



## Effects of *Hibiscus rosa sinensis* water extract on liver function of BALB/c mice

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### Introduction

Plants are one of important source of drugs. It have been used medicinally for thousands of years and some of the most common practices involve the use of crude plant extracts, which may contain a broad diversity of molecules with often unknown biological effects. For these reasons, traditional medicines have become the focus of numerous studies in order to evaluate their safety, efficacy and validate traditional uses. Malaysia has a great abundance of tropical plants which served as an important repository of medicinal plants for millennia. Today, many medical practitioners trained in pharmacology and pharmacognosy is well aware of the number of modern therapeutic agents that have been derived from the tropical species of Malaysia. Currently, there has been a strong trend in seeking plants as sources of novel pharmaceutical agents.

The *Hibiscus rosa-sinensis* L. (Malvaceae), commonly known as bunga raya by Malaysian, is a large shrub that can grow up to 15 feet in frost free climates. The flowers are glorious and can bloom up to 6 inches in diameter. It can also be of different colours, from scarlet to orange to yellow to white. The leaves are shiny dark green and large with toothed edges. Besides being used as a decorative purpose, this plant has many medicinal uses for healthy body system and skin. It is known to have hair growth promoting properties (Adhirajan *et al.*, 2003) and wound healing (Ali *et al.*, 2014). Even though the therapeutics effects have been proven, its toxicity is still a question mark. Therefore, the aim of this study was to investigate the effect of daily intake of *Hibiscus rosa-sinensis* leaves water extract on liver function of BALB/c mice.

### Methods

#### Preparation of extracts

The leaves of *Hibiscus rosa sinensis* were collected from areas around Universiti Teknologi Mara (UiTM), Puncak Alam, Selangor, Malaysia. 700g of fresh leaves of *Hibiscus rosa sinensis* were rinsed using running tap water to removed unnecessary dirt. The leaves were blended by using heavy duty blender. Blended leaves were immersed in water bath at 70oC for 3 hours. The water extract were filtered and dried by using freeze dryer at -80°C. The crude extract was stored in air tight container with silica gel.

#### Experimental design

24 mice were randomly divided into 4 groups (n=6). The treated groups were given through oral gavage 50mg/kg, 300mg/kg and 2000mg/kg of *Hibiscus rosa-sinensis* leaves water extract respectively for 28 days. For the control group, tap water was given. Body weight and food intake were recorded weekly. At the end of study, blood samples and liver tissues were collected for analysis. Experimental animals were treated in accordance with criteria outlined in UiTM Animal Care Guidelines, and the protocols were approved by the UiTM Animal Research and Ethics Committee (Reference No:195/2017).

#### Biochemical analysis

For biochemical analysis, at the end of the sub-acute study, after the mice were euthanized, their blood were taken by cardiac puncture and transferred into red vacuitaner. The blood was centrifuged for 10 to separate serum from the blood cells. The obtained blood serum was used for assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The assay was conducted by using Rat ALT and AST ELISA kit.

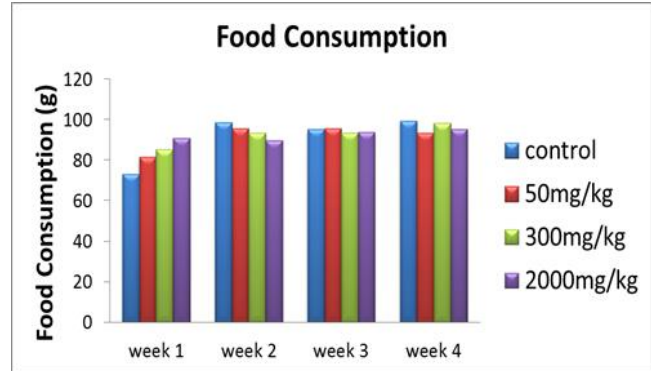
### Histological evaluation

The livers were preserved in neutral buffered formalin and were processed for paraffin embedding, following the standard microtechnique. Five micron sections of livers, stained with haemotoxylin and eosin (H&E) were observed under microscope for the histopathological changes.

## Results

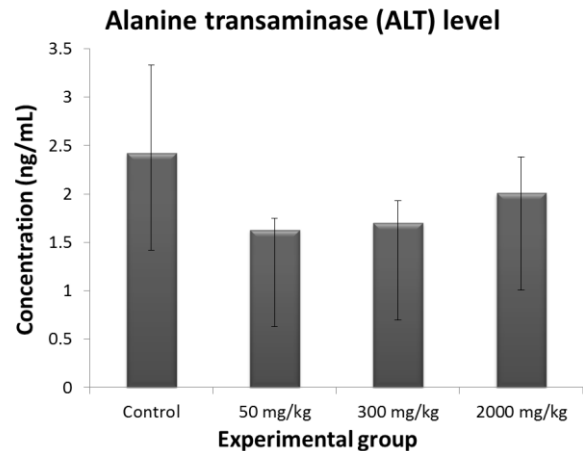
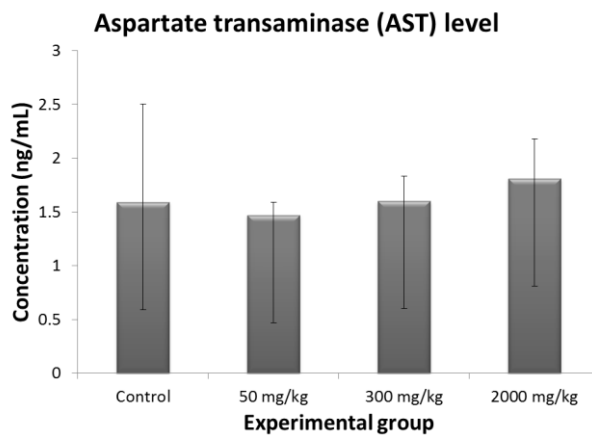
### Body weight and food intake

Groups	Initial BW (g)	Final BW (g)	Weight gain (g)
Control	24.33 ± 3.57	27.90 ± 3.70	3.28 ± 3.70
50 mg/kg	25.13 ± 3.57	27.32 ± 2.31	2.20 ± 2.31
300 mg/kg	26.43 ± 4.90	29.91 ± 5.83	3.48 ± 2.58
2000 mg/kg	25.92 ± 3.91	27.20 ± 4.81	1.28 ± 2.30



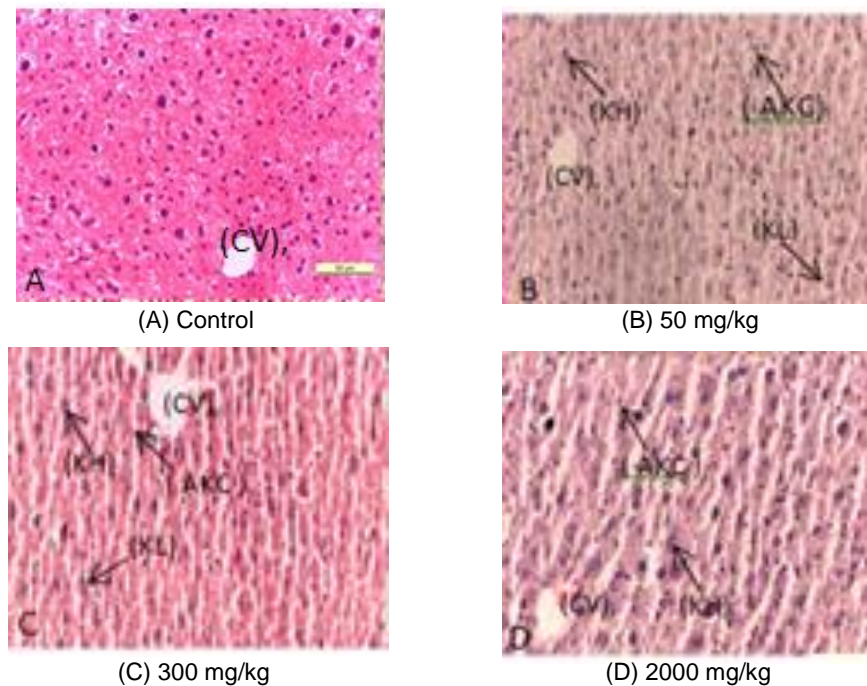
All groups gained weight after 28 days. No significant different ( $p > 0.05$ ) compared to the control group. The mice consume approximately the same amount of food throughout the 28 days. Statistical test showed no significant difference ( $p > 0.05$ ) in food consumption between groups.

### Serum aspartate transaminase (AST) and alanine transaminase (ALT) level



Concentration of AST and ALT in the serum after 28 days repeated doses. Results are presented as mean ± S.D. No significant different ( $p > 0.05$ ) compared to the control group were observed indicates no hepatotoxicity.

### Histology evaluation



Photomicrograph 1: Liver sections of BALB/c mice received repeated doses of *Hibiscus rosa-sinensis* leaves water extract in 28-day duration (H&E stained). CV: central vein; AKC: activated kupffer cells; KL: karyorrhesis; KH: karyorrhexis

The histopathological analysis showed that administration of *Hibiscus rosa-sinensis* water extract caused presence of cytoplasmic vacuolation with consistent karyorrhesis, and karyorrhexis of cell nuclei which indicate that irreversible liver damage after mild subacute liver injury. Presence of activated kupffer cell in group (B),(C) and (D) can indicated that there is an activation in innate immune response. However no change was recorded in all treated groups.

### Discussion

Body weight and weekly food intake were used as the parameter for evaluating health status of the animals toxicity study (Oecd/Ocde, 2008). In this experiment, no significant difference ( $P>0.05$ ) were observed in the weekly average body weight between the group. The average food intake was continuously increased from week 1 until week 4.

No significant difference on AST and ALT level suggests no observed adverse effect experienced by the mice. According to study conducted by Menegati et al.(2016), at highest dose which was 1000 mg/kg of aqueous extract of *Alibertia edulis* showed no significant different in AST and ALT level and they concluded that it indicates no liver damage in the experimental animals. Thus, in this current study, we suggest no liver damage occur after repeated dose of *Hibiscus rosa sinensis* water extract intake.

In this study, histopathological analysis revealed that daily administration of *Hibiscus rosa-sinensis* water extract for 28 days induced mild histopathological changes in the liver for group 2 (50mg/kg group), group 3(300mg/kg) and group 4(2000mg/kg) compared to control (Photomicrograph 1). Based on the result, there were significant different ( $P<0.05$ ) among group compared to control group. Cytoplasmic vacuolation of diffusely distributed hepatocyte is seen consistently with mild liver injury. This change may reflect cellular adaptation rather than a degenerative change. This showed that cytoplasmic vacuolation of the liver was due to excess accumulation of glycogen, predominantly monoparticulate form due to exposed highly dose of xenobiotics (Nayak et al., 1996).

The histological architecture of the liver in control group, showed the normal feature having hexagonal shaped cells (hepatocytes) which were arranged as a cord along with the sinusoids present in between the cords of the hexagonal hepatocytes and the central vein was at the center of the hepatic cord. Presence of activated kupffer cell in group (B), (C) and (D) can indicated that there is activation in innate immune

response. This is because kupffer cells thus play a major anti-inflammatory role by preventing the movement of these gut-derived immunoreactive substances from travelling past the hepatic sinusoid (Dixon et al. 2013). It can be protective in a number of situations, including drug-induced liver injury and toxin-induced fibrosis dysregulation in the precise control of inflammatory responses in kupffer cells can contribute to chronic inflammation in the liver (Dixon et al. 2013).

The present of karyorrhysis, and karyorrhexis of cell nuclei indicate that liver tissue damage that illustrate with degenerative changes of cell nuclei (Nassar et al. 2010). Karyolysis was characterized by a nuclear dissolution (karyolysis) that is associated to random DNA cleavage. This lysis of chromatin is due to the action of endonucleases in cellular apoptosis (Damjanov, 2009). When DNA from necrotic cells appear as a smear, which contrasts with the classical DNA ladder observed with apoptotic cells (Lecoeur et al. 2008). Karyorrhexis denotes the fragmentation of nuclear material, colloquially as formation of nuclear dust. However, the liver is capable of regenerating damaged tissue hence the liver function may not be impaired early following an insult from toxicant.

## Conclusion

In conclusion, although there were mild changes in the hepatocyte, the liver function remain unchanged as reflected by the level of ALT and AST.

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