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Soursop *(Anona muricata* L.): Blood hematology and serum biochemistry of Sprague-Dawley rats

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Abstract: This study was aimed to evaluate the effect of soursop (*Annona muricata* L.) extract on Sprague-Dawley rats subjected to *in vivo* 28-day repeated doses. The extract was given to the study group via force feeding. In the 28-day study, *Annona muricata* L. extract was dosed at 0 (CD, control dose), 0.5 (LD, low dose), 1.0 (MD, medium dose), 2.0g/kg (HD, high dose) body weight. For control group, distilled water was given to the animals. Administration of *Annona muricata* L. extract did not cause negative effect in blood hematology even though a statistically significant ($p<0.05$) increase in platelet level was noted. Result from serum biochemical test showed that the consumption of the extract did not result in liver and kidney failure. The total antioxidant status (TAS) increased significantly as the dosages increased. However the incresase were within the normal laboratory limits.

Keywords: Soursop, blood hematology, serum biochemistry, Sprague-Dawley

Introduction

Fruits are rich with antioxidants that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (Shi *et al*., 2001). The most abundant antioxidants in fruits are polyphenols and vitamins C, A, B and E; while carotenoids are present to a lesser extent in some fruits. These polyphenols with antioxidant activities are mostly belong to flavonoids (Fleuriet and Macheix, 2003).

Annona muricata L. is one of the tropical fruits that demonstrate antioxidant properties. This plant contains annonaceous acetogenins in the twigs, unripe fruit, seeds, roots, and bark tissues, which display antitumor, pesticidal, antimalarial, antihelmintic, piscicidal, antiviral, and antimicrobial effects, thus suggesting many potentially useful applications. Ripe *Annona muricata* L. pulp extract contains three prominent acetogenins: asimicin, bullatacin, and bullatalicin. Previous research on *Annona muricata* L. was focused on the leaves, seeds and roots for pharmaceutical purposes (Gleeve *et al*., 1997; Jaramillo *et al*., 2000; Onimawo, 2002). Little attention has been paid to the study of the pulp of *Annona muricata* L. fruit. This study was therefore conducted to evaluate the effect of *Annona muricata* L. pulp extract on blood hematology and serum biochemistry of Sprague-Dawley rats.

Materials and Methods

Annona muricata L. extract

Extraction of *Annona muricata* L. was prepared by blending the fruit pulp (without the seeds) with the ratio of 1:4 (pulp:distilled water) by using a Warring blender. The mixture was filtered by vacuum filtration. The filtrate was then concentrated in a rotary evaporator at 40°C before being used for animal study.

Animal husbandry

All procedures concerning the use of animals were approved by the University Kebangsaan Malaysia Animal Ethic Committee (UKMAEC). The animals were housed in a controlled environment, with temperature of 24 ± 2°C and a relative humidity of 30-70%. The rooms were illuminated with 12 hours artificial fluorescent light and 12 hours darkness per day. The animals were provided with a standard

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pelleted (Australian Rat Pellet) and distilled water via *ad libitum*. The animals were allowed to acclimatize for 7 days before the treatment started.

Twenty eight day study

The 28-day repeated dose study was performed based on Ryu *et al*. (2004). Five male Sprague-Dawley rats weighing 200-250g per group were given distilled water (CD, control dose), 0.5 (LD, low dose), 1.0 (MD, medium dose) and 2.0g/kg (HD, high dose) of *Annona muricata* L. extract via force feeding. The physical conditions and behavior of each animal were monitored daily during the 28-day study. The body weight was measured weekly. On day 29, all rats were sacrificed and blood samples were collected from posterior vena cava.

Hematology and biochemistry

Animals were fasted for approximately 12 h and blood samples were withdrawn from posterior vena cava. Samples of blood for hematological and biochemistry analyses were withdrawn under light ether anesthesia. For the evaluation of haematological parameters, an aliquot of blood per animal was placed in a 3ml ethylen-diamino-tetracetic-acid (K_3-EDTA) tube (Bacton Dickinson, BD Vaccutainer). Blood sample collected were then analyzed for complete blood profile: red blood cell (RBC), white blood cell (WBC), platelet, hematocrit and hemoglobin and glucose level. The measurements were performed by Hematology Analyzer (Medonic CA530).

For the evaluation of biochemical parameters, one aliquot of blood per animal was placed in a 5 ml Z-serum tube (Bacton Dickinson, BD Vaccutainer) and centrifuged at 15,000 rpm for 20 mins. Serum aliquots were subjected to evaluation of alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities, total protein, albumin, globulin, albumin/globulin (A/G) ratio, urea, creatinine and total antioxidant status (TAS). All parameters were measured using Blood Clinical Analyzer (Vitalab Selectra E). The reagents for the tests were obtained from Randox (Randox Laboratories Ltd, Antrim, United Kingdom).

Statistical analysis

All data were subjected to one-way analysis of varians (ANOVA) and Duncan by using The statistical analysis system (SAS v.11).

Results

Blood hematology

All animals survived until the scheduled necropsy in all study groups. Dietary administration of *Annona muricata* L. extract produced no clinical signs and did not affect the normal physical and behavior of animal. The result of blood hematology (Table 1) showed that there were no effects on parameter evaluated. However, the platelet level was increased significantly ($p<0.05$) as the dosage of *Annona muricata* L. were increased up to 2 g/kg. Other parameters did not show any significant differences. Blood glucose contents (Figure 1) were increased as the dosage was increased, but no significant difference were noted. However, the glucose levels were still within the normal laboratory range of 5.0 to 11.2 mmol/l (Petterino and Argentini-Storino, 2006).

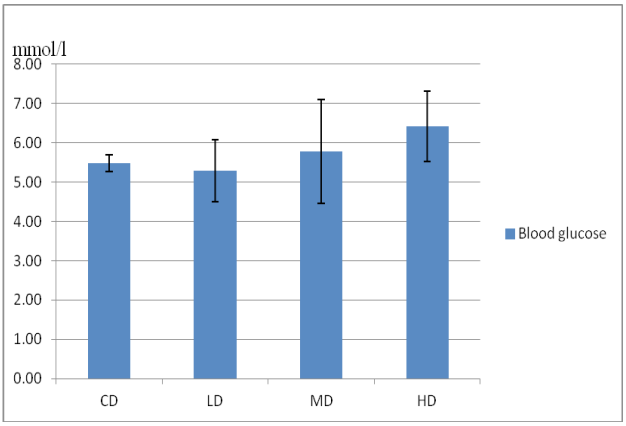

Figure 1. Blood glucose level after 28-day repeated dose CD, control dose; LD, low dose; MD, medium dose; HD, high doseValues are mean ± sd for 5 rats in each group.

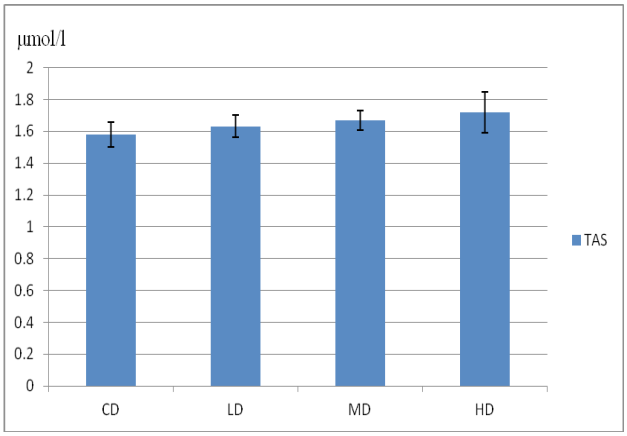

Figure 2. Serum total antioxidant level after 28-day repeated dose. CD - control dose; LD- low dose; MD- medium dose; HD- high dose; TAS- total antioxidant status, Values are mean ± sd for 5 rats in each group.

Table 1. Level of blood hematology parameter after 28-day repeated dose

Parameters	CD (distilled water)	LD (5%)	MD (10%)	HD (20%)
RBC (10^{12}/l)	8.90 ± 0.56 [a]	8.86 ± 0.48 [a]	8.86 ± 0.47 [a]	8.98 ±0.41 [a]
WBC (10^9/l)	16.24 ± 1.57 [a]	15.60 ± 3.01 [a]	15.30 ± 3.27 [a]	14.42 ± 0.68 [a]
Platelet (10^9/l)	952.80 ± 137.67 [b]	1091.40 ± 85.55 [ab]	1093.20 ± 24.59 [ab]	1231.80 ± 43.78 [a]
Hematocrit (%)	47.36 ± 2.14 [a]	47.24 ± 2.34 [a]	47.04 ± 2.86 [a]	43.20 ± 7.05 [a]
Hemoglobin (mmol/l)	167.40 ±5.77 [a]	158.60 ± 9.89 [a]	162.60 ± 8.26 [a]	164.40 ±11.35 [a]

Means with the same letter in same row are not significantly different ($p<0.05$). Values are mean±sd for 5 rats in each group, CD, control dose; LD, low dose; MD, medium dose; HD, high dose; RBC, red blood cell; WBC, white blood cell.

Table 2. Blood biochemistry parameter for liver function after 28-day repeated dose

Parameters	CD (distilled water)	LD (5%)	MD (10%)	HD (20%)
ALT (U/l)	55.40 ± 13.35 [a]	58.60 ± 9.44 [a]	56.20 ± 9.20 [a]	53.60 ± 12.10 [a]
AST (U/l)	129.80 ± 28.26 [a]	128.60 ± 30.27 [a]	122.00 ± 27.81 [a]	112.8 ± 12.15 [a]
LDH (U/l)	1074.00 ± 583.95 [a]	1118.20 ± 637.19 [a]	884.60 ± 881.59 [a]	776.60 ± 417.62 [a]
GGT (U/l)	10.80 ± 0.45 [a]	11.00 ± 1.87 [a]	9.67 ± 2.31 [ab]	7.80 ± 1.10 [b]
ALP (U/l)	156.20 ± 24.84 [a]	198.20 ± 41.64 [a]	189.80 ± 40.13 [a]	168.80 ± 36.99 [a]
Total protein (g/l)	75.37 ± 4.37 [a]	73.35 ± 2.18 [a]	73.44 ± 1.67 [a]	72.63 ± 2.51 [a]
Albumin (g/l)	38.24 ± 2.18 [a]	37.36 ± 0.57 [a]	37.18 ± 1.24 [a]	37.14 ± 0.63 [a]
Globulin (g/l)	37.20 ± 2.95 [a]	36.00 ± 1.87 [a]	36.20 ± 1.79 [a]	35.40 ± 2.07 [a]
A/G ratio	1.03 ± 0.08 [a]	1.04 ± 0.04 [a]	1.03 ± 0.07 [a]	1.05 ± 0.05 [a]

Means with the same letter in same row are not significantly different ($p<0.05$). Values are mean±sd for 5 rats in each group , CD, control dose; LD, low dose; MD, medium dose; HD, high dose; ALT- alanine amino transferase; AST - aspartate amino transferase; LDH - lactate dehydrogenase; ALP - alkaline phosphatase; A/G ratio- albumin/globulin ratio.

Serum biochemistry

It is evident from Table 2 that the extracts and their interactions exhibited non-significant differences in liver functioning tests, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehidrogenase (LDH) and alkaline phosphatase (ALP) activities with the range from 53.60 ± 12.10 to 58.60 ± 9.44, 112.8 ± 12.15 to 129.80 ± 28.26, 776.60 ± 417.62 to 1118.20 ± 637.19 and 156.20 ± 24.84 to 198.20 ± 41.64 U/l, respectively in different dosage groups. While for γ-glutamyl tranferase (GGT) activities, the results ranged from 7.80±1.10 to 10.80±0.45 U/l. Increasing the dosage from LD to HD reduce a increasing trend in GGT where at HD, the value of GGT was significantly ($p<0.05$) lower than CD. Results on serum protein profiles showed that the mean values of total protein, albumin, globulin and A/G ratio varied from 72.63 ± 2.51 to 75.37 ± 4.37, 37.14 ± 0.63 to 38.24 ± 2.18, 35.40 ± 2.07 to 37.20 ± 2.95 g/l and 1.03 ± 0.07 to 1.05 ± 0.05, respectively. The decrease in activities of liver enzymes and protein profile indicated that there was no liver damage or dysfunction caused by the administration of the extracts.

Table 3. Blood biochemistry parameter for kidney function after 28-day repeated dose.

	CD	LD	MD	HD
Urea (mmol/l)	5.59 ± 0.74 [a]	5.46 ± 0.27 [a]	5.74 ± 0.60 [a]	5.05 ± 0.61 [a]
Creatinin (umol/l)	57.78 ± 5.42[a]	50.46 ± 2.13[b]	52.92 ± 6.55[ab]	54.04 ± 3.63[ab]

Means with the same letter in same row are not significantly different ($p<0.05$), Values are mean±sd for 5 rats in each group, CD, control dose; LD, low dose; MD, medium dose; HD, high dose

Results from kidney function test (urea and creatinin) are showed in Table 3. There was no significant difference noted in urea (range from 5.46± 0.27 to 5.59± 0.74 mmol/l) when dosage was increased from LD to HD. For creatinin (50.46± 2.13 to 57.78±5.42 umol/l) a significant differences was observed for LD, MD and HD when compared to the CD. These results also suggested no kidney failure was caused by the administration of the extracts. After 28 days repeated dose, the total antioxidant status (TAS) in serum (Figure 2) were significantly increased (range from 1.58±0.08 to 1.72±0.13) as the dosage was increased. It was therefore evident that the antioxidants present in the extract were responsible for the increase.

Discussions

Similar to the results of this study, Cerda *et al.* (2003) also did not observed any significant difference in blood parameters analyzed. From Table 1, although platelet showed a significant increase with increasing dosage, levels for all parameters were within the normal range as reported by Petterino and Argentini-Storino (2006). With regards to serum biochemistry, a significant decrease in liver function test parameters such as serum ALT, AST, LDH, GGT and ALP were noted. The total protein, albumin, globulin and A/G ratio showed almost similar value with control group and were within the normal laboratory range. As these parameters represent liver function, the increase in their levels will indicate liver damage. A decrease in the activities of these liver enzymes is not considered to give any toxicological significance. Kidney function of the study group was not affected by the administration of extract. The level of urea and creatinin were within the normal range. For serum TAS , the increasing level was noted as the dosage was increased. It has been shown that *Annona muricata* L. extract contains high total antioxidant which is good in promoting health. Blood hematology results in the present study did not show any abnormalities. The increase in blood glucose level was also noted as the dosage was increased. However, the value was still within the normal range. Liver and kidney functions tests and serum protein profile are important parameters in determining the safety of functional ingredient or final product (Farag *et al.*, 2006; Patel *et al*., 2008). The findings from present studies suggested that the administration of soursop extract did not cause any toxicological effect since the values were in the normal range as reported by Chengelis *et al*. (2008), Morita *et al*. (2008) and Petterino and Argentini-Storino (2006). Further study need to be carried out to evaluate the effectiveness of the extract on treatment of metabolic syndrome disease in rats.

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