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ORIGINAL ARTICLE

COMPARISON BETWEEN THE EFFECT OF SOYBEAN AND GOAT'S MILK ON TUMOR-MARKER ENZYME ACTIVITIES DURING HEPATOCARCINOGENESIS IN RATS

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Milk is a physiological fluid which has high nutritional value and soybean has strong antioxidant characteristics which is believed to inhibit carcinogenesis. The objective of this study was to investigate the effects of administration of soybean and goat's milk on hepatocarcinogenesis in rats (fed with diethylnitrosamine; DEN and acetylaminofluorene; AAF) by determining the activities of plasma gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP). Thirty-six rats from the species Sprague-Dawley were divided into 6 groups : control, DEN/AAF, soybean, DEN/AAF with soybean treatment, goat's milk and DEN/AAF with goat's milk treatment. Soybean and goat's milk administrations were given 5 ml/day. The rats were sacrificed after 8 weeks and the blood was collected. Treatment with DEN/AAF caused an increase in ALP and GGT levels and a decrease in weight significantly ($p<0.05$). ALP and GGT activities decreased significantly after administration of soybean and goat's milk ($p<0.05$). Administration of goat's milk and soybean alone did not cause any changes in the enzyme activities. Comparison between the effect of soybean and goat's milk in reducing the enzyme activities (ALP and GGT) did not give significant values ($p>0.05$). However, a decrease in weight was observed in the rats given soybean as well as goat's milk. The results obtained suggested that soybean and goat's milk may work as anti cancer agents in hepatocarcinogenesis although further studies are required to further elucidate this aspect.

Key words : *soybean, goat's milk and hepatocarcinogenesis*

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Introduction

Milk is a physiological fluid which has high nutritional value as it is naturally rich in energy, proteins, vitamins and minerals. The whey protein component in human milk has been reported to pose antitumor activity(1).One study(2)showed that milk fat contains a number of potential anticarcinogenic components including conjugated linoleic acid, sphingomyelin, butyric acid and ether lipids. Conjugated linoleic acid inhibited proliferation of human malignant melanoma, colorectal, breast and lung cancer cell lines. In animals, it reduced the incidence of chemically induced mouse epidermal tumors, stomach neoplasia in mouse and aberrant crypt foci in the rat colon(1).

Soybean possess several naturally occuring phenolic and flavonoids that have strong antioxidant characteristics and are believed to inhibit carcinogenesis. Much attention has focused upon genistein and daidzein, the predominant isoflavones found in soy(3). Epidemiologic, *in vitro* and laboratory animal studies provide evidence for the hypothesis that phytochemicals in soy products have

anticarcinogenic properties(4,5). In addition, protease inhibitors, the Bowman-Birk inhibitor, inositol hexaphosphate (phytic acid), lignans, phytosterols and saponins found in soy products may also have bioactivities relevant to the inhibition of carcinogenesis(4-7).

γ-glutamyl transpeptidase (GGT, E.C 2.3.2.2) and alkaline phosphatase (ALP, E.C 3.1.3.1) are amongst marker enzymes that have been monitored during carcinogenesis (8,9). GGT is a marker enzyme for liver cancer. ALP, a marker enzyme in the liver function test has been reported to be useful as a marker of neoplastic transformation and in hepatocarcinogenesis(10). The objective of this study was to compare the effects of administration of soybean and goat's milk on ALP and GGT activities in hepatocarcinogenesis induced rats using diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF).

Materials and Methods

Chemicals

Diethylnitrosamine (DEN) (Sigma Chemical Co, USA), 2-acetylaminofluorene (AAF) (Sigma Chemical Co, USA), γ-glutamyl carboxynitroanilide (Sigma Chemical Co, USA), p-nitrophenyl phosphate disodium (Sigma Chemical Co, USA) and all other chemicals and other reagents used were of the highest grade commercially available. A basal diet of rat chow was purchased from Gold Coin Co. Ltd. (Malaysia).

Animal Treatment

Male Sprague-Dawley rats (Animal House, Universiti Putra Malaysia), 7-8 week old, weighing 120-150 g, were used. The rats were housed individually in a well-ventilated room (30^0∞C), maintained on normal or treated rat chow and given water *ad libitum*. The rats were divided into six groups of 5-8 rats each. The control group (group 1) was fed a basal diet for the whole duration of the experiment. The soybean and goat's milk control groups (groups 3 and 5) were fed a basal diet and supplemented either soybean or goat's milk 5 ml/day for every rat. Groups 2, 4, and 6 were induced with hepatocarcinogen; the rats in group 2 were fed a basal diet, the rats in group 4 and 6 were supplemented either soybean or goat's milk at the doses stated previously.

Induction of Hepatocarcinogenesis

Chemically induced hepatocarcinogenesis was carried out using the method of Solt and Farber(11). The rats were injected intraperitoneally with a single dose of DEN at 200 mg/kg body weight. After a 2-week recovery period, the rats were fed a diet (w/w) containing 0.02% (w/w) AAF for 2 weeks. Soybean and goat's milk supplementation

Table 1. Effects of soybean and goat's milk on the activity of Gamma-glutamyl Transpeptidase and Alkaline Phosphatase in plasma induced cancer rats.

Groups	Plasma GGT (IU/L)	Plasma ALP (IU/L)
1. Control	6.19 ± 0.50	343.80 ± 21.98
2. DEN/AAF	10.15 ± 0.66[a]	490.85 ± 36.50[a]
3. Soybean	7.31 ± 0.65[b]	338.32 ± 17.64[b]
4. DEN/AAF + soybean treatment	8.05 ± 0.45[b]	312.68 ± 28.57[b]
5. Goat's milk	6.95 ± 0.37[b]	292.74 ± 29.97[b]
6. DEN/AAF + goat's milk treatment	8.47 ± 0.26[b]	333.29 ± 37.54[b]

Values are means ± SE

a : $p<0.05$ vs control group

b : $p<0.05$ vs induced cancer groups

in the hepatocarcinogen-treated rats was started at the beginning of the DEN administration. The supplementation was continued for a total of 8 weeks after which the rats were killed.

Determination of Marker Enzyme Activities

Blood was collected from the cardiac puncture. Plasma GGT and ALP activities were determined according to the methods described by Jacobs(12) (with some modifications) and Jahan and Butterworth(13), respectively. Both plasma GGT and ALP activities were expressed as IU/L.

Statistical Analysis

The results obtained were analysed using analysis of variance and student's t test. A value of $p<0.05$ was considered as significant.

Results

The results from Table 1 shows that treatment with DEN/AAF caused an increase in ALP and GGT levels and a decrease in weight significantly ($p<0.05$) (Table 2). ALP and GGT activities decreased significantly in DEN/AAF treated rats after administration of soybean and goat's milk ($p<0.05$). Administration of goat's milk and soybean alone did not cause much changes in the enzyme activities when compared to control values. There were no significant differences of ALP and GGT activities ($p>0.05$) among the two treatments. However, a decrease in weight was also observed in the rats given soybean as well as goat's milk (Table 2).

Discussion

The optimum approach to conquering cancer is prevention. Although human diet contains components which promote cancer, it also contains components which may prevent it (2). Observations from animal studies have shown that milk fat reduced the incidence of chemically induced tumors(14). So far, the determination of enzymes in animals supplemented with soy milk or goat's milk with experimentally induced hepatocarcinogenesis has not been reported. It is implied from the results obtained in the present work that soybean and goat's milk reduced the severity of carcinogenesis, as reflected in the reduction of the plasma tumor marker enzymes activities i.e GGT and ALP(8-10). The body weights were affected by the different treatments. A decrease in weight was also observed in rats given soybean as well as goat's milk alone. This may suggest that soybean and goat's milk can be used to decrease or to maintain body weight.

The dairy cow or goat has the ability to extract potential anticarcinogenic agents such as beta-carotene, beta ionone and gossypol from its feed and transfer them to milk(2). In animal studies comparing the tumorigenic potential of milk fat or butter with linoleic acid rich vegetable oils or margarines showed less tumor development with dairy products(2).

Soy protein isolate has been shown to inhibit the growth of human prostate tumors transplanted in mice(14). The *in vivo* inhibition of cancer

Table 2. Effect of different treatment on body weight gained

Groups	Weight Gained (g)
1. Control	218.33 ± 2.19
2. DEN/AAF	170.00 ± 9.36[a]
3. Soybean	171.25 ± 9.04[a]
4. DEN/AAF + soybean treatment	177.67 ± 3.51[a]
5. Goat's milk	160.80 ± 6.87[a]
6. DEN/AAF + goat's milk treatment	177.67 ± 7.47[a]

Values are means ± SE

a : $p<0.05$ vs control group

incidence or progression of cancer by soy products or pure isoflavone has been reported for gastric cancer(15) leukemia(16), breast cancer(17) and others(18). In contrast, some studies have not found *in vivo* inhibitory effects of soy on tumorigenesis. Mc Intosh et al(19)and Rao(20) reported in their studies that soy-based dietary treatments had tumor-promoting effects. Here we report the effect of the soybean supplementation in chemically induced hepatocarcinogenesis. Comparison between the effect of soybean and goat's milk gives not much difference. The differences of the activities of ALP and GGT in both treatments were not significant. It is interesting to note that the soybean exerted an effect similar to goat's milk.

The mechanism of action proposed for the protective effect of soybean and goat's milk during carcinogenesis is similar for vitamin E or C and other antioxidants. The potential mechanisms include scavenging of free radicals produced in hepatocarcinogenesis. Antioxidants are well known to delay or inhibit oxidation and lipid peroxidation.

Conclusion

In conclusion, soybean and goat's milk supplementation caused a reduction in the severity of carcinogenesis, indicating a protective effect of soybean and goat's milk in hepatocarcinogenesis induced rat. The results obtained suggested that soybean and goat's milk worked effectively as anti cancer agents in hepatocarcinogenesis although further studies are required.

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References

1. Hakansson, A., Boris, Z., Sten, O. and Hemant, S. Apoptosis induced by a human milk protein. *Proceeding National Academic Sciences USA.* 1995; **92**: 8064-68.
2. Parodi, P.W. Cow's milk fat components as potential anticarcinogenis agents. *The Journal of Nutrition*.1997; **127** (6): 1055-60.
3. Wang, H. and Murphy, P.A. Isoflavone composition of American and Japanese soybean in Iowa : effects of variety, crop year, and location. *J. Agric. Food Chem.* 1999; **42** : 1647-77.
4. Kennedy, A.R. The evidence for soybean products as cancer preventive agents. *J. Nutr.* 1995; **125** : 733S - 743S.
5. Messina, M.J., Persky, V., Setchell, K.D. and Barnes, S. Soy intake and cancer risk : a review of the *in vitro* and *in vivo* data. *Nutr. Cancer*.1994; **21** : 113-31.
6. Rao, A.V. and Sung, M.K. Saponin as anticarcinogens. *J. Nutr.* 1995; **125**: 717S - 724 S.
7. Shamsuddin, A.M. Inositol phosphatase have novel anticancer function. *J.Nutr.* 1995; **125** : 725S-732S.
8. Hendrich, S. and Pitot, H.C. Enzymes of glutathione metabolism as biochemical markers during hepatocarcinogenesis. *Cancer and Metastasis Reviews.* 1987; **6** : 155-78.
9. Moss, D.W. Multiple forms of acid and alkaline phosphatase: genetics, expression and tissue specific modification. *Clin Chim Acta*. 1986; **161**: 123-35.
10. Asmah Rahmat, Wan Zurinah Wan Ngah, Nor Aripin Shamaan, Abdul Gapor,. and Khalid Abdul Kadir. Long term administration of tocotrienols and tumor-marker enzyme activities during hepatocarcinogenesis in rats. *Nutrition*. 1993; **9**: 229-32.
11. Solt, D. and Farber, E.. New principle for the analysis of chemical carcinogens. *Nature*. 1976; **263** : 701-3.
12. Jacobs, W.L.W. A colorimetric assay for gamma-glutamyl transpeptidase. *Clin Chim Acta*.1971; **31** : 175-79.
13. Jahan, M. and Butterworth, P.J. Alkaline phosphatase of chick kidney. *Enzyme*. 1986; **35** : 61-69.
14. Zhou, J.R., Gugger, E.T., Tanakan, T., Guo, Y., Blackburn, G.L. and Clinton, S.K. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J. Nutr.* 1999; **129** : 1628-35.
15. Yanagihara, K., Ito, A., Toge, T. and Numoto, M. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res.* 1993; **53** : 5815-21.
16. Jing, Y., Nakaya, K. and Han, R. Differentiation of promyelocytic leukemia cells HL-60 induced by daidzein in *vitro* and in *vivo*. *Anticancer Res*. 1993; **13**: 1049-54.
17. Hawrylewicz, E. J., Zapata, J.J and Blair, W.H. Soy and experimental cancer: animal studies. *J. Nutr.*1995; **125** : 698S-708S.
18. Clinton, S.K., Destree, R., Anderson, D.B., Truex, C.R.,

Imrey, P.B. and Visek W.J. 1,2-dimethylhydrazine-induced colon cancer in rats fed beef or vegetable protein. *Nutr. Rep. Int.* 1979; **20** : 335-42.

19. McIntosh, G.H., Regester, G.O., Le Leu, R.K., Royle, P.J. and Smithers, G.W. Dairy proteins protect against dimethylhydrazine-induced intestinal cancers in rats. *J.Nutr.* 1995; **125** : 809-16.

20. Rao, C.V., Wang, C.X., Simi, B., Lubet, R., Kelloff, G., Steele, V and Reddy, B.S. Enhancement of experimental colon cancer by genistein. *Cancer Res.* 1997; **57** : 3717-22.