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EFFECT OF NATURAL PRODUCTS WITH CANCER CHEMOPREVENTIVE AGENTS ON ENDOGENOUS ANTIOXIDANT ENZYMES IN THE LIVER OF ALBINO MICE

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ABSTRACT

Five food substances rich in chemopreventive agents were assessed for their potential to increase the activity of endogenous antioxidant enzymes in the liver. The dietary supplements used were, onion, cabbage, honey, green tea and their combination. The effects of these supplements on the activities of catalase (CAT), glutathione-s-transferase (GST), glutathione reductase (GR), cytosolic superoxide dismutase (SOD1) and mitochondrial superoxide dismutase (SOD2) were examined. The result showed that the combination of these products increased the antioxidant enzymes activities significantly ($p < 0.05$) and more potently than individual food supplement compared which animals on normal diet. Supplementation with the individual diet supplement significantly increased the activities of all the enzymes ($p < 0.05$) except SOD1 and SOD2. However, combination of the supplements significantly increased the activities of SOD1 and SOD2 ($p < 0.05$) in addition to the activities of the enzymes increased by individual food supplement ($p < 0.05$)

Keywords: Antioxidants, Onion, Cabbage, Honey, Green Tea

INTRODUCTION

The role of reactive oxygen species (ROS) in the pathogenesis of many diseases affecting organs has been well documented (Roberts *et al.*, 2009). Epidemiological studies have shown that the ingestion of diet rich in fruit and vegetable may decrease the risk of cancer ((Steinmetz and Potter, 1991,1996; Block *et al.*, 1992& Jeanelle *et al.*; 2004). Much of the protective effects of fruits and vegetable have been attributed to phytochemicals with the ability to protect against oxidative stress induced damage (Nakazawa and Oshizawa, 1998). The chemopreventive agents commonly found in fruits and vegetables are mainly flavonoids such as Quercetin found in (*Allium cepa*) (Boyer *et al.*, 2004), Indole – 3 – carbinol in *Brassica oleracea* (Brandi *et al.*, 2005). Epigallo catechin gallate (EGCG) in green tea and caffeic acid phenethyl ester in honey (Cavine *et al.*; 2006). However, there are strong lines of evidence supporting a lower cancer risk associated with diet high in fruits and vegetables, which is due to complex combination of phytochemicals rather than action of a single compound (Schoelleer, 1990). The chemopreventive agents largely elicit their effect in preventing reactive oxygen species damage by inducing endogenous antioxidants proteins such as glutathione reductase, scavenging of free radical and singlet oxygen by SOD, destruction of hydrogen peroxide by catalase (Gutteridge and Halliwell, 2002). Oxidative damage may occur when antioxidant potential is decreased or when oxidative stress is increased (Gutteridge and Halliwell, 2002).

Liver is the largest organ in the body weighing approximately 1.2-1.5 kg in adults. It performs multiple diverse functions essential for life,

such functions as processing and storage of material absorbed from the digestive tract. Some of the absorbed materials include amino acids, carbohydrates, lipids and vitamins. Another critical function of the liver is the detoxification of xenobiotics. Thus, since liver is the first contact organ in the body after food absorption, it is potentially exposed to damage by these agents and their metabolites (Jaeschke *et al.*, 2002). Esterification of free fatty acids to triglycerides by the microsomal fractions in the liver, consists of enzyme such as NADPH, oxidase, cytochrome C reductase and cytochrome P450 leading to the generation of free radicals. Similarly, cytochrome P450 in its role of hydroxylation of drugs, steroids and xenobiotics leads to free radicals and reactive oxygen species formation (Jaeschke *et al.*, 2002). Deliberate induction of endogenous antioxidant in dietary therapy has been found to be beneficial in attenuating the effect of these reactive oxygen species (Pana and Ho, 2008)

Therefore induction of endogenous antioxidants by using combination of natural diet containing different phytochemical compounds may constitute a new complementary approach in attenuating the effect of the xenobiotics or their metabolites. Induced antioxidants can directly participate in the removal of the ROS or culminates in the synthesis of reduced glutathione used by the liver in the conjugation of xenobiotic metabolites. Hence this work assessed the potential of commonly consumed food substances for their potential to increase the activities of antioxidant enzymes necessary for the protection of liver against oxidative damage.

MATERIALS AND METHODS

Chemical

. All chemicals unless otherwise stated were purchased from Sigma Chemical Company (St Louis, MO) USA.

Animals

The experiments were performed on Swiss Albino mice obtained from National Institute of Veterinary Research Vom, Jos., Plateau State, Nigeria. All aspects of animal care complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee of the Department of Biochemistry, Bayero University, Kano, Nigeria.

Animals were housed in cages in an environmentally controlled animal facility (room temperature, 12 h light: 12 h dark cycle) with free access to a standard commercial diet and water *ad libitum*. The weight gain, food and water intake were determined daily in the morning.

Forty Eight (48) albino mice weighing 22g on the average were used for the study. The mice were housed in six groups of eight. Each group contains two sub-groups separated on sexes (4 males, 4 females) to prevent mating. Animals were housed in room with 12 hour light dark cycle. Mice were allowed free access to standard drinking water and powdered control diet *ad libitum* for 10 days for acclimatization. After this period, the animals were then fed on food supplements for a total period of 3 months (12 weeks) with the food sources containing chemopreventive agents with the exception of group V1, the control group. After this period animals were sacrificed.

Treatment

Group I (Control): Powdered diet *ad libitum* and standard drinking water administered for three months.

Group II: Powdered diet *ad libitum* mixed with raw onions at a concentration of 1.4g per 100g diet and standard drinking water for three months.

Group III: Standard drinking water containing 0.93g per 100 cm³ of Green tea and adjusted to 1:3 dilutions with standard drinking water was administered. The animals were also fed on powdered diet *ad libitum* of 3 months.

Group IV: The animals were fed on diet *ad libitum* mixed with 1.71g cabbage per 100g of diet for 3 months were administered. They were allowed free access to standard drinking water. The cabbage was dried in shade after which it was made into powder.

Group V: Powdered diet *ad libitum* was mixed with 1.4g raw onion, 1.71g cabbage per 100g diet. Also green tea at concentration of 0.93g per 100 cm³ of water was boiled and allowed to stand at room temperature for 24 hr. It was then adjusted to 1:3 dilution with standard drinking water containing pure honey at a concentration of 1 cm³ per 50 cm³ of the diluted green tea extract for three months. Honey and

green tea mixture was freshly prepared everyday to avoid fermentation of sugar in the honey

Group VI: Honey at a concentration of 1 cm³ per 50 cm³ standard drinking water was administered as drinking water. Powdered diet *ad libitum* was also provided as feed for 3 months.

Sacrificing Procedure

The mice were sacrificed by utilizing a reaction of calcium trioxocarbonate (iv) and concentrated hydrochloric acid to produce carbon iv oxide to make the animal unconscious, before being sacrificed by surgical dislocation utilizing a stick. Three animals of each sex were sacrificed and organs removed and frozen at -40°C, while one animal of each sex was sacrificed and organs preserved in 10% formal saline.

Procedure for Homogenization

The homogenizing buffer prepared was Tris (HCl) concentration 80mM containing sucrose 250mM and potassium chloride (KCl) 0.25mM, adjusted to a final pH of 7.4. Four (4) cm³ homogenization buffer was added to 1g of liver. Sample was homogenized in a hard glass ware (Pestle and Mortar). Homogenates were centrifuged at 20,000g using ultracentrifuge to obtain the cytosolic fractions. Sample (resultant supernatant) were then preserved at -40°C.

Total Protein Estimation

Estimation of protein concentration was carried out by the method of Bradford (1976).

Glutathione – s – Transferases (GST) Assay

GST activity was measured by the method of McClellan *et al.* (1991) in absorbance at 320nm caused by GST catalyzed conjugation of reduced glutathione (GSH) to CDNB.

Calculation of enzyme activity was based on the molar extinction coefficient \cdot CDNB = 9,600 L mol⁻¹ cm⁻¹

Superoxide Dismutase (SOD): The method of McCord and Fridovich (1969) as modified by Gwarzo and Muhammad (2011) was used in assaying for the activity of SOD1 and SOD2..

Glutathione Reductase Assay

Glutathione reductase activity was assayed by the methods of Castro *et al.* (1990). Calculation of enzymes activity was based on consumption of NADH (ϵ NADPH = 6300 Lmol⁻¹cm⁻¹)

Catalase

Catalase activity was determined by the method of Aebi (1983) by measuring the UV absorbance change of H₂O₂ at 240 nm.

Histochemical Analysis

Histochemical analysis was done according to Bancroft and Stevens (1982).

Statistical Analysis of Data

Standard error of mean (SEM) from the mean standard deviation were obtained for all data collected during analysis and experiment. The differences between the groups were analysed by one-way analysis of variance (ANOVA) followed by post tests of Scheffe and Duncan. Value of $p < 0.05$ was taken to imply statistical significance.

RESULTS

Daily food consumption and gain in body weight are given in table 1. In the treated group food consumption was significantly increased, thus suggesting increased intake of the phytochemical compounds contained in the diet. However, at the termination of the experiment there was no significant difference in weight gain between the control group and those receiving dietary supplementation.

Table 2 shows the effect of the phytochemicals contained in the various food materials on the activity of the endogenous antioxidant enzymes such as Glutathione S-transferases (GST), Glutathione Reductase (GR), Catalase (CAT), cytosolic superoxide dismutase (SOD₁) and Mitochondrial superoxide

dismutase (SOD₂). Dietary supplementation had significant effect on the enzymes activity compared with control. Increase in activity of endogenous antioxidant enzymes was observed for all the dietary treated groups (p<0.05) except SOD₁ and SOD₂ compared with control untreated group. Combination of food supplement resulted in the highest increase in enzymes activities. Furthermore, it was only the combination of the supplements that increased the activities of SOD1 and SOD2. Histochemical study (Fig 1) indicated that supplementation did not affect liver cell integrity. Therefore, food supplementation at the concentration give had no adverse effect on the liver.

Table 1: Mean weekly body weights of albino mice fed with natural products

Week	Control	Onion	Cabbage	Green tea	Combination	Honey
0	21.5±1.25	21.5±1.25	20.5±1.25	22.0±1.0	20.0±1.25	19.0±2.25
1	23.0±0.25	24.0±1.5	22.0±2.25	24.5±0.25	21.5±1.25	20.0±1.25
2	23.4±1.0	24.2±0.25	22.5±0.25	25.0±1.25	21.8±0.5	20.8±0.8
3	23.8±1.5	24.8±0.25	23.0±1.5	25.2±0.5	22.2±2.0	21.3±0.5
4	23.9±0.25	25.0±0.75	23.3±1.5	25.6±0.5	22.5±2.2	21.6±2.0
5	24.5±1.0	25.4±0.85	23.8±1.5	25.9±1.5	23.0±0.5	21.8±0.5
6	24.9±1.0	25.8±1.5	24.1±9.45	26.0±1.5	23.7±1.25	22.0±0.25
7	25.0±1.5	25.9±1.75	24.5±1.5	26.6±0.25	24.0±1.5	22.2±0.25
8	25.1±1.5	25.9±2.0	24.7±1.0	26.7±0.85	24.4±1.5	22.5±0.25
9	25.2±0.5	26.0±1.5	25.1±1.75	26.7±1.5	24.8±1.5	22.9±1.0
10	25.4±0.85	26.1±0.85	25.4±1.5	27.2±1.5	25.5±0.25	23.2±0.75
11	25.7±1.25	25.4±1.5	25.6±0.5	27.4±0.25	25.8±0.75	23.5±1.0
12	25.8±0.5	26.5±1.5	25.8±0.5	27.5±1.0	26.3±1.0	23.6±0.25

Results are Mean ± Standard Deviation

Table 2: The effect of natural product on the induction of endogenous antioxidant enzyme in mouse liver

Treatment Group	Catalase ·mol/min/ mg protein	Glutathione Reductase ·mol/min/mg protein	Glutathione Transferase ·mol/min/mg protein	SOD1 U/mg protein	SOD2 U/mg protein
Control	851±82	192±5	1200.1±1.22	85.2±7.2	32.3±3.5
Onion	842±68 ^a	220±5 ^a	2605±160 ^a	84.3±8.2	38.2±3.2
Green Tea	892±99 ^a	263±4 ^a	2501±152 ^a	83.9±7.7	40.3±4.6
Green Tea	892±99 ^a	263±4 ^a	2501±152 ^a	83.9±7.7	40.3±4.6
Cabbage	910±66 ^a	275±9 ^a	2901±105 ^a	84.5±5.3	39.2±3.8
Combination	1107±102 ^a	310±31 ^a	4672±175 ^a	86.5±10.2 ^a	43.3±6.7 ^a
Honey	895±50 ^a	240±16 ^a	1875±95 ^a	85.2±8.2	36.2±3.4

Result are expressed as mean ± SEM

a= indicate (P<0.05)

A statistical significance p<0.05

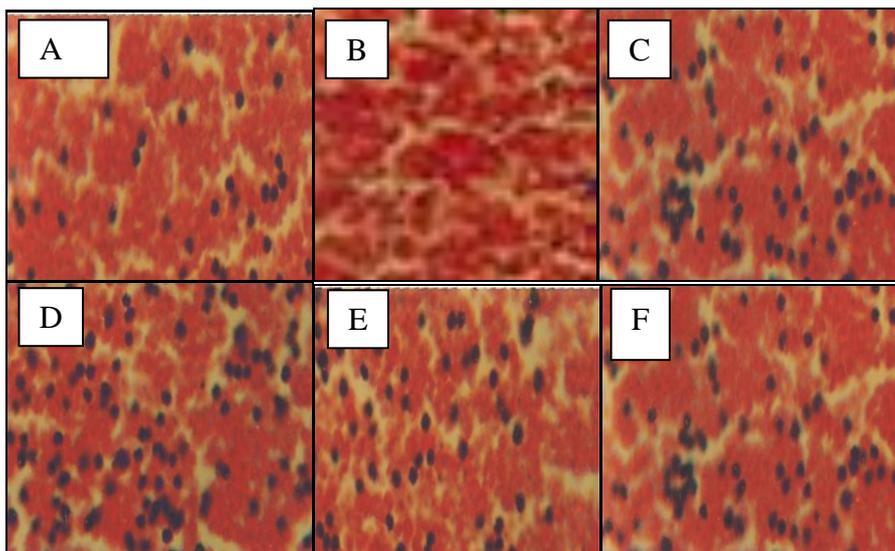


Figure 1: Histochemical stain of liver tissue using Haematoxylin X 40 (A) Control, (B) Onion, (C) Green Tea (D) Cabbage, (E) Combination, (F) Honey. The integrity of the tissue was not affected by dietary supplementation

DISCUSSION

This study was divided into two parts with a common objective of understanding the effect of administration of combination of food supplements on endogenous antioxidant enzyme in liver. The first part was an attempt to know if the administration of the supplement at the concentration given would have any adverse effect on the tissue. In this regard histological study (Fig 1) has shown that the administration of the of the supplements had no adverse effect on this tissue.

The second part of the study evaluated the potential of the supplements to increase the activity of endogenous antioxidant enzymes.

Induction of endogenous enzymes in tissue is generally accompanied by an increase tolerance to the toxic agents that cause oxidative stress (Levin, 1980). Glutathione plays both detoxification role and scavenging of free radical generated by pro-oxidant (Chasseud 1979). A diverse number of naturally occurring compounds possess the capacity of inducing proteins of intracellular defence mechanism involved in detoxification and scavenging of free radical (Hayes *et al.*, 1999). Oxidized glutathione (GSSG) produced by glutathione peroxidase 1 (GPX 1) can form mixed disulphide with proteins, and hence is potentially toxic. It is therefore reduced by glutathione reductase (GR), which utilizes NADPH as a co factor and regenerates GSH.

Glutathione reductase activity was increased by all food supplements in the liver with highest increase in the rats administered with the combination of the supplements. Since liver is involved in the detoxification of xenobiotics, it is possible that induction of antioxidant enzyme would enhance detoxification process as more oxidized glutathione is

recycled back to reduced form by glutathione reductase.

SOD1 is considered as the first line of defence against ROS (Van Loon *et al.*; 1984) and SOD₂ is by far the most important member of the SOD family in aerobic organisms, because superoxide radicals are mainly generated on the matrix side of the liver mitochondrial membrane (Balzan *et al.*, 1999). The combination of dietary supplements significantly increased the activity of both SOD1 and SOD2. Thus it is conceivable that increase in SOD₂ activity may provide increased protection against ROS. Though GSTs do not necessarily form the first line of defence against ROS, they confer a major role of protection against oxidative stress generated by electrophilic Xenobiotics or products of lipid peroxidation such as 4-Hydroxy- 2-enals (Esterbauer *et al.*, 1991; Schaur *et al.*, 1991). The glutathione transferases are ubiquitous enzymes, being particularly rich in hepatocytes. Certain GST isozymes (GST-A and GST-M) show catalytic activity of Conjugation of 4-Hydroxy- 2-enals (HNE) to GSH in rat and human liver cells as well as related endogenous electrophiles (Esterbauer *et al.*, 1991; Schaur *et al.*, 1991).

GST activity in the liver tissues was significantly increased by the supplement, with highest increase in combination group compared with non supplemented control. Catalase like glutathione peroxidase acts on hydrogen peroxide to produce water. Increase in activity of catalase may likely confer additional protection on the liver, since some xenobiotics generate ROS during detoxification process. Detoxification may involve making the compounds water soluble before conjugation. This process involves hydroxylation by cytochrome P450, which is known to generate free radical.

The result obtained showed the effectiveness of combination of food supplements containing chemopreventive agents as potent inducers of endogenous antioxidant enzymes, in which the activities of catalase, glutathione reductase, glutathione transferase, SOD₁ and SOD₂ were increased significantly compared with group on normal diet.

Liver has higher levels of glutathione than other tissues (Kopolowitz *et al.*, 1985) but in disease condition, the glutathione level is depleted (Olinski *et al.*, 2002), thus compromising its major functions. Though reduced glutathione was not estimated, but

the activity of glutathione reductase, a key player in the glutathione recycle was increased significantly.

CONCLUSION

Individual chemopreventive agents present in the food supplements used in this study have been established to attenuate carcinogenesis and free radical induced cellular damage (Hayes *et al.*, 1999). The combination of these food supplements at the concentration used in this study had no adverse effect as demonstrated by the histological study. Therefore, their consumption should be encouraged in order to reduce the incidence of liver diseases resulting from chemical damage.

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