



Original Article

Hepatoprotective Effect Of Hibiscus Sabdariffa L Extract On 7,12-dimethylbenz(α) anthracene (dmba) Induced Rat

Nurkhasanah^{a*}, Muhammad Ryan Radix Rahardhian^{a,b}

^aFaculty of Pharmacy, Ahmad Dahlan University, Jl Prof. Soepomo, Janturan, Yogyakarta, Indonesia

^bSchool of Pharmaceutical Sciences STIFAR, Jl Letnan Jendral Sarwo Edie Wibowo Km. 1, Semarang, Indonesia

ARTICLE INFO

Keywords:

Hibiscus sabdariffa
hepatoprotective
SGPT
SGOT
ALP

ABSTRACT

Rosella (*H.sabdariffa L*) extract has been reported to have antioxidants effect. Antioxidant has important role on detoxification and metabolism of xenobiotic. The objective of this study was to to investigate the effect of ethanolic extract from Roselle Calyx on activity of Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) and liver histopathologic profile of Sprague Dawley (SD) rats induced by DMBA. The study found that activity of SGPT, SGOT and ALP were increase after DMBA treatment. The rosella extract showed to reduce the activity of SGPT, SGOT and ALP indicating hepatoprotective effect. The histopathology of liver rat induced by DMBA after 33 days treatment of rosella extract showed repairing effect on dependent manner.

©Copyright 2010 BioMedSciDirect Publications IJBMR - ISSN: 0976:6685. All rights reserved.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants generated by incomplete combustion of fuels wood, and other organic matter. PAHs are present in cigarette smoke, grilled foods, automobile exhaust, and woodsmoke. DMBA is a prototypic PAH with carcinogenic, immunosuppressive effects in various species. DMBA has been reported to be hepatotoxic. The conversion of DMBA to its ultimate carcinogenic metabolites is mainly accomplished by the cytochrome P450 (CYP)1 family enzymes. In particular, CYP1A isoforms are responsible for its bioactivation in the liver, the major organ of DMBA metabolism, while CYP1B enzymes are reported to exert their activity predominantly in extra-hepatic tissues, such as the mammary gland.

Liver cells or hepatocytes contain various of enzymes, some of which are important for the diagnostic of liver damage. This enzyme also excreted and found in the blood vessels. The enzyme aminotransferase including Serum Glutamic Pyruvic Transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) are specific indicators to determine the damage of the liver cells. Alkaline phosphatase (ALP) is enzyme produced by hepatocyte and hydrolyze ester phosphate on alkaline condition.. Increasing levels of these enzymes reflect the existence of damage to the liver cells.

Rosella (*H. sabdariffa*) has been reported to have an antioxidant activity, This antioxidant properties expected to protect the liver damage caused by free radical (DMBA). Rosella also reported to have hepatoprotective effect against paracetamol

, carbon tetrachloride, streptozotocin. The objective of this study was to explore the hepatoprotective effect of rosella extract on DMBA induced rat.

2. MATERIAL AND METHODS

2.1 Collection of material

The rosella calyx were collected from Madiun (East Java, Indonesia). After it had been dried were pulverized and stored in a desiccator before extraction.

2.2 Extraction

2.2 Determination of Total Phenolic Content (TPC).

Determination of TPC was carried out by Folin Ciocalteu method with gallic acid as a standard. The amount of 50.0 mg of ethanol extract of rosella was diluted to a volume of 50.0 ml with a 50% ethanol. Then, 300 μ L solution were added with 1.5 ml of Folin-Ciocalteu reagent. After shaking the solution were then added by 1.2 ml of 7.5% Na₂CO₃ solution and allowed to stand in 10 minutes at room temperature. Absorbance was then measured by UV-Vis spektrofotometer at a 730 nm.

2.3 Determination of Total Flavonoid Content (TFC).

Determination of total flavonoids content was carried out using AlCl₃ as reported by Chang et al. (2002) using quercetin as a standard. The amount of 1 gram rosella extract was added to a volume of 100 ml of ethanol. The solution of 0.5 ml was added with 1.5 ml ethanol, 0.1 ml of 10% AlCl₃ reagent, 0.1 ml of Na-acetate, and 2.8 ml of distilled water. The mixture were stand at room temperature for 30 minutes. Furthermore the absorbance were determined using UV-Vis spectrophotometer at 430 nm.

2.4 Animal treatment

* Corresponding Author: Nurkhasanah

Faculty of Pharmacy, Ahmad Dahlan University

Jl Prof Soepomo,

Janturan, Yogyakarta, Indonesia

E-mail: nurkhas@gmail.com

HP: +62 85878322867

©Copyright 2010 BioMedSciDirect Publications. All rights reserved.

The experiment was performed on Sprague Dawley rats with 4 weeks age. The animal tests were adapted with the research environment for 1 week and fed ad libitum with commercially available feed and water. A total of 30 animals were equally divided in to five groups, each group consist of 6 animals. Group I was normal (base line), untreated, fed adlibitum with standard feed and water. Group II was induced with DMBA 15 mg/rat single dose through intragastric. Group III, IV and V are the treatment groups, induced with DMBA 15mg/rat and followed by administration of rosella extract with dose of, 10, 50 and 100 mg/kg BW respectively. The extract were administered for 33 days.

2.5 SGPT, SGOT and ALP assay

Determination of SGPT, SGOT and ALP activities were carried out by enzymatic reaction using the reagent kit Dyasis®.

2.6 Histopathological observation

Histopathological examination of organs was conducted to determine the possibility of damage or changes in the organ tissue structure due to administration of the rosella extract. The histopathological observation was carried out using Haematoxylin & Eosin staining. The extraction of dried rosella calyx were performed using maceration method with 70% ethanol and followed by evaporation to get the concentrated extract.

3. RESULTS AND DISCUSSION

3.1 Total Flavonoid and Total Phenolic content of *H sabdariffa* L extract

Anthocyanin one of the flavonoids group member is the major compound of *H sabdariffa* and various color fruit and vegetables. Flavonoids have been reported to have antioxidant effect. The antioxidant properties of flavonoids were consider to deactivate the reactive metabolite of DMBA.

The previous research reported the high level of flavonoids and phenolics compound on *H. sabdariffa* L extract. It has been recognized that flavonoids show antioxidant activity. The mechanisms of action of flavonoids are through scavenging or chelating process. Phenolic compounds are a class of antioxidant agents which act as free radical scavenger. The total phenolic content was expressed as gallic acid equivalent. It was found that total phenolic content of rosella extract was 6.03 ± 0.21 GAE/100g. The flavonoid contents of the extracts in terms of quercetin equivalent was 2.47 ± 0.11 %. Flavonoid is also reported to have a hepatoprotective activity.

Table I. Total phenolic and flavonoid content of *H sabdariffa* extract

Sample	Total phenolic	Total flavonoid
Rosella extract	6.03 ± 0.21 GAE/100g	2.47 ± 0.11 %.

3.2 SGPT, SGOT and ALP level

Enzymatic activation of PAHs leads to the generation of active oxygen species such as peroxides and superoxide anion radicals, which induce oxidative stress in the form of lipid peroxidation. Certain aldehyde such as malondialdehyde (MDA), the end product of lipid peroxidation (LPO) arising from the free radical

generation leading to the degradation of polyunsaturated fatty acids. Formation of reactive oxygen species (ROS) in hepatocytes is reported to undergo hepatotoxicity. The oxidative stress play an important role in liver injury. Many of the members of the PAH family are toxic, carcinogenic and immunotoxic. The DMBA was metabolized in hepar into a more toxic form and involved the activation of the cytochrome P450 enzyme into a reactive intermediate, epoxides dihydrodiol and radical cations. Epoxide dihydrodiol exocyclic will bind the amino group of purine DNA adducts covalently into a stable form, while the radical cation would bind purine N7 or C8 becomes unstable form adducts. Cytochrome P450 enzyme CYP1A1 or CYP1B1 and microsomal hydrolase enzyme change into DMBA-3,4-diol-1,2-epoxide (DMBA-DE) in the metabolism of DMBA. These enzymes were found in hepatic cells and extra hepatic such as lung, mammary gland, spleen, kidney, prostate, uterus, and heart. DMBA-DE elimination from the body is accelerated in the presence of the antioxidant enzyme glutathione-s-transferase (GST).

The result of rosella extract on SGPT, SGOT and ALP level on DMBA induced rat were shown on Table II.

Table II. The effect of rosella extract on SGPT, SGOT and ALP level on DMBA induced rat

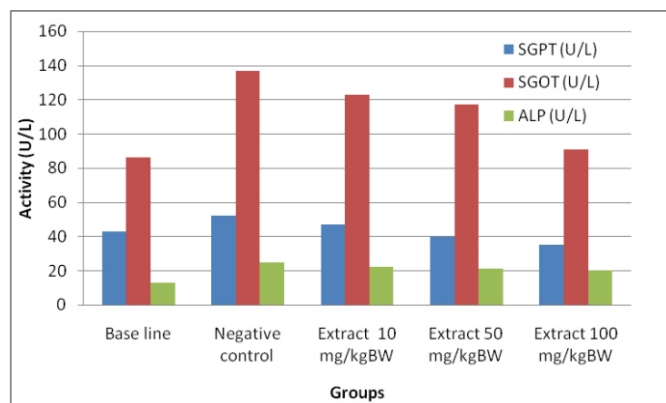
Group	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
Base line	43 ± 2.9	86 ± 10	13 ± 6.9
Negative control	52 ± 2.2	137 ± 15	25 ± 12
Extract 10 mg/kgBW	47 ± 5.8	123 ± 5	22 ± 7.1
Extract 50 mg/kgBW	40 ± 2.1	117 ± 6.7	21 ± 11
Extract 100 mg/kgBW	35 ± 4.4	91 ± 15	20 ± 3.4

The result showed that DMBA caused the hepatotoxicity as the SGPT, SGOT and ALP level after DMBA induced was increase. The SGPT activity on negative control was increase 21% after DMBA administration. Treatment with rosella extract 10, 50 and 100mg/kg BW were decrease the SGPT 10%, 23%, 33% respectively. The hepatoprotective effect of rosella extract was shown on dose dependent manner.

The SGOT level also was found to increase after DMBA administration. The SGOT activity of negative control was 59% more than base line group. Increasing of SGOT activity was more than SGPT due to SGOT enzyme was found in some tissues including heart, muscle, kidney and brain. The SGPT is more specific indicator to liver function than SGOT. Treatment with rosella extract was found to decrease the SGOT level get near to the base line group.

ALP is the prototype of the enzymes that reflects the pathological alteration in the biliary flow. DMBA induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The ALP level was found to increase after DMBA administration by 92%. The treatment of rosella extract for 33 days cannot decrease ALP activity significantly.

Figure 1. The activity of SGPT, SGOT and ALP after rosella extract treatment for 33 days on DMBA induced rat



The hepatic toxicity of DMBA was also reported . They reported that chronic DMBA treatment increase the SGPT, SGOT, and ALP level, and treatment with turmeric was decrease the SGPT, SGOT and ALP level by 22.5%, 21.4%, 19% respectively. Oral administration of ethanolic extract of rosella was reported to decrease of SGPT, SGOT, and ALP on CCl₄-induced liver damage mice . They also reported that rosella extract also increase the activity of endogenous antioxidants including, SOD, Catalase and GSH and conclude that hepatoprotective activities observed in the study could be due to the ability of phenolic compounds to neutralize the free radicals produced from the metabolism of CCl₄. The hepatoprotective of rosella extract on streptozotocin induced rat was related to the antioxidant activity and flavonoids content .

Some of Hibiscus family plant were also reported to have hepatoprotective activity. Hibiscus esculentus Linn ethanolic extract was reported to have a hepatoprotective activity by lowering ALP activity . Hibiscus hispidissimus ethanolic extract was reported to reduce ALP, bilirubin on CCl₄-induced rat. In the in vitro test this extract also has antioxidant activity by reducing DPPH .

3.3 Histopathology of hepar

Histopathological observation of hepar was shown on Figure 2. Histopathological observation was carried out by Haematoxilin & Eosin staining.

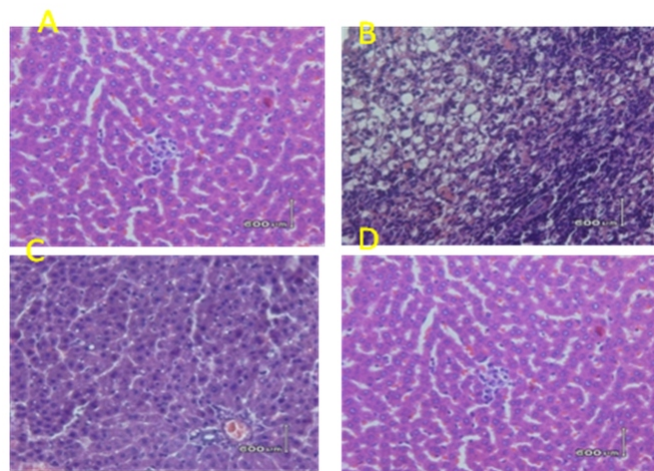


Figure 2. The histopathological observation (magnification 400x) of liver after rosella extract treatment on DMBA induced rat, A, DMBA (negative control) showed inflammation, characterized by infiltration of inflammatory cells (lymphocytes) in the liver, B, DMBA (negative control) group marked degradation of fat-vacuoles C. rosella extract group of 100 mg/kg BW, no specific pathological changes, D. rosella extract group of 50 mg/kg BW have foci necrotic

The result showed that DMBA (negative control) showed inflammation, characterized by infiltration of inflammatory cells (lymphocytes) in the liver (Fig 2A). The DMBA (negative control) group also marked degradation of fat-vacuoles characterized by vacuoles of various sizes with clear boundaries (Fig 2B). Treatment wit rosella extract was returned the damage of the cells, which there is no specific pathological changes after treatment with rosella extract of 100 mg/kg BW (Fig 2C). The antihepatotoxic effect of rosella extract showed to be dose dependent due to the treatment with lower dose, (50 mg/kg BW) cannot repair the liver damage completely (Fig 2D). The result showed foci necrotic, marked by cell death in the core area of the hepatocytes, lymphocytic infiltration and picnotic.

4. CONCLUSION

Rosella extract showed the hepatoprotective activity on DMBA induction by lowering the SGPT, SGOT and ALP level.

ACKNOWLEDGEMENTS

The authors thanks to the Indonesian Directorate of Higher Education for funding through the Postgraduate Scheme.

REFERENCES

- Burchiel SW, Hadley WM, Barton SL, Fincher RH, Lauer LD, Dean JH. Persistent suppression of humoral immunity produced by 7,12-dimethylbenz(a)anthracene (DMBA) in B6C3F1 mice: Correlation with changes in spleen cell surface markers detected by flow cytometry. Int J Immunopharmacol [Internet]. 1988 Jan [cited 2014 Oct 22];10(4):369-76. Available from : <http://www.sciencedirect.com/science/article/pii/0192056188901233>
- Gao J, Lauer FT, Dunaway S, Burchiel SW. Cytochrome P450 1B1 is required for 7,12-dimethylbenz(a)-anthracene (DMBA) induced spleen cell immunotoxicity. Toxicol Sci [Internet]. 2005 [cited 2014 Oct 22];86. Available from: <http://www.readcube.com/articles/15843505>
- Gao J, Mitchell LA, Lauer FT, Burchiel SW. p53 and ATM / ATR Regulate 7, 12-Dimethylbenz [a] anthracene- Induced Immunosuppression. Mol Pharmacol. 2008;73(1):137-46.
- Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci. 2004;95(1):1-6.
- Buters J, Quintanilla-martinez L, Schober W, Soballa VJ, Hintermair J, Wolff T, et al. CYP1B1 determines susceptibility to low doses of 7 , 12-dimethylbenz [a] anthracene- induced ovarian cancers in mice: correlation of CYP1B1-mediated DNA adducts with carcinogenicity. 2003;24(2):327-34.
- Singh H, Bedi PS, Singh B. Hepatoprotective Activity of Turmeric and Garlic against 7-12 , Dimethylbenzanthracene Induced Liver Damage in Wistar Albino Rats. European J Med Plants. 2011;1(4):162-70.
- Ali DA, Ismail MF, Badr HA. Hepatoprotective effect of ginger extract against the toxicity of 7,12-dimethylbenz (a) anthracene (DMBA) in albino rats. World J Pharm Sci. 2013;1(3):61-71.
- Koul A, Mohan V BS. Azadirachta indica mitigates DMBA-induced hepatotoxicity: a biochemical and radiometric study. Indian J Biochem Biophys. 2014;51(1):37-45.

9. El-gerbed MSA. Research Journal of Pharmaceutical , Biological and Chemical. Res J Pharm Biol Chem Sci. 2013;4(3):1534–48.
10. Singh A, Bhat TK, Sharma OP. Clinical Biochemistry of Hepatotoxicity. Clin Toxicol. 2011;S4:001:1–19.
11. Anokwuru PC, Ijeoma E, Ajibaye O, Adesuyi OA. Polyphenolic content and antioxidant activity of hibiscus sabdariffa calyx. Res J Med Plant. 2011;5(5):557–66.
12. Sujono TA, Widiatmoko YW. Influence Dried Flower of Hibiscus sabdariffa Linn Infusion on Serum Glutamate Pyruvate Transaminase (SGPT) Level against Paracetamol Induced Liver Injury in Rats. Int Conf Res Appl Tradit Complement Altern Med Heal Care. 2012;22–3.
13. Adetutu A, Owoade AO. Hepatoprotective and Antioxidant Effect of Hibiscus Polyphenol Rich Extract (HPE) Against Carbon Tetrachloride (CCL 4) – Induced Damage in Rats. Br J Med Med Res. 2013;3(4):1574–86.
14. Adeyemi DO, Ukwenya VO, Obuotor EM, Adewole SO. Anti-hepatotoxic activities of Hibiscus sabdariffa L . in animal model of streptozotocin diabetes-induced liver damage. BMC Complement Altern Med. 2014;14(277):1–11.
15. Einbond LS, Reynertson K a., Luo X-D, Basile MJ, Kennelly EJ. Anthocyanin antioxidants from edible fruits. Food Chem [Internet]. 2004 Jan [cited 2014 Oct 22] ; 8 4 (1) : 2 3 – 8 . Available from : <http://linkinghub.elsevier.com/retrieve/pii/S0308814603001626>
16. Miguel MG. Anthocyanins : Antioxidant and / or anti-inflammatory activities. J Appl Pharm Sci. 2011;01(06):7–15.
17. Guerrero CJ, Luigi CP, Castilla CA, Medel SF, Schalchli H, Hormazabal U E, et al. Antioxidant capacity , anthocyanins , and total phenols of wild and cultivated berries in Chile. Chil J Agric Res. 2010;70(December):537–44.
18. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids : a review of probable mechanisms of action and. Am J Clin Nutr. 2001;74:418–25.
19. Al-jumaily EF, Abdulla RSAJM. Hepatoprotective Activity of Flavonoids Purified and Ethanolic Extract from Iraqi Propolis Against Carbon tetrachloride- Induced Liver Damage In Male Mice. IOSR J Pharm. 2014;4(3):22–7.
20. Galván N, Page TJ, Czuprynski CJ, Jefcoate CR. Benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene differentially affect bone marrow cells of the lymphoid and myeloid lineages. Toxicol Appl Pharmacol [Internet]. 2006 Jul 1 [cited 2014 Oct 31];213(2):105–16. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16307768>
21. Gao J, Lauer FT, Dunaway S, Burchiel SW. Cytochrome P450 1B1 is required for 7,12-dimethylbenz(a)-anthracene (DMBA) induced spleen cell immunotoxicity. Toxicol Sci [Internet]. 2005 Jul [cited 2014 Oct 22] ; 8 6 (1) : 6 8 – 7 4 . Available from : <http://www.ncbi.nlm.nih.gov/pubmed/15843505>
22. Hodgson E. A Textbook of modern toxicology. third. Hodgson Ernest, editor: North Carolina; 2004.
23. Bedi P, Priyanka S. Effect of garlic against 7,12, dimethylbenz anthracene induced toxicity in wistar albino rats. Acad Sci. 2012;5(3):4–7.
24. Dahiru D, Obi OJ, Umaru H. Effect of Hibiscus sabdariffa calyx extract on carbon tetrachloride induced liver damage. Biokemistri. 2003;15(June):27–33.
25. Sunilson J Anbu Jeba, P Jayaraj, Mohan M Syam, Kumari A Anita Gnana RV. Antioxidant and hepatoprotective effect of the roots of Hibiscus esculentus Linn. Int J Green Pharm. 2008;2(4):200–3.
26. Krishnakumar NM, Latha PG, Suja SR, Shine VJ, Shyamal S, Anuja GI, et al. Hepatoprotective effect of Hibiscus hispidissimus Griffith , ethanolic extract in paracetamol and CCl 4 induced hepatotoxicity in Wistar rats. Indian J Exp Biol. 2008;46(September):653–9.