. Hortic., 129: 247-252.

Veerapur, V.P., K.R. Prabhakar, B.S. Thippeswamy, P. Bansal, K.K. Srinivasan and M.K. Unnikrishnan, 2012. Antidiabetic effect of *Ficus racemosa* Linn. stem bark in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats: A mechanistic study. Food Chem., 132: 186-193.

Whiting, D.R., L. Guariguata, C. Weil and J. Shaw, 2011. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract., 94: 311-321.

Yang, C.M., H.Y. Cheng, T.C. Lin, L.C. Chiang and C.C. Lin, 2007. The *in vitro* activity of geraniin and 1,3,4,6-tetra-O-galloyl-β-d-glucose isolated from *Phyllanthus urinaria* against herpes simplex virus type 1 and type 2 infection. J. Ethnopharmacol., 110: 555-558.

Yang, Y., L. Zhang, X. Fan, C. Qin and J. Liu, 2012. Antiviral effect of geraniin on human enterovirus 71 *in vitro* and *in vivo*. Bioorg. Med. Chem. Lett., 22: 2209-2211.

Zheng, T., G. Shu, Z. Yang, S. Mo, Y. Zhao and Z. Mei, 2012. Antidiabetic effect of total saponins from *Entada phaseoloides* (L.) merr. In type 2 diabetic rats. J. Ethnopharmacol., 139: 814-821.