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SUBACUTE TOXICITY OF *Berberis vulgaris* L. AQUEOUS EXTRACT IN ATHEROSCLEROTIC-INDUCED RABBIT

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ABSTRACT

Objective: *Berberis vulgaris* L is extensively used as medicinal herb to treat many diseases. Assessment of it's toxic properties is crucial when considering the protection of public health, as exposure to any plant extracts can have unwanted effects on consumers. **Methods:** In this study, the subacute oral toxicity of *B. vulgaris* aqueous extract was investigated in atherosclerotic-induced rabbit for ten weeks. This include to determine toxicity effect of BVAE on liver function test (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase), renal function (urea and creatinine) and histopathological changes. **Result:** Oral administration of aqueous extract at 25mg / kg and 50mg / kg for ten weeks did not result in any adverse effects and mortality, implying that *B. vulgaris* is non-toxic. No significant elevations were observed in biochemical analysis. Further, histopathological examination revealed normal architecture and no significant adverse effects observed on renal and liver. **Conclusion:** Overall, the results suggest that, the oral administration of *B. vulgaris* aqueous extract did not produce any significant toxic effect in atherosclerotic-induced rabbit. Hence, the extract can be utilized for pharmaceutical formulations at these concentrations.

Keywords: *Berberis vulgaris* L, subacute toxicity, atherosclerosis

1. Introduction

The use of medicinal herbs is a common practiced in Asian culture since years ago before the introduction of modern medicine. *B. vulgaris* from the family Berberidaceae also known as "barberry" is a thorny shrub with yellow flowers and small scarlet red fruits. It is a native herb to Europe and Asia that grows in a varied soils with cooler climate (Imanshahidi & Hosseinzadeh, 2008). In traditional Chinese medicine, *B. vulgaris* was mentioned more than 3,000 years ago to have varied medicinal properties, including antimicrobial, antiemetic, antipyretic and antipruritic effects (Zarei et al., 2015). It has been used as treatment for cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones (Srivastava, Bordia, & Verma, 1995). Besides, it also been use as antimalarial (Mahmoudvand et al., 2014), anti-rheumatic (Suau et al,. 1998), antiseptic (Javadzadeh & Fallah, 2012), diuretic (El-Wahab et al., 2013) and dysmenorrhoea (Imanshahidi & Hosseinzadeh, 2016).

Therapeutic uses of *B. vulgaris* have been a focus for experimental research in both animals and human trials. It have been concluded that its extracts have antioxidant, anti-inflammatory, anti-mutagenic, antimicrobial and anti-parasitic activity (Mohammadi et al., 2014). *B. vulgaris* extract has also been shown to play an important role in promoting apoptosis in the treatment of hepatocarcinogenic rats (Motalleb et al., 2012). Many compounds have been identified from *B. vulgaris*. Three important alkaloid compounds, berberine, berbamine and oxycanthine and phenolic compound including N-tyramine, cannabin G and lyniresinol. All these important compounds stated responsible in anticancer, anti-inflammatory, anti-atherosclerosis and

antioxidant using animal and cell lines studies (Chi et al, 2014; Hadaruga et al., 2010; Ivanovska & Philipov, 1996).

However, the concentration-related toxicity of medicinal plants, especially the histological side, has not been much known. Bioactive compounds derived from medicinal plants may be helpful but may have severe side effects (An, Sohn, & Kim, 2006). Therefore, given their widespread usage for alternative medicine, toxicological assessment is a must in order to be considered as safe formulation for clinically efficient remedies. As no work has been done on ingestion of *B. vulgaris* L at high doses, the systemic approach in evaluating their efficacy and safety profile is needed. Therefore, the present study was aimed to evaluate the safety of *B. vulgaris* L leaves extract with sub-acute toxicity tests in atherosclerotic-induced rabbit.

2. Materials and Method

2.1. Plant material

BV fruits was purchased and imported from certified herbal marketing company in Iran. The voucher specimens were identified by Dr. Mohd Firdaus Ismail of Institute of Biosciences, UPM. The herbarium of the plants was deposited at the Herbarium Biodiversity Unit, Institute of Biosciences, UPM under the reference number SK3208/17 for BV. The plant was washed with distilled water, dried in an oven at 60°C for three consecutive days.

2.2. Preparation of aqueous extract (BVAE)

Preparation for aqueous extract follow common decoction method with slight modification (Bilia et al, 2008). The dried powder of BV, TP and OS were put into 10 L beaker separately. For each 100 g of dried powder, 4000 mL of distilled water was added. Then the mixtures were heated up to 70 ^{o}C to decrease the water content to 1000 mL through evaporation. After these steps, the residues were filtered, and the crude extracts were subjected to lyophilise and stored at -20 ^{o}C until further used.

2.3. Target animal

Sixteen male New Zealand white rabbits weighing 1.5 to 2 kg were obtained for this study. The rabbits were housed in animal cages (1 rabbit per cage) at animal house in Experimental Animal Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The rabbits had been acclimatised for seven days before starting the experimental studies. They were maintained on regular commercial diet (rabbits chow) and tap water *ad labitum* at constant temperature 25±5 °C, relatively 70±5% humidity, and on a light cycle of 12h light, 12h dark. The study protocol was design as such to fulfil the requirements by Institutional Animal Care and Use Committee (IACUC), Faculty Medicine and Health Sciences, UPM before the initiation of the study (Approval Number for Animal Ethic: UPM/IACUC/AUP-R077/2013).

The preparation of 2% cholesterol diet was previously described (Soma et al., 2009). The experiment groups consisted of 16 male New Zealand white rabbits, randomly divided equally into four groups with four rabbits per group (Rosenfeld & Ross, 1990). Control group (Normal group) diet was a normal commercial diet (Havens Premium, Malaysia) contained 15.5% wheat bran, 18.6% crude fibre and 4% crude fat and the other eight groups diet were contained additional 2% cholesterol (0.4g) in each 20 g rabbit pellets.

The rabbits were divided into four groups and fed with 2 % cholesterol diet only, 2% cholesterol and difference dosage of BVAE (25 mg/kg and 50 mg/kg) and normal diet for ten weeks in a row.

2.4. Blood biochemical assay

Blood samples from auricular vein was taken prior to treatment of week 0, week 5 and week 10 for blood parameters (renal profile and liver function test). At the end of the experiment (week 10), Normal, Control, BVAE 25 and BVAE 50 groups were euthanised via intravascular pentobarbital 200 mg/kg.

2.5. Collection of organ samples

At the end of study period, kidney and liver were resected. These organs were weighed and examined macroscopically for relevant anatomical changes. They were sliced and incubated in 10% formalin for more than 48 hours. Subsequently, the vital organs were cut by using microtome and stained with haematoxylin and eosin (H&E).

2.6. Histology of kidney and liver

The liver and kidney of rabbits were exclusively used for histopathological analysis. Thin sections (5µm) of tissues were cut and fixed on slides. Liver and kidney sections were stained with haematoxylin and eosin stain. The haematoxylin and eosin stain were used to

visualise and score the toxicity effect of BVAE on kidney and liver. From each tissue sample, three slides were prepared, and two random, non- overlapping fields were selected from each slide. A representative picture was randomly selected from each group.

H&E staining technique was used to stain the tissue using auto-stainer XL (Leica, Germany) as described by McManus and Mowry (1960). The slide underwent hydration, colorization and dehydration processes. After that, slides were mounted with cover slips using DPX gum (R&M, United Kingdom).

The slides were viewed under 40x, 100x and 200x magnification using an Olympus BX51 and DP72 and captured using Olympus Cell^F (Olympus, USA). The lesion scoring of the liver and kidney were done according to Knodell et al. (1981) and Ramli (2005) methods respectively as in Table 1 by two independence viewers and the result calculated in mean ± SEM.

2.7. Statistical analysis

The data were analysed with SPSS version 22.0 (SPSS inc., Chicago, USA). An one-way ANOVA was performed and followed by LSD's post-hoc test. A *p* value <0.05 was considered as significant. Data are reported as mean ± SEM

3. Results

3.1. Liver function tests

Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are liver enzymes that were assessed after ten weeks of experimental period. Elevated levels of AST, ALT and ALP injury to the liver cells. Control group showed no significant reduction of ALT, AST and ALP when compared to control (p>0.05). However, AST and ALT values showed a significant reduction in BVAE 50-treated group compared to normal group as shown in Table 2 (p<0.05). The ALP level showed no significant difference between all groups (p>0.05).

3.2. Renal profile

Serum urea (Figure 1) and creatinine levels (Figure 2) were assessed at week 0, week 5 and at the end of the study (week 10). Elevated of urea and creatinine level indicated renal injury. Serum urea and creatinine level in the current study showed no significant difference between all groups except for the creatinine level in non-treated atherosclerosis-induced rabbits (control) group at week 10 with mean value of 295.7 umol/L (p<0.05). The increasing creatinine value might indicate kidney injury. However, histological analysis might confirm the finding of the creatinine result.

Table 1: Lesion scoring of the liver and kidney

Tissue	Score	Descriptions
Liver	0	Normal
	1	Mild piecemeal necrosis
	2	Moderate piecemeal necrosis Involved less than 50% of the circumstance of most portal tracts
	3	Marked piecemeal necrosis Involves more than 50% of the circumstance of most portal tracts (Knodell et al., 1981)
Kidney	0	Normal
	1	There was slight hyperemia The epithelium was slightly detached from the basement membrane of the parietal layer of Bowman's capsule Glomerulus was slightly shrunken
	2	Moderate hyperemia The epithelium was moderately detached from the basement membrane of the parietal layer of Bowman's capsule Glomerulus was moderately shrunken with damaged basement membrane
	3	Severe hyperemia The epithelium was highly detached from the basement membrane of the parietal layer of Bowman's capsule Glomerulus was severely shrunken with damaged basement membrane (Ramli, 2005)

Table 2: The Effects of BVAE on Liver Function Enzymes of Preventive Study.

Group	AST (u/l) Mean ± SEM	ALT (u/l) Mean ± SEM	ALP(u/l) Mean ± SEM
Normal	37.0 ± 7.2	31.4 ± 6.1	49.3 ± 5.1
Control	28.9 ± 5.1	24.2 ± 1.4	46.1 ± 11.3
BVAE 25	26.5 ± 5.3	18.9 ± 9.3*	52.1 ± 10.4
BVAE 50	9.5 ± 1.5*	7.45 ± 1.8*	51.0 ± 8.7

* p is significant when <0.05

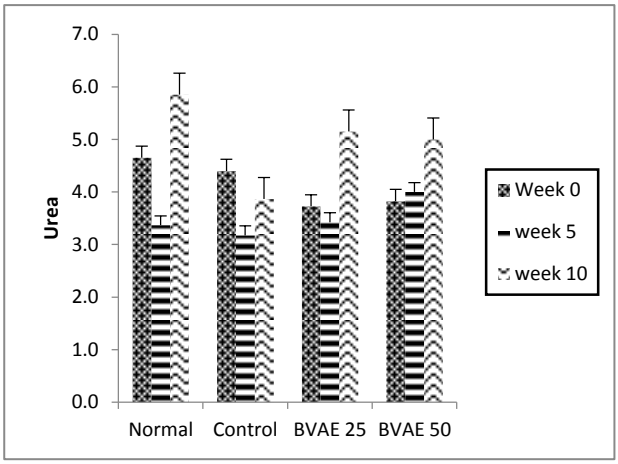

Figure 1: The Effects of BVAE on Urea of Atherosclerotic-induced Rabbits.

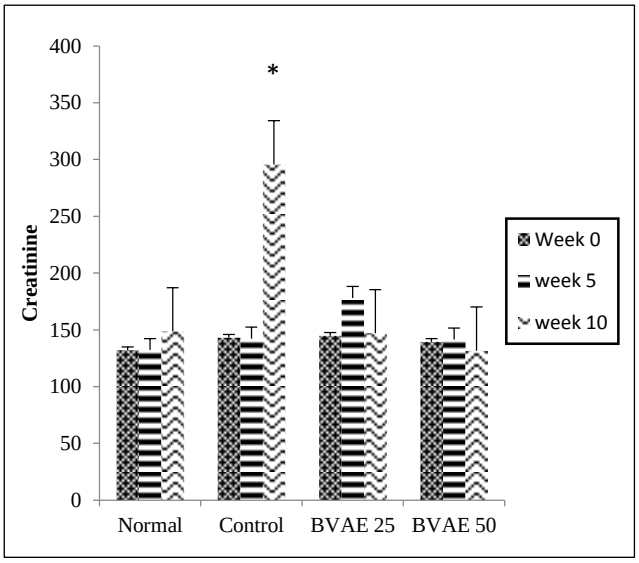

Figure 2: The Effects of BVAE on Creatinine Level of Atherosclerotic-induced Rabbits. * p is significant when <0.05

3.3. Mean lesion scoring for kidney

The morphology of kidney was scored by two independent viewers after H&E stained and the score was approximately zero. This interpreted that normal, control, BVAE 25 and BVAE 50 treated groups showed normal morphology of kidney as shown in Figure 3.

3.4. Mean lesion scoring for liver

There was no abnormality detected in histology slides of all groups viewed by independent viewer with score of approximately zero. All groups showed no significant changes compared to the normal group. There was no pathological change as shown in Figure 4.

4. Discussion

Biochemical evaluation done in this study expressed the toxicity assessment of BVAE in atherosclerosis-induced rabbits. Toxicology can be assessed biochemically using liver function test and renal profile as these two organs functions are bio transforming of xenobiotics, endogenous compounds, removal and excretion of toxic metabolic waster from blood. Parameters measured were ALT, AST and ALP for LFT; and urea and creatinine for kidney function.

Serum ALP, AST and ALT levels were the most common and important liver function markers. Normally, liver enzyme will be very low in the blood circulation. Increasing levels of those markers are indicative of liver cell abnormality especially in hepatitis or injury (Hoekstra et al., 2013). In the present study, ALT and AST enzymes were within the normal range and did not significantly raised in the treated group. There were also no significant changes in the histological tissue examination and the value obtained is less than three times of normal range (Fitzgerald, 2000).

There was also no abnormality detected in the renal function assessment for all groups. Kidney function test is one of the simple tests that can be conducted to assess kidney function (Burtis & Ashwood, 1999). Urea is a biological complex in nitrogen-containing compounds metabolism. Meanwhile, creatinine is a by-product of muscle energy metabolism that had been filtered from the blood. High urea or creatinine level is an indication of kidney function problem (Levey et al., 2003). Urea and creatinine of all group showed no significant change except a slight elevation of creatinine level in control group. However, no pathological changes in histological seen in the glomerulus of kidney. This normal biochemical result of urea and creatinine was corresponding with normal histological finding of the glomerulus of kidney.

Based on the findings in this study, no histological change was observed although significant differences were found in BVAE on renal function test. This finding was supported by study on *B. vulgaris* that showed this plant had very low toxicity and side effects (Imenshahidi & Hosseinzadeh, 2016). The cytotoxicity of MTT assay measures the cell viability in percentage for the *B. vulgaris* treated cell and the untreated cell (negative control). Calculated from concentration-response curves, the cytotoxic activity was defined as a concentration in which cell growth was inhibited by 50 percent (IC_{50}).The study was done on cytotoxicity of *B. vulgaris*

on normal 3T3 cell lines for 24 and 48 hours, with IC_{50} of 13mg/ml (Saedi, 2015).

4. Conclusion

Histologically and biochemically of aqueous extract of *B. vulgaris* revealed no subacute toxicity in *in vivo* model . Hence, *B. vulgaris* can be consume as a medicinal agent at known dosages. Further experimental analysis of its chronic toxicity is crucial in order to confirm the safeness of this medicinal herb usage.

Acknowledgements

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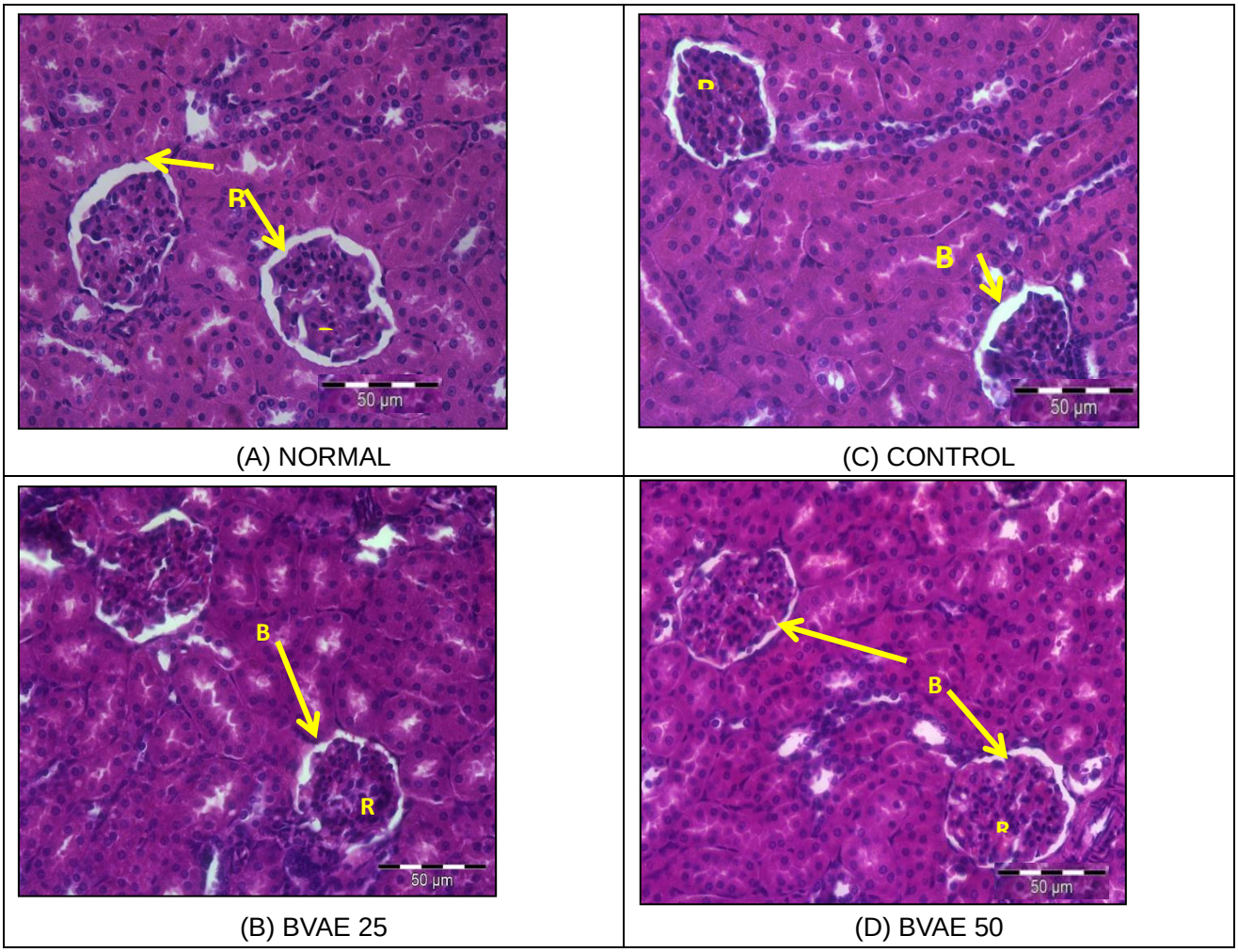
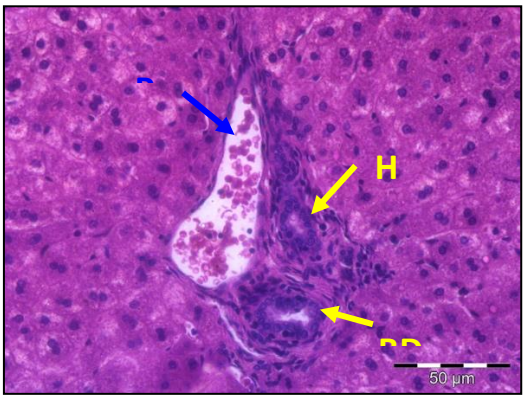
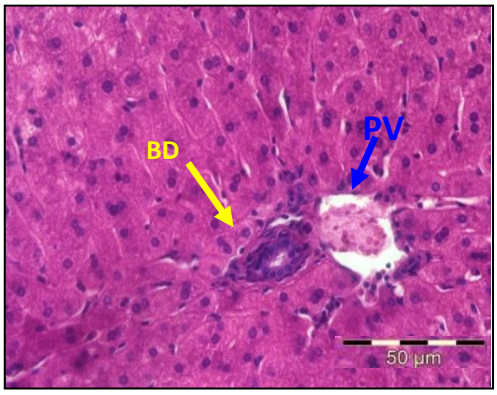


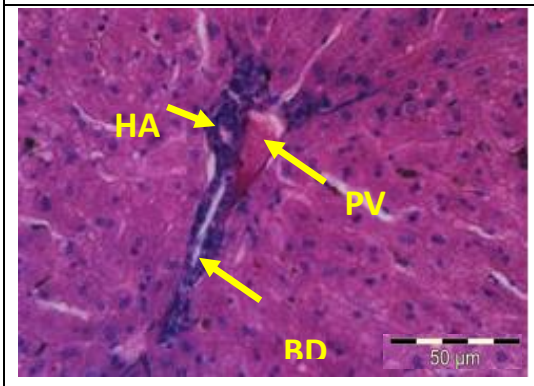
Figure 3: Photomicrographs of Renal Corpuscle in Kidney in Preventive Study**.** Renal corpuscle composed of capillaries, glomerulus, surrounded by Bowman's capsule. B: Bowman's capsule, R: renal corpuscles. (H&E X400)



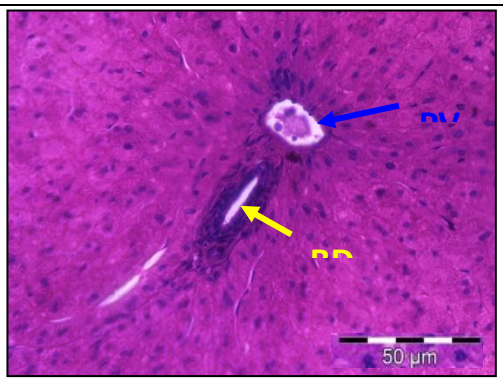
(A) NORMAL



(c) CONTROL



(B) BVAE25



(D) BVAE 50

Figure 4: Photomicrograph of Portal Triad in Liver in Preventive Study. BD: bile duct, PV: portal vein, HA: hepatic artery. (H&E X400)