International Journal of Cancer Research 1 (2-3): 109-112, 2005
ISSN 1811-9727
© 2005 Asian Network for Scientific Information

Reducing Effect of *Strobilanthes crispus* Leaf Extract in Hepatocarcinogenesis Rats

O. Fauziah, P. Hanachi, S. Yogespiriya and R. Asmah
Department of Biomedical Science, Faculty of Medicine and Health Sciences
Universiti Putra Malaysia, Serdang, Malaysia

Abstract: The effect of *Strobilanthes crispus* extract and glycyrrhizin in on rats induced with carcinoma is reported. The results showed significant, increase of liver microsome glutathione s-transferase (GST) in rats after 12 weeks. Treatment with glycyrrhizin caused decrease in liver GST activity compared to control. Treatment with *Strobilanthes crispus* extract caused overall decrease in liver GST activity almost close to control groups. The microscopic observation of the lesion score during hepatocarcinogenesis revealed that cells of cancer group without treatment were severely necrotic at week 12. However, cells of cancer group with *S. crispus* treatment appeared normal at the same period.

Key words: *Strobilanthes crispus,* hepatocarcinogenesis, lesion score, glutathione s-transferase

INTRODUCTION

There has been considerable interest developed in studies on hepatocellular carcinoma (HCC) in developing countries, because it accounts for 15% of total cancer mortality[1]. Accumulating epidemiological and experimental evidence revealed the influence of a number of natural and synthetic compounds on drug detoxification and HCC incidence[1]. Hepatocarcinogenesis induced by diethylnitrosamine (DEN) and acetylaminofluorene (AAF) is a common model in rats to study the mechanism of chemical carcinogenesis and the use of HCC in anticancer drug therapy.

Since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, there is a worldwide trend to go back to natural resources (medicinal plants) which are therapeutically effective, culturally acceptable and economically within the reach the poor people.

Over the centuries no fewer than 3000 plant species have been used to treat cancer[2]. Many new plants were studied to identify natural cancer chemotherapeutic agents[3]. *Strobilanthes crispus* (L) Bremek or *Saricocalyx crispus* (L) Bremek (Acanthaceae) is native of countries from Madagascar to Indonesia which is commonly known as picah beling in Jakarta or kejibeling in Java[4]. *Strobilanthes crispus* plant was recorded by Thomas Anderson (1832-1870)[5]. The plant is an erect shrub which is 0.5-1 m high. The young parts of this plant are hairy and the leaves are longish, roughly serrated. The leaves are covered with short hairs on both surfaces. This plant can be found in shaded terrain, in Indonesia, in coconut tree gardens, at roadsides and in the woods[6]. The infusion of the dried leaves of this plant species was used as antidiabetic, diuretic, antilitic and laxative[4].

Liver is the principal site of many metabolizing activities, biochemical patterns and the biotransformation enzymes are analysed. Diversity of biochemical pathway and mechanism of cancer development are understood. The anticancer potency of *S. crispus* (SC) extract is reported in this study using certain experimental methods and models.

MATERIALS AND METHODS

Chemicals: Diethylnitrosamine, Acetylaminofluorene, Aniline and all other reagents used were of highest grade commercially obtained (Sigma Chemical Co., St. Louis, Mo, USA

Animals: Thirty male 200-250 g (6-8 weeks) Sprague-Dawley rats (*Rattus norwegicus*) were purchased from the animal colony unit, UPM. These were maintained in animal house of Faculty of Medicine and Health Sciences for 12 weeks and acclimatized for at least a week before use. They were kept in separate cages in a ventilated room with equal periods of day light and darkness with temperature (32±2°C). Rat chow (Ridley Rat Chow, Australia) and water *ad libitum* were given to these rats daily. Each cage was cleaned every week and bedded with wood chip for urine absorption.

Corresponding Author: Dr. Parichehr Hanachi, Department of Biomedical Sciences, Faculty of Medicine and Health Science, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia Tel: 0060389568440 Fax: 0060389436178 E-mail: paricheh@medic.upm.edu.my, parichehr78@yahoo.com

Preparation of *S. crispus* extract for rat bioassay: The leaves of *Strobilanthes crispus* were collected from the Herbs Garden at animal house of Faculty of Medicine and Health Sciences, University Putra Malaysia. Crude extract of SC was prepared using the green tea extraction method[7]. In this experiment, 5.0% w/v of leaf extract was used.

Animals treatment: Protocol for the rat hepatocellular carcinoma induction was basically according to Soft and Farber[8] method. The method was modified, as the rats were not subjected to the partial hepatectomy (selective pressure) stage. Rats were divided to 6 groups, each group with 5 rats. Rats in Group 1, 2 and 3 were injected with 200 mg/kg/body wt. of diethylnitrosamine (DEN) intraperitoneally as an initiator to hepatocarcinogenesis and after 2 weeks, the rat chow which was mixed with acetylaminofluorene (AAF) were given to these rats as promoter of hepatocarcinogenesis. But rats in Group 4, 5 and 6 were not induced to develop liver cancer. Rats in group 1 and 4 were given *ad libitum* distilled water as positive and negative control. However, rats in group 2 and 5 were given *ad libitum* 50 mL of 5% (w/v) *Strobilanthes crispus* extract daily. And also 50 mL of 0.005% (w/v) of glycyrrhizin were given to rats in group 3 and 6 daily.

Preparation of cytosol: The cytosolic preparation was carried out essentially following the method of Hashem *et al.*[9].

Lesion scoring was done according to the modified method: The toluidine blue stained sections were used for lesion scoring by using digital light microscope Leica DMRA II equipped with Qwin and Qfluoro software under power x100 and x200. The severity was based on inflammation and necrosis grade using standard criteria[10].

Enzyme assays: Glutathione S-Transferase (GST) assay was measured using the method of Habig *at al.*[11] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate and in the presence of 1 mM glutathione. Protein determination was carried out according to the method of Bradford[12].

Statistical analysis: Lesion scoring data was analyzed by using Mann-Whitney test to compare between group. However, the enzyme assay data was compared between groups by using 1-way ANOVA. The level of significance was 0.05 or differences with $p<0.05$ were considered to be significant.

RESULTS

Lesion scoring analysis: In untreated cancer induced group, the grade of inflammation or necrosis was 2.3 and

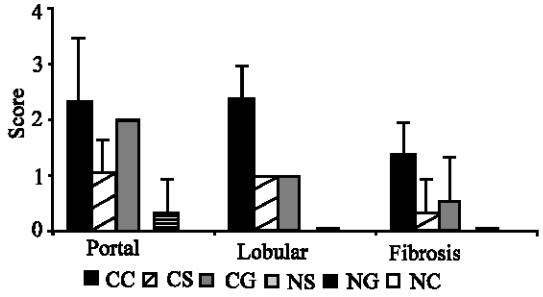

Fig.1: Lesion scoring of control and SC treated rat liver. (CC: cancer control, CS: cancer with (SC) treatment, CG: cancer with glycyrrhizin treatment, NS: normal with (SC) treatment, NG: normal with glycyrrhizin treatment and NC: normal without treatment)

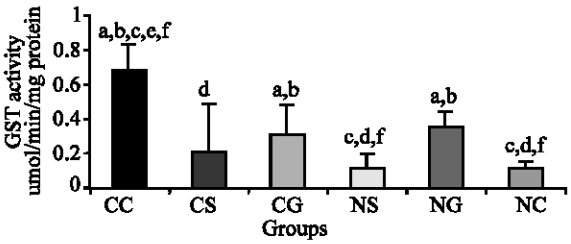

Fig. 2: Detoxification activity of enzyme glutathione s-transferase in experimental and control groups. (CC: cancer control, CS: cancer with *Strobilanthes crispus* treatment, CG: cancer with glycyrrhizin treatment, NS: normal with *Strobilanthes crispus* treatment, NG: normal with glycyrrhizin treatment and NC : normal control).

a: Significant ($p<0.05$) compared to normal
b: Significant ($p<0.05$) compared to normal *Strobilanthes crispus*
c: Significant ($p<0.05$) compared to normal glycyrrhizin
d: Significant ($p<0.05$) compared to cancer
e: Significant ($p<0.05$) compared to cancer-*Strobilanthes crispus*
f: Significant ($p<0.05$) compared to cancer-glycyrrhizin

higher compared to other groups. However, the score is not significantly different when compared with cancer induced rats treated with SC and glycyrrhizin group and normal rat treated with glycyrrhizin group. Hepatocyte with necrosis were seen at portal area in the former group. However, the grade of cancer with *Strobilanthes treatment* group is 1.0 and this group did not showed any significant difference compared to normal rats and normal rats with SC and glycyrrhizin treatment groups. The portal

of this group was inflamed but necrotic cells were not found (Fig. 1).

In rats induced hepatocarcinogenesis, the score of inflammation or necrosis of liver lobular was found to be at 2.3 and found to be at significant changes in hepatic lesion when compared with normal groups and cancer with SC treatment group. Moderate and severe focal necrotic cells were seen in this group. However, cancer with (SC) group differs significantly when compared to normal, normal with *Strobilanthes* and cancer group. In this group, inflammatory cells without necrosis were seen and in some area normal cells without inflammation were seen.

Cancer group showed the highest stage of fibrosis and showed significant different between cancer with *Strobilanthes* group and normal (SC) group. In this group, fibrosis at portal area was seen.

Glutathione s-transferase activity: Oral administration of *Strobilanthes crispus* 5.0% w/v extract was effective in reducing glutathione s-transferase activity near to normal level. The results showed decrease ($p<0.05$) of GST activity in liver cytosol of DEN/AAF induced and treated with *Strobilanthes crispus* group after 12 (Fig. 2).

DISCUSSION

The effect of *S. crispus* extract in DEN/AAF induced hepatocellular carcinoma, was studied in male Sprague Dawley rats. Histological evaluation of rat liver revealed DEN/AAF induced and untreated rats group showed higher score of inflammation or necrosis at portal, lobular and stages of fibrosis compared to all the other groups. 5% (w/v) SC extract administration successfully reduced the score of inflammation or necrosis at portal, lobular and stages of fibrosis.

Glycyrrhizin also found to be reduced the histopathological changes during hepatocarcinogenesis in rats but not effective as SC treatment. Five percent (w/v) of SC did not cause any side effect towards normal cells. SC did not fully recovered the histopathological changes during hepatocarcinogenesis. This could be due to short experimental duration. In this study, SC might act as antioxidant agent which can inhibit or slow down histopathological changes which induced DEN/AAF.

Glutathione s-transferase is a detoxification enzyme which enhanced the metabolic conjugation and played a role in the protective mechanism. This might be of interest in view of the importance of the liver in detoxification process[13]. In addition, GST activity was also used as a tumor marker enzyme in the study of hepatocarcinogenesis in mice[14].

The *S. crispus* extract reduced the enzyme activity to near normal levels after 12 weeks. Glutathione conjugation catalyzed by glutathione s-transferase is an important phase II in biotransformation reactions of reduced glutathione with a variety to endo and exogenous electrophiles. The high intracellular levels of glutathione, acted as a noncritical nucleophile scavanger to inactivate the toxins, protect cells against the very reactive electrophilic metabolites of carcinogens[15]. From this study, it can be concluded that *S.crispus* extract may contains phase II enzyme repressors and it may also contain compounds capable of repressing GST activities.

ACKNOWLEDGEMENTS

The authors acknowledge the help given by Universiti Putra Malaysia and IRPA for supporting this study.

REFERENCES

1. Premalatha, B. and P. Sachdanandam, 2000. Potency of *Semecarpus anacarddium* nut milk extract against aflatoxin B1-induced hepatocarcinogenesis: Reflection on microsomal biotransformation enzymes. Pharmacol. Res., 42: 161-166.
2. Lewis, W.H. and M.P.F. Elvin-Lewis, 1997. Plants Affecting Man's Health. 105-148. In: Medical Botany. Wiley, A. (Ed.) Interscience Publication, New York.
3. Rates, S.M.K., 2001. Plants as source of drugs. Toxicon, 39: 603-613.
4. Sunarto, P.A., 1977. In Materia Medika Indonesia. Penerbit Ditektoral Pengawasan Obat dan Makanan, Jakarta, pp: 95-99.
5. Brummit, R.K and C.E. Powell, 1992. In Authors of Plant Names. Royal Botanic Garden Kew, Britain.
6. Heyne, K., 1987. In Tumbuhan Berguna Indonesia III. 1754. Badan Litbang Kehutanan Yayasan Sarana Wana Jaya, Jakarta.
7. Conney, A.H., Z.Y. Wang, M.T. Huang, C.T. Ho and C.S. Yang, 1992. Inhibitory Effect of Oral Administration of Green Tea on Tumourigenesis by Ultraviolet Light, 12-0-tetradecanoylphorbol-13-acetate and N-nitrosodiethylamine in Mice. 362. In: Cancer Chemoprevention. Lee, W., L. Martin, B.W. Charles and K. Gary, (Eds.) CRC Press Inc., New York.
8. Solt, D. and E. Farber, 1976. New principle for the analysis of chemical carcinogenesis. Nature, 263: 701-703.
9. Hesham, M.S., H.M. Mostafa, P.J. O'Connor, J.A. Rafferty and J.D. Michael, 2002. Evidence for the presence of active cytochrome P450 systems in *Schistosoma mansoni* and *Schistosoma haematobium* adult worms. FEBS Lett., 519: 205-209.

10. Stevens, A., J.S. Lowe and B. Young, 2002. Staging and Grading of Chronic Hepatitis. In: Wheater's Basic Histopathology, Churchill Livingstone, Toronto, pp: 157.
11. Habig, W.H., M.J. Pabst and W.B. Jacoby, 1974. Glutathione s-transferases, The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
12. Bradford, M.M.A., 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 50: 249-253.
13. Hanachi, P., N.A. Shamaan, J. Ramli, J.H. Arshad and M.A. Syed, 2003. The effect of Benzo(a)pyrene on glutathione s-transferase, glutathione peroxidase in the liver and kidney mouse *Mus musculus*. Pak. J. Med. Sci., 19: 197-202.
14. Sato, K., 1989. Glutathione s-transferases as markers of preneoplasia and neoplasia. Adv. Cancer. Res. 52: 205.
15. Talalay, P., 1992. Chemical Protection Against Cancer by Induction of Electrophile Detoxication (Phase II) Enzymes. In: Cellular Target for Chemoprotection. V.E. Steele, G.D. Stoner, C.W. Boone and G.J. Kelloff (Eds.): CRC Press, Boca Raton, Ann Arbor, London, Tokyo, pp: 193-205.