International Journal of Tropical Medicine 8 (3): 62-67, 2013
ISSN: 1816-3319

Protective Properties of Tofu (Curdled Soymilk) Against Acetaminophen-Induced Oxidative Stress in Rats

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Abstract: The study was designed to assess the effect of various coagulants on the protective properties of tofu against acetaminophen induced oxidative stress. Tofu was prepared using different coagulants (calcium salt, alum and steep water (effluent of pap produced from maize). The protective ability of tofu was assessed by treating acetaminophen (50 mg/rat/day) stressed rats with the tofu and water *ad libitum* for 2 weeks. Oral administration of rats fed basal diet with mega dose of paracetamol (50 mg/100 g) caused a significant decrease in the total feed intake (141.8±5.31 g/rats/days), total weight gain (9.1±1.35 g/rats/days), PCV (33±2.60 g L^{-1}), Hb (10.9±1.48 g L^{-1}), RBC (4.1±0.91 ×10^{6} mm^{2}), MCV (66.5±3.65 fl) and WBC (1900±19.49 mm^{3}) compared with those on the basal diet without paracetamol. However, those rats fed with various coagulated tofu with paracetamol had a significant increase in the PCV (34.0-35.5 g L^{-1}), Hb (11.3±1.50-11.8±1.54 g L^{-1}), RBC (4.2±0.92-4.8±0.98×10^{2} mm^{2}), MCV (70.5±3.75-74.8±3.87 fl), WBC (2000±20.00-2800±23.66 mm^{3}) and MCH (9.4±1.37-9.8±1.40×10^{-12}), compared to those fed basal diet with paracetamol. Also, a significant increase of the serum ALP (122±4.93 mg dL^{-1}), ALT (31±2.49 mg dL^{-1}) and AST (101±4.49 mg dL^{-1}) were observed in rats fed basal diet with paracetamol, compared to those fed basal diet without paracetamol. While those fed tofu with paracetamol had significantly lower value in serum AST (94±4.34-80±4.0 mg dL^{-1}), ALT (30±2.45-26±2.28 mg dL^{-1}) and ALP (120±4.90-1104.70 mg dL^{-1}) compared to those fed basal diet with paracetamol. Furthermore, a significant increase in the serum cholesterol (172±5.87 mg dL^{-1}), LDL (83±4.07 mg dL^{-1}), HDL (89±4.22 mg dL^{-1}) and triglyceride (84.3±4.11 mg dL^{-1}) levels of rats fed basal diet with paracetamol, compared with those fed those fed basal diet without paracetamol while those fed tofu with paracetamol had a significant lower values in serum cholesterol, HDL, LDL and triglyceride, compared to those fed basal diet with paracetamol orally administered.

Key words: Antioxidant, acetaminophen, coagulants, oxidative stress, tofu

INTRODUCTION

Over two thirds of the population in the developing world suffers hunger and malnutrition due to lack of access to good quality and quantity of food. Poverty and over population have made it difficult to improve on nutritional status (Edward, 1980). Soybeans are inexpensive and serve as high quality protein source. Soymilk and tofu consumption is increasing in Nigeria due to animals diseases such as mad cow disease, global shortage of animal protein, strong demand for healthy (cholesterol free and low in saturated fat) and religious halal food and economic reasons (Asgar *et al*., 2010). The greater acceptance of soy foods by the general population is due to increase recognition of the health benefits of soy foods, especially by those who want to reduce their consumption of animal products (Poysa and Woodrow, 2002). Tofu is popularly consumed in Nigeria because of the various nutritional and medical attributes associated with soybeans products such as reduction of cardiovascular disease, osteoporosis and cancer risks (FDA, 1999). Tofu, also known as soybean curd is a soft cheese-like food made by curdling fresh hot soymilk with a coagulant. Traditionally, in Nigeria, it is produced by curdling fresh hot soymilk with either $CaCl_2$, $MgSO_4$, alum or steep water (effluence from pap produced from maize) (Descheemaeker and Debruyne, 2002; Murphy and Wilson, 1997; Poysa and Woodrow, 2002). Tofu is rich in proteins, low in saturated fats, higher in polyunsaturated fatty acids and cholesterol free, a good source of

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β-vitamins, minerals and isoflavones, antioxidants (carotenoids, vitamin C, E, phenolic and thiol (SH) compounds and essential amino acids (Poysa and Woodrow, 2002; Paganga *et al*., 1999). Coagulants have been reported to modulate hypocholesterolemic effect on experimental rats. Soybean products reduce the risk of heart diseases by lowering levels of oxidized cholesterol which is taken up more rapidly by coronary artery walls to form dangerous plaques. Previous research has shown that soy consumption reduces cholesterol in general while also decreasing the amount of bad cholesterol (low density lipoprotein) in the body and maintaining the amount of good cholesterol (high density lipoprotein) (Parma *et al*., 2007). Acetaminophen is a commonly used and safe analgestic drug which is known to cause centribular necrosis upon overdose (Davidson and Esther, 1996). Its toxicity accounts for many emergency hospital admission and continues to be associated with high mortality. The hepatoxic effect of acute paracetamol overdose is well known and has been extensively reviewed (Liu, 1997). Since, tofu (soybean product) contain compounds that are valuable antioxidants and protecting molecules which can trap or destroy free radicals and subsequently protect us from damage due to oxidative stress. In view of this, people have started to take an interest in tofu consumption due to its good nutritional and health benefit to human. Therefore, this research is designed to evaluate the protective effect of tofu produced using different coagulants against acetaminophen induced oxidative stress.

MATERIALS AND METHODS

The soybeans (*Glycine max*), TGX923-1E variety were obtained directly from Ibrahim Badamasi Babangida University, Lapai experimental farm in Niger State of Nigeria. The alum, calcium salt ($CaCl_2$) and magnesium salt ($MgCl_2$) were industrial grade while the steep water was collected from domestically processed pap. The water used in the analysis was glass distilled. The weanling albino rats used were of the same litter origin obtained from the animal house, College of Medicine, University of Ilorin.

Tofu preparation: Soybeans (2.0 kg) were soaked in water (6 L) at 20-40°C for 9 h. The soaked beans were drained, weighed and ground with grinder, tap water added at a ratio of 6:1 with raw bean and then filtered to separate soy cake from soymilk. The soymilk was subsequently heated to 98°C and maintained for 1 min before delivering to the mix tank. When cooled to 87°C, 1 L of soymilk was mixed at 420 rpm with each of the coagulants (50 mL). The mixed solutions were held for 5 sec and then filled on to tofu trays and allowed to coagulate for 10 min. The bean curd was pressed after which the tofu weight was recorded. Tofu was stored in water at 4°C overnight prior to analysis.

Animals: Twenty five of 3 weeks old, strain albino rats with an average of 50.2 g were used in the study. They were obtained from the animal house of the college of medicine, University of Ilorin, Kwara State, Nigeria. The animals were housed in metabolic cage in the laboratory under ambient temperature and 12 h light and dark periodicity. They were fed commercial rat pellets (Niemeth livestock Feeds, Ltd. Ikeja), water *ad libitum* and allowed to acclimatize for 2 weeks. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care.

Experimental design: Animals were weighed and randomly assigned in to five groups, viz:

- Normal control group (n = 5) was placed on a basal diet of 20 g day^{-1} only
- Negative control group (n = 5) was placed on 20 g day^{-1} and 50 mg/100 g/day of basal diet and acetaminophen orally administered
- Treated group (n = 5) was placed on steep water tofu (20 g) and acetaminophen (50 mg/100 g/day) orally administered
- Treated group (n = 5) was placed on alum tofu (20 g) and acetaminophen (50 mg/100 g/day orally administered
- Treated group (n = 6) was placed on calcium tofu (20 g day^{-1}) and acetaminophen (50 mg/100 g/day) orally administered

The experimental period was 14 days. During the cause of the experiment; the weight of the rats and the leftover feed were measured at 2 days interval. On the 15th day, the rats were killed by cervical dislocation and plasma (using EDTA) were collected for PCV, WBC, RBC, Hb and MCV analysis and serum for serum enzyme markers (ALP, AST, ALT) and lipid profiles (cholesterol, HDL and LDL levels) analysis.

Measurements: The mean average weight of the rats was determined at the beginning of the experiment at every 2 days. The weight of the rats was determined using weighing scale (Ohaus Model Cs 5000, Capacity 500×2 g). This was done by placing a container on the scale and the balance adjusted to zero after which the rats (Aderemi *et al*., 2004) in each group were placed into

container and the measurement taken (Ayoola, 2010). Total feed consumed was determined using the differences between the feed supplied (20 g) and leftover, Total Weight Gained (TWG) was calculated using final weight gained subtracted from initial weight gained.

Haematological studies: Blood samples of about 6 mL were collected with the aid of 2 cm^3 plastic syringe, 3 mL of the heparininzed blood were analyzed for Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (Hb) concentration, White Blood Cell (WBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) using methods described by Dacie and Lewis (1994).

Serum lipids profile and marker enzymes: The 3 mL of blood samples in the non-heparinized bottle was allowed to clot at room temperature, centrifuged at 2,500 g for 10 min to obtain the serum and stored in the biofreezer until analyzed. The serum samples from each group were subjected to triglycerides, High-Density Lipoprotein (HDL) cholesterol and total cholesterol analysis using Randox Laboratory kit reagents. Low-Density Lipoprotein (LDL) cholesterol was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol (Tietz, 1990; Yakubu *et al*., 2006). Another 3 mL of non-heparinized blood samples were subjected to enzyme markers (ALP, AST and ALT) analysis using the Method of Reinhold (1953).

Analysis of data: Data were presented as mean of three replicates. One way Analysis of Variance (ANOVA) and Least Significant Difference (LSD) was applied. Significant was accepted at $p<0.05$ probability.

RESULTS AND DISCUSSION

The result of total feed intake and total weight gain are shown in the Table 1 and revealed that there was decreased in total feed intake (141.8±5.31 g/rats/day) and total weight gain (9.11±1.35 mg/rats/day) of rats fed basal diet with acetaminophen when compared with those rats fed basal diet without acetaminophen (total feed intake (443.8±9.40 g/rats/days) and total weight gain (14.5±1.70). However, there was a significant increase ($p<0.05$) in total feed intake (44.2±2.10-73.5±3.80) of rats fed tofu curdled with various coagulants with acetaminophen orally administered when compared with those rats fed basal diet with acetaminophen.

In addition, the total weight gain of rats fed tofu curdled with various coagulants plus acetaminophen decreased significantly (-5.1±1.01-2.1±0.60) when compared with those fed diet with acetaminophen (9.1±1.35 g/rats/days). The results of haematology of rats offered different treatments are presented in Table 2. It is revealed that there was a significant decreased ($p\leq0.05$) in PCV (33.0±2.60 g L^{-1}), Hb (10.9±1.48 g L^{-1}), RBC (4.1±0.91×10^6/mm^2), MCV (66.5±3.65 fl) and WBC (1900±19.49 mm^3) of rats fed basal diet with acetaminophen when compared with those fed basal diet without acetaminophen orally administered (PVC (34.5±2.63, Hb (11.4±1.51), RBC (4.2±0.92), MCV (78.4±1.23) and WBC (3000±24.49 mm^3)). However, there was a significant increase ($p<0.05$) in the PVC (34.0±2.61-35.5±2.66), Hb (11.3±1.50-11.8±1.54), RBC (4.2±0.92-4.8±0.98), MCV (70.5±3.79-74.8±3.87) and WBC (2000±20.00-2800±23.66) of rats fed tofu curdled with various coagulants with acetaminophen when compared to those fed basal diet with acetaminophen orally administered Table 3 shows the results of serum lipid levels of rats fed diet with/without acetaminophen orally administered. It shows that there was a significant increase ($p<0.05$) in the serum total cholesterol (172±5.87 mg dL^{-1}), LDL (83±4.07 mg dL^{-1}) levels of rats fed basal diet with acetaminophen when compared to

Table 1: Total feed intake and weight gain of albino rats fed with commercial diet and various coagulated tofu (g/rats/days)

Samples	Total food Taken (g/rats/days)	Total weight gained (g/rats/days)
BDA	141.8±5.31^b	9.11±1.35^b
BDWA	443.8±9.40^a	14.5±1.70^a
CST	64.7±3.60^d	-3.2±0.80^d
SWT	44.2±2.10^e	-2.1±0.60^e
ALT	73.5±3.80^c	-5.1±1.01^c

Value represents mean of triplicate reading. Means with the same superscript letter (s) within the same column are not significantly different ($p\leq0.05$). ALT: Alum coagulated Tofu, SWT: Tofu coagulated with effluent from pap, CST: Calcium coagulated Tofu

Table 2: Hematology of rats studied

Sample (rats)	PCV (g L^{-1})	Hb (g L^{-1})	RBC (×10^6 mm^2)	MCV (fl)	WBC (mm^3)	MCH (×10^{-12})
BDA	33.0±2.60^c	10.9±1.48^c	4.1.0±0.91^c	66.5±3.65^c	1900.0±19.49^c	9.10±1.35^c
BDWA	34.5±2.63^b	11.4±1.51^b	4.2.0±0.92^c	78.4±1.23^a	3000.0±24.49^a	10.40±1.44^a
SWTA	35.5±2.66^a	11.8±1.54^a	4.8.0±0.98^a	74.8±3.87^b	2800.0±23.66^b	9.80±1.40^a
ALTA	34.0±2.61^b	11.3±1.50^b	4.5.0±0.95^b	70.5±3.75^b	2700.0±23.24^b	9.60±1.39^b
CSTA	35.5±2.66^a	11.3±1.50^b	4.2.0±0.92^c	72.5±3.81^b	2000.0±20.00^c	9.40±1.37^b

Means with the same superscript letter (s) along the same column are not significant different ($p\leq0.05$); BDA: Basal Diet with acetaminophen orally administered; BDWA: Basal Diet Without Acetaminophen orally administered; SWTA: Steep Water coagulated Tofu with Acetaminophen orally administered; ALTA: Alum coagulated Tofu with Acetaminophen orally administered and CSTA: Calcium chloride coagulated Tofu with Acetaminophen orally administered

Table 3: Serum lipid profile of rat studied

Sample (rats)	Total cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	Triglyceride (mg dL^{-1})
BDA	172.0±5.87^{a1}	89.0±4.22^{c}	83.0±4.07^{a}	84.3±4.11^{a}
BDWA	154.0±5.45^{b}	90.0±4.24^{b}	64.0±3.58^{b}	78.5±3.96^{b}
SWTA	121.9±4.94^{c}	90.6±4.26^{a}	31.3±2.50^{e}	45.1±3.00^{e}
ALTA	122.3±4.95^{c}	90.1±4.24^{b}	31.2±2.50^{e}	44.9±3.00^{e}
CSTA	121.8±4.40^{c}	90.3±4.25^{a}	31.5±2.51^{c}	45.3±3.01^{c}

Table 4: Serum enzyme marker level of rats studied

Sample (rats)	ALP (mg dL^{-1})	AST (mg dL^{-1})	ALT (mg dL^{-1})
BDA	122.0±4.93^{a}	101.0±4.49^{a}	31.0±2.49^{a}
BDWA	90.0±4.24^{c}	77.0±3.92^{c}	26.0±2.28^{b}
SWTA	110.0±4.70^{b}	82.0±4.05^{b}	26.0±2.28^{b}
ALTA	120.0±4.90^{a}	94.0±4.34^{b}	27.0±2.32^{b}
CSTA	113.0±4.75^{b}	80.0±4.00^{c}	30.0±2.45^{a}

Means with the same superscript letter (s) along the same column are not significant different ($p \leq 0.05$). BDA: Basal Diet with Acetaminophen orally administered; BDWA: Basal Diet Without Acetaminophen orally administered; SWTA: Steep Water coagulated Tofu with Acetaminophen orally administered; ALTA: Alum coagulated Tofu with Acetaminophen orally administered and CSTA: Calcium chloride coagulated Tofu with acetaminophen orally administered

those rats fed basal diet without acetaminophen orally administered {serum total cholesterol (154±5.45 mg dL^{-1}), LDL (64±3.58 mg dL^{-1})}. Also, there was a significant decrease ($p>0.05$) in the serum HDL (89±4.22 mg dL^{-1}) of rats fed basal diet with acetaminophen, compared to those fed basal diet without acetaminophen (HDL) (90±4.24 mg dL^{-1}).

However, there was a significant decrease ($p>0.05$) in the serum total cholesterol (122.3±4.95-121.8±mg dL^{-1}), LDL (31.5±2.51-31.2±2.50 mg dL^{-1}) of rats fed tofu curdled with various coagulants with acetaminophen when compared to rats fed basal diet with acetaminophen orally administered.

Hence, there was a significant increase in the serum HDL (90.1±4.24-90.6±4.26 mg dL^{-1}) of rats fed tofu curdled with various coagulants with acetaminophen when compared with rats fed basal diet with acetaminophen orally administered, LDL (83.0±4.07 mg dL^{-1}). The result of serum enzyme marker levels of the albino rats fed diet with/without acetaminophen orally administered are presented in Table 4.

The results revealed that there was a significant increase ($p<0.05$) in the serum ALP (122±4.93 mg dL^{-1}), AST (101±4.49 mg dL^{-1}) and ALT (31±2.49 mg dL^{-1}) levels of rats fed basal diet with acetaminophen when compared to those rats fed basal diet without acetaminophen serum ALP (90±4.24 mg dL^{-1}), AST (77±3.92 mg dL^{-1}) and ALT (26±2.28 mg dL^{-1}) orally administered. However, there was a significant decrease in the serum ALP (120±4.90-110±4.70 mg dL^{-1}), AST (94±4.34-80±4.0 mg dL^{-1}) and ALT (30±2.45-26±2.28 mg dL^{-1}) of rats fed tofu curdled with various coagulants with acetaminophen orally administered. Table 1 shows the results of total feed intake and total weight gain of rats studied. The result revealed that there was a decreasedin total feed intact (141.8±5.31 g/rats/days and total weight gain (9.11±1.35 g/rats/days) of the rats fed basal diet with acetaminophen when compared with those rats fed basal diet without acetaminophen (total feed intake (443.8±9.40 g/rats/days) and total weight gain (14.5±1.70 g/rats/days)). This drastic reduction in feed intake and total weight gain could be because of over dose paracetamol intake that generated oxidative stress in rats. The free radicals produced in rats might caused certain abnormality against food consumed and weight gain. It was also observed that rats fed with alum-coagulated tofu consumed the highest amount of tofu in the duration of the experiment (73.5±3.80 g/rats/days) follow by those fed with calcium chloride coagulated tofu (64.7±3.60 g/rats/days) while those fed with tofu coagulated with steep water consumed the lowest amount of tofu (44.2±2.10 g/rats/days).

The total weight gain of rats fed tofu produced using different coagulants was significantly higher ($p<0.05$) in alum-coagulated tofu (-5.1 g/rats/days). Tofu coagulated with steep water significantly had the least weight gain (-2.1 g/rats/days). The higher consumption of alum and calcium chloride coagulated tofu might be because of good taste and odour as indicated in the tofu and the negative value of weight gain recorded by the rats fed with steep water tofu could be because of very low feed intake. While the low intake of the tofu coagulated with steep water could be because of the unpleasant odor imparted by the steep water to the tofu. The wide variation for tofu consumed by the various rats could be because of the difference in the taste, nutritional quality and acceptability of the various coagulated tofu (Aning *et al.*, 1998).

The results of hematology is shown in Table 2 which revealed that there was a significant different ($p<0.05$) in the PCV (33.0±2.60), Hb (10.9±1.48 g L^{-1}), WBC (1900±19.49 mm^3), MCV (66.5±3.65 fl) and MCH (9.1±1.35) of the rats fed basal diet with acetaminophen orally administered when compared to those fed basal diet without acetaminophen orally administered (PCV (35.5±2.66 g L^{-1}), Hb (11.4±1.51 g dL^{-1}), MCV (78.4±1.23 fl), WBC (3000±24.49 mm^3) and MCH (10.4±1.44×10^{-12}). The reduction in hematological parameters is an indicative of blood loss and could be attributed to the ability of the acetaminophen to induce the production of free radicals. This also agreed with earlier report by Yong in that over consumption of garlic induces the production of free radicals.

However, oral administration of acetaminophen with tofu using various coagulants caused a significant increase ($p<0.05$) in the PCV (34.0±2.61-35.5±2.66 g L^{-1}),

Hb (11.3±1.50-11.8±1.54 g L^{-1}), RBC (4.2±0.92-4.8±0.98×10^6/mm^2), MCV (70.5±3.75-74.8±3.87 fl) and WBC (2000±20.00-2800±2366 mm^3) of rats when compared with those fed basal diet with acetaminophen orally administered. This indicated that tofu curdled with various coagulants were capable of preventing acetaminophen induced free radical production. This could be attributed to the high antioxidant properties of soy product which serve as an extracellular neutralizer of free radicals (Anderson and Theron, 1990). In addition, soy food and vegetables had been reported to be rich in many phenols such as flavonoid (Alia *et al*., 2003). Flavonoids have antioxidants capacity that is much stronger than those of vitamin C and E are which are reportedly used to prevent free radical production (Alia *et al*., 2003). It was reported that flavonoids could protect membrane lipids from oxidation and a major source of flavonoids vare vegetables, fruits and soybeans (Alia *et al*., 2003).

The result of serum total cholesterol and LDL levels as shown in Table 3, revealed that there is a significant increase intotal cholesterol (172±5.87 mg dL^{-1}) and LDL (83±4.07 mg dL^{-1}) and triglyceride (84.3±4.11 mg dL^{-1}) levels of rats fed basal diet with acetaminophen orally administered when compared to those fed basal diet without acetaminophen orally administered (cholesterol (154±5.45 mg dL^{-1}), LDL (64±3.58 mg dL^{-1}), triglyceride (78.5±3.96 mg dL^{-1}). This elevation in serum cholesterol and LDL levels could be attributed to the ability of acetaminophen to induce the production of free radicals which result in hypercholesterolemia and the atherosclerosis. This correlates with the findings of Abdel-Wahab *et al*. (2002) that an increase in the serum levels of cholesterol and LDL is associated with hypercholesterolemia and atherosclerosis, respectively. However, supplementation of tofu curdled with various coagulants with acetaminophen orally administered causes a significant decrease ($p>0.05$) in serum cholesterol (121.8±4.40, 122.3±4.95 mg dL^{-1}), LDL (31.2±2.50-31.5±2.51 mg dL^{-1}) and triglyceride (44.9±3.00-45.3±3.01 mg dL^{-1}), compared to those fed basal diet with acetaminophen orally administered. It indicated that tofu produced using three coagulated agents are capable of preventing acetaminophen induces oxidative stress. This could be attributed to high antioxidant potential of soybeans which serve as an extracellular neutralizer of free radicals (Anderson and Theron, 1990). This finding is in line with the study of Anderson and Theron (1990) that the anti-hypercholesterolemic effect of soy protein was found to decrease the plasma concentrations of LDL as well as the ratio of plasma LDL to HDL.

However, there was a significant decrease ($p<0.05$) in serum HDL (89.0±4.22 mg dL^{-1}) levels of rats fed basal diet with acetaminophen orally administered when compared to those fed basal diet without acetaminophen orally administered (90.0±4.24 mg dL^{-1}). The decrease levels of HDL could be attributed to the ability of acetaminophen to induce the production of free radicals which result in oxidative damage. This correlates with the findings of Abdel-Wahab *et al*. (2002) that a decrease in the serum HDL is an indicative of liver damage. However, the supplementation of tofu produced using various coagulants with acetaminophen orally administered causes a significant increase ($p<0.05$) in serum HDL levels (90.1±4.24-90.6±4.26 mg dL^{-1}), compared to those fed basal diet with acetaminophen orally administered. The elevation of serum HDL levels could be attributed to the high antioxidant properties of soybeans which prevent and reduce the activity of free radicals. This is in line with the research of Abdel-Wahab *et al*. (2002) that high level of serum HDL has been proved to protect LDL from oxidation. Therefore, based on this finding the antioxidant properties of tofu is able to control oxidative damage and effectively lower serum levels of cholesterol. The results ofenzyme markers (AST, ALT and ALP) levels are shown in Table 4 and revealed that rats on basal diet with acetaminophen orally administered had the AST (101±44.49 mg dL^{-1}) while it was (77±3.92 mg dL^{-1}) on those fed with basal diet without acetaminophen orally administered. Increased in AST levels signified liver damage. This finding suggested that the mega doses of acetaminophen administered induces the production of free radicals which causes damage on the hepatocytes of rats. This result correlates with the finding of Abdel-Wahab *et al*. (2002) that toxicity with acetaminophen occurs when too much of it is taken. Also, the results of ALT and ALP levels of experimented rats revealed that there was increased in serum ALT (31±2.49 mg dL^{-1}) and ALP (122±4.93 mg dL^{-1}) in the sera of rats on basal diet with acetaminophen orally administered when compared with those (ALT (26±2.28 mg dL^{-1}) and ALP (90±4.24 mg dL^{-1})) of the rats on basal diet without acetaminophen orally administered. The elevations of serum liver enzymes indicate liver damage. This correlates with the report of Sai *et al*. (1992) that a significant increase in serum AST, ALT and ALP levels suggest the liver damage. Conversely, a significant decrease ($p\leq0.05$) was observed in serum AST (94±4.34-80±4.0 mg dL^{-1}), ALT (30±2.45-26±2.28 mg dL^{-1}) and ALP (120±4.90-110±4.70 mg dL^{-1}) of the rats on tofu with acetaminophen, compared with those of rats on basal diet with acetaminophen (AST (101±4.49 mg dL^{-1}), ALT (31±2.49 mg dL^{-1}) and ALP (122±4.93 mg dL^{-1}).

Decreased in serum enzyme marker levels (liver enzymes) is an indication of repairing liver damage caused by acetaminophen orally administered. Therefore, lower level of serum enzyme markers observed in rats on tofu coagulated with various coagulants with acetaminophen orally administered suggest liver repair but steep water coagulated tofu looked more promising in term of liver repair than other form of tofu diet. Therefore, tofus curdled with various coagulants have proven to prevent hypercholesterolemia, atherosclerosis and liver damage caused by high doses of acetaminophen orally administered. In addition, the intake of acetaminophen in excess should be discouraged in homes because of its destructive effects on the liver. The tofu consumption should be encouraged in diets as it can be used as a functional food to prevent oxidative stress due to its antioxidant properties and in the treatment of liver damage, hypercholesterolemia and atherosclerosis. Therefore, tofu can be recommended for clinical trials.

CONCLUSION

The results of the study show that all tofu used proved to be good but steep water tofu appeared to be more promising in preventing paracetamol-induced oxidative stress on rats hepatocytes.

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