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Effect of peroxidized soyabeans oil on acid and alkaline phosphatase activities of some rat bones

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Abstract

Soyabean oil was deep fried in an open air for two hours daily for ten days at a temperature of 200^0c ± 20^0c. The peroxide value and Free Fatty acids of the deep fried soyabean oil was determined and found to be higher 35.00 ± 0.02 and 5.04 ± 0.40 than the fresh soyabean oil 20.00 ± 7.07 and 2.24 ± 0.01 respectively. This is an index of peroxidation of the soyabean oil. The peroxidized soyabean oil was used in the formulation of diet at 5% and 15% levels and fed to male albino rats of wistar strain for six weeks. The bone (Tibia and Femur) of the animals were then studied weekly for alkaline and acid phospatase enzyme activities. The activities of these enzymes at 5% level were reduced when compared to the control groups. At 15% level, the activity was higher. There were no significant difference between the activities of the enzymes studied at all levels when compared to the control group ($p>0.05$). This shows that there is little or no effect due to toxic intake of peroxidized soyabean oil on the enzyme activities in bone of the animals.

Keywords: Alkaline phospatase, Acid phospatase, Femur, Tibia, Peroxidation

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INTRODUCTION

Ingestion of mildly oxidized oils at 15% level of the diet have indicated typical milder symptoms which included depressant effects on growth or steatorrhea with no change in survival and morphological appearance (1). The lipid peroxidation products, including hydroperoxyl cyclic epoxides react with DNA in the presence of metals and ascorbic acid (2). Such oxidation products have been shown to be formed from highly oxidized linoleate and linolenate esters at peroxides value of between 800 and 3000 (3). Recent studies have shown moderate mutagenicity in the polar fat fractions containing oxidative degradation products of fatty acids of deep frying fat samples (4). The fractions contained low levels of thiobarbituric acid reactive substances which suggested that mutagenic compounds other than liquid peroxidation products may have been responsible for part of the mutagenic activity by the difference of the polar fractions (5). Alkaline phospatase in bone have been reported to be glycoprotein containing sialic acid (6). These glycoprotein enzymes contain glucosamine, galactosamine and the sacharides.

The research is designed to expose the effects of peroxidized soyabean oil on the activities of alkali and acid phosphatase in bone. The effect of supplementation with peroxidized soyabean-oil in diet on the activities of enzymes has not been previously reported. The biological significance of repeatedly used frying oils is more often determined by toxicological or pathological studies (7). The measurement of the enzyme activity in bones will provide information on the identity of the bone damage or damaged bone cells. Apart from its usefulness in toxicity testing, it also provides a significant aid in the diagnosis of bone diseases (2).

MATERIALS AND METHOD

Soyabean-oil (Louis Carter PLC KMC 97) was obtained from Fas supermaket in Ilorin, Nigeria. The soyabean-oil was three weeks old from the registered date of manufacture to the date it was used. Male albino rats (wistar strain) of twenty-five days old with average weight of forty-five grammes were obtained from the Department of Biochemsitry, University of Ilorin, Ilorin, Nigeria. Sodium acetate buffer (0.1m, PH = 4.5), P-nitrophenyl phosphate (19mM), sodium hydroxide (1M), carbonate buffer (pH = 10.1), Magnesium sulphate (0.1M) were products of sigma chemical company limited, London. The reagents used were prepared in volumetric flasks and stored in reagent bottles before use.

Treatment of soyabean-oil

The fresh and peroxidized soyabean oils were studied using Hofmann and Green method (8). The method provide the basis for comparing the peroxide value, Acid value, Ester value, percentage free fatty acid as well as the saponification value of the fresh and peroxidized soyabean oil as shown in Table 1. Two liters of fresh soyabean-oil was measured and kept in a stainless steel fry pan. It was deep-fried in an open air at a temperature of 200^0c $\pm$ 20^0c. The temperatures were monitored using industrial thermometer. The deep-frying was done for two hours daily for ten-days and left to cool in an open space each day to simulated repeated use of frying oil. The peroxidized soyabean-oil was used in formulation of diet for feeding experiment at 5% and 15% levels (9)

Diet composition

The diet was composed using Gabriel and Co-workers method of feed formulation (9). The composition of the diet is as shown in Table 2. Each diet was kept in reagent bottles in a cool dry place all through the research period.

Table 1: Chemical analysis of Soyabean-oil (8).

Parameters	Fresh oil	Peroxidized oil
Acid value	1.12 $\pm$ 0.01	2.52 $\pm$ 0.20
Iodine value	35.00$\pm$ 0.02	24.00 $\pm$ 0.10
Ester value	41.68$\pm$ 4.96	37.48 $\pm$ 8.92
% free fatty acid	2.24 $\pm$ 0.01	5.04 $\pm$ 0.40
Saponification value	42.80$\pm$ 4.96	40.00 $\pm$ 8.92
Peroxide value	20.00$\pm$ 7.07	35.00 $\pm$ 0.02

Each value is the mean of 5 determinations $\pm$ SD ($p<0.05$).

Table 2: Composition of diets (g/kg dry weight basis) (9):

Ingredients	A	B	C	D
Casein (defatted)	250	250	250	250
DL-Methionine	4	4	4	4
DL-lysine	4	4	4	4
Corn starch	412	412	250	250
Cellulose	130	130	255	255
Sucrose	100	100	37	37
Soyabean-oil	50	50	150	150
Mineral mix	40	40	40	40
Vitamin mix	10	10	10	10

A = 5% Fresh soyabean oil diet; B = 5% Peroxidized soyabean oil diet; C = 15% fresh soyabean oil diet; D = 15% peroxidized soyabean oil diet. Fourty-eight male albino rats (wistar strain) were used for the experiment. They were divided into four groups, each containing twelve animals as follows: Group A fed on 5% fresh soyabean oil diet; Group B fed on 5% peroxidized soyaben oil diet; Group C fed on 15% fresh soyabean oil diet; Group D fed on 15% peroxidized soyabean oil diet. The diets and water were given ad.libitum. The animals were fed for six weeks. All animals were weighed and two from each group sacrificed on weekly basis until the end of the experiment for protein and enzyme analysis. Analysis of variance and correlation coefficient were used to compare result at p = 0.05.

Preparation of homogenates

The rats were anaethesized in jars containing cotton wool soaked with chloroform. They were allowed to go into unconscious state, brought out quickly and dissected. The bones (Femur and Tibia) were removed and homogenized in ice cold 0.25M sucrose solution using mortal and pestle. The homogenates were frozen overnight before enzyme assay to allow unbroken cells to lyse (10). The homogenate were diluted using 0.25M sucrose solution as diluents; 1 in 30 for protein determination and phosphatase enzymes because phosphatase are glycoproteins.

Enzyme activities determination

The enzymes assay was done using the spectronic – 21A spectrophotometer and glass cuvette with 1.00cm light path throughout. The activities of phospatase enzymes were done using the procedure of Wright and co-workers (11). The homogenates were incubated with P-Nitrophenyl, phospate, buffered at 37^0c. the hydrolysis of the product then react with sodium hydroxide to give yellow colour which was measured spectrophotometrically at 400nm.

RESULTS AND DISCUSSION

The results on Table1 show that, the peroxide value in peroxidized soyabean-oil (35.00) is higher than the Fresh soyabean oil (20.00). The considerably higher peroxide value in peroxidized soyabean oil meant higher oxidation value. The values are significantly different ($P<0.05$) confirming the peroxidized soyabean-oil as deteriorated and no longer acceptable. The free fatty acid of the peroxidized soyabean-oil were higher 5.04% than the fresh soyabean-oil 2.24% which shows that there was complete hydrolysis during frying possibly leading to reduction in some essential fatty acid in the oil. The saponification values show no essential difference but the iodine values in peroxidized soyabean oil were reduced indicating the degree of degradation due to oxygen attack (12). Yoshika and co-workers (13); associated high carbonyl values of oxidized fats with their toxicity, because the rat liver enzymes thiokinase and Succinyldehydrogenase had low activities when such fats were fed.

Figures 1, 2, 3 and 4 shows the activities of Alkaline and Acid phospatase in bones (Tibia and Femur) of animals following the administration of diets supplemented with peroxidized soyabean-oil. Those fed 5% peroxidized soyabean oil showed decreased enzyme activities throughout the experimental period when compared to their respective control groups. This result agreed with Yoshika and co-workers (13) that associated the reduced activities of such enzymes to toxic effect of high carbonyl values generated during the deep frying procedure of the soyabean-oil. There was no significant difference however established between the activities of the enzymes at this level ($P>0.05$). Those fed the 15% peroxidized soyabean oil however showed variation in the enzyme activities when compared to the control. The alkaline phospatase activities were higher than the control

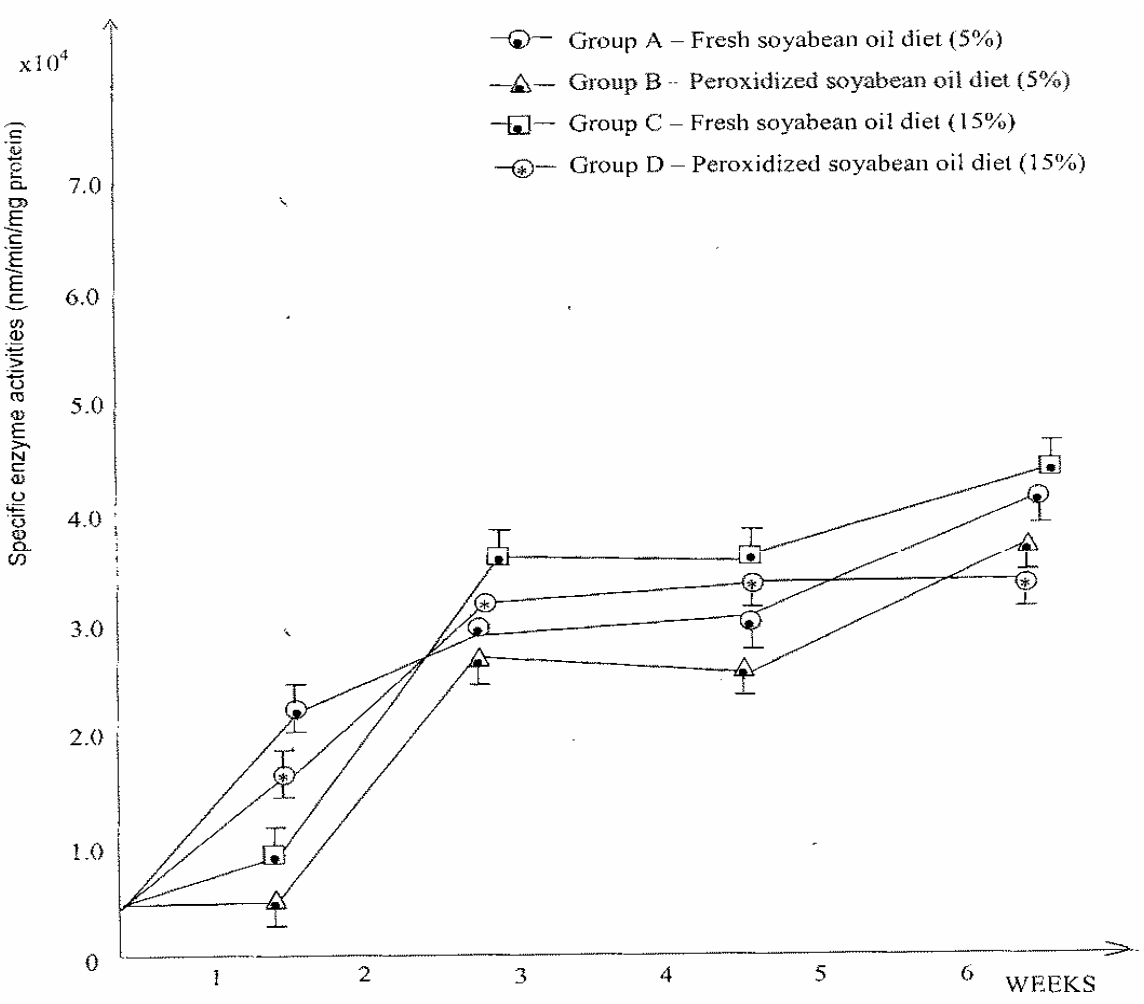

Fig. 1: Alkaline phosphatase specific activity of rat tibia following supplementation with peroxidized soyabean oil.

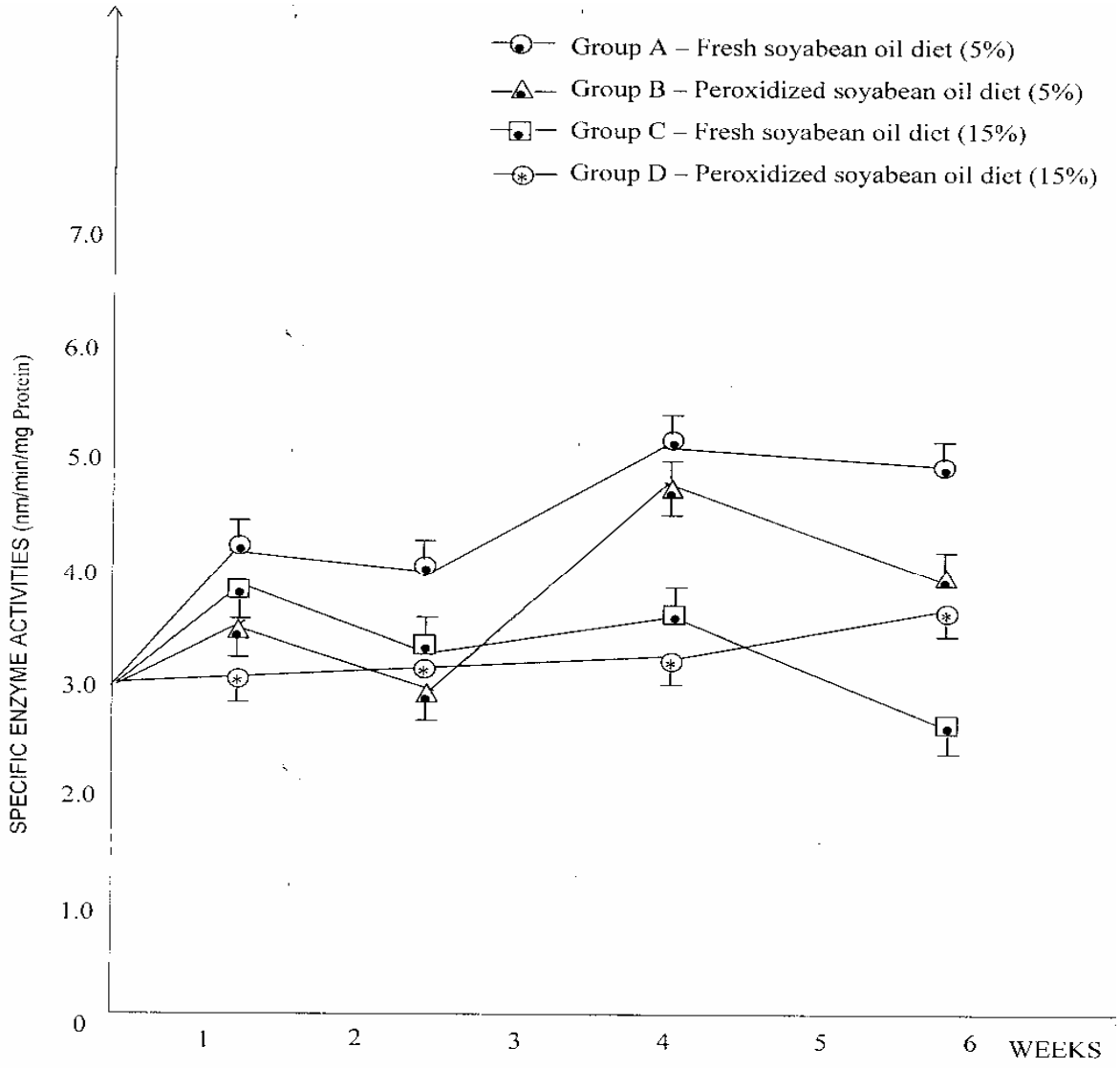

FIG. 2: Alkaline phosphatase specific activity of rat Femur following supplementation with peroxidized soyabean oil.

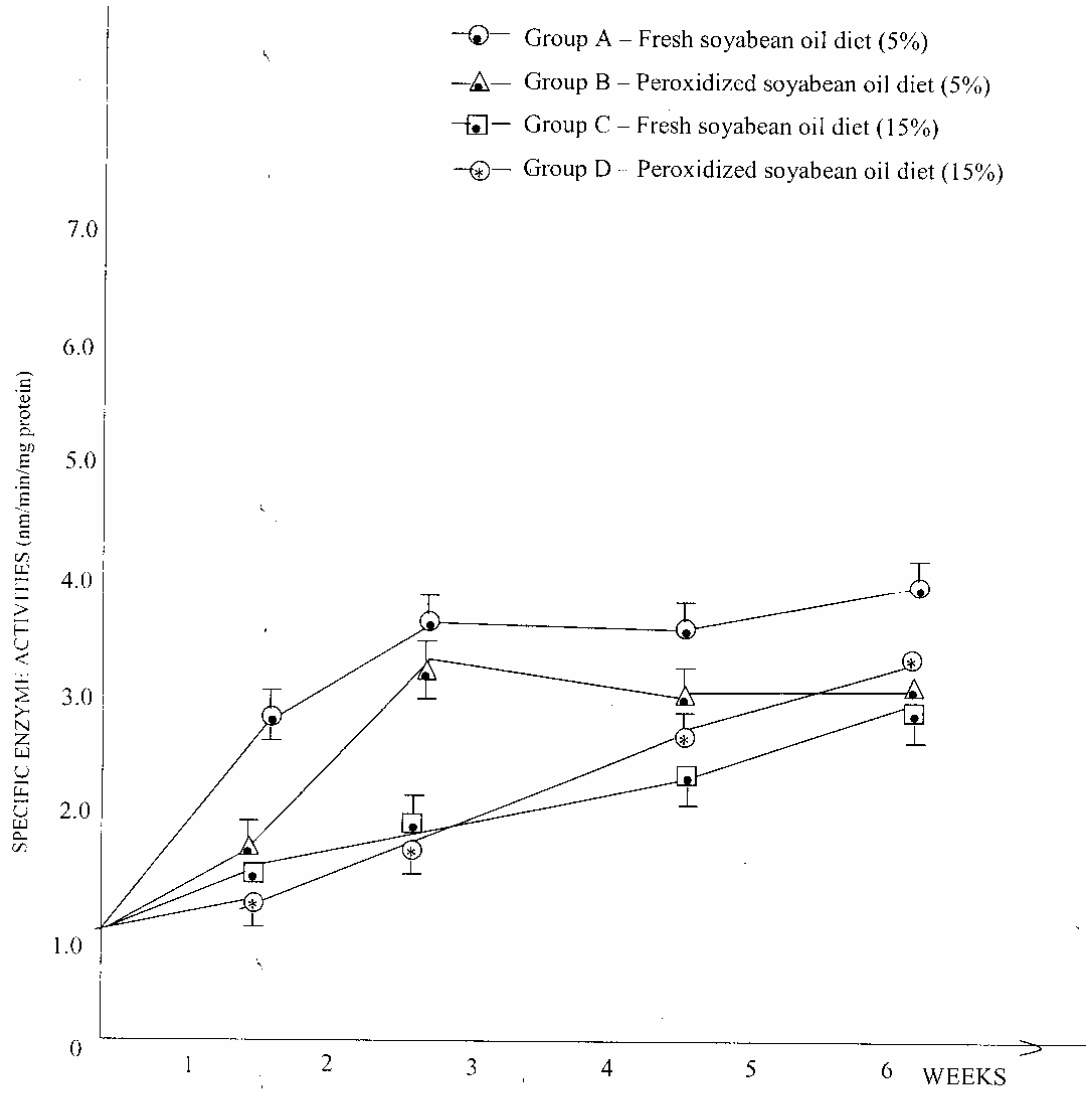

Fig. 3: Acid phosphatase specific activity of rat tibia following supplementation with peroxidized soyabean oil

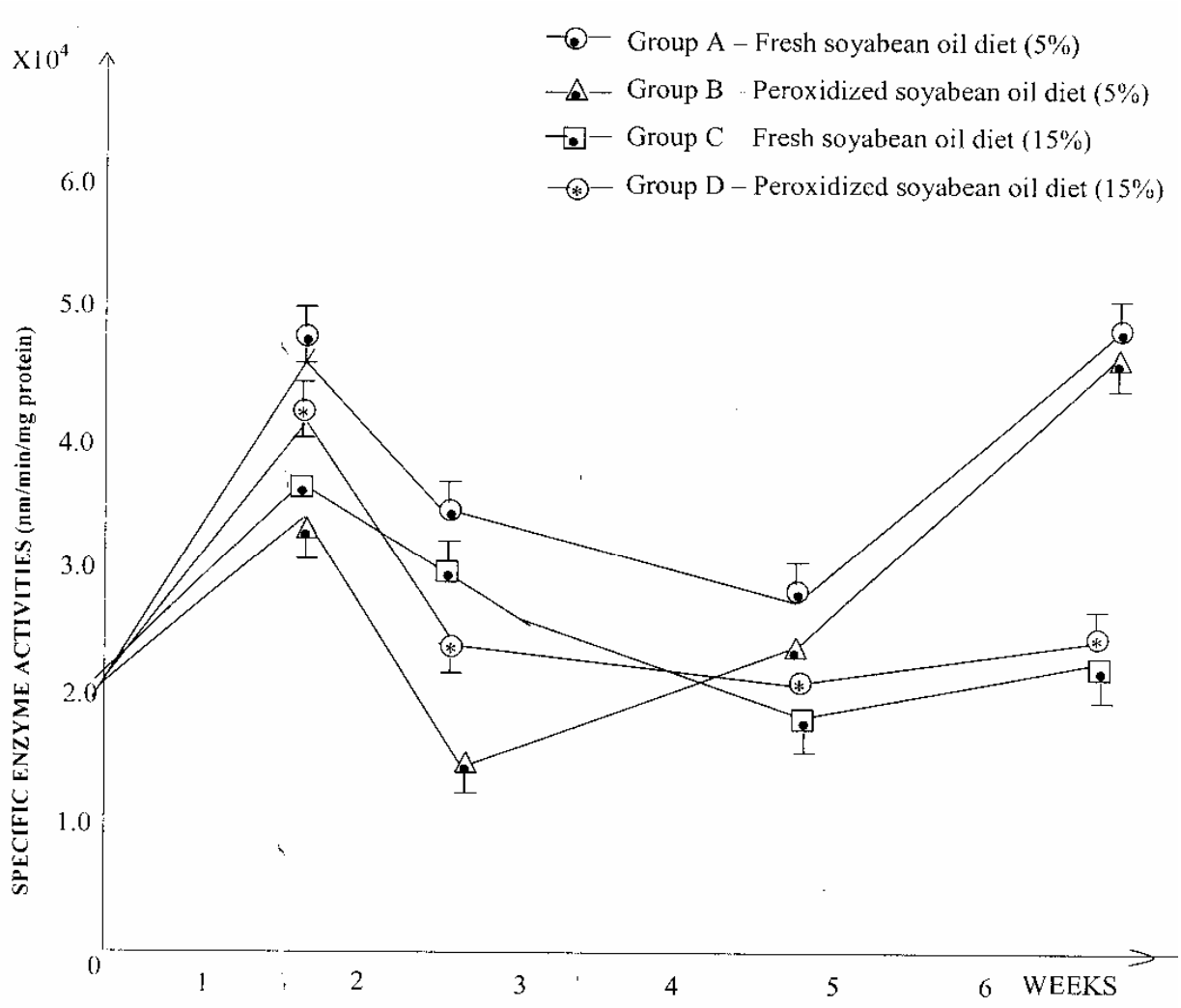

Fig. 4: Acid phosphatase specific activity of rat Femur following supplementation with peroxidized soyabean oil.

only in the first and sixth week in tibia and femur respectively. The acid phosphatase activities was increased in tibia during the fourth and sixth week whereas higher activities were found in femur in the first, third and sixth week of the experiment when compared to the control group. This result is not surprising because specific enzymatic reactions have been demonstrated to be capable of releasing peroxidized fatty acids from membranes and hence preventing the reduction of the enzyme activities. There was no significant difference in the enzyme activities (P>0.05). The correlation that exists between the activities of phospatase enzymes in peroxidized soyabean-oil groups and their respective control at 5% and 15% level indicates the degree of bone healthiness as a result of little or no effect due to the toxic intake of peroxidized soyabean oil products in the diets. The lack of serious adverse consequences observed in the activities of the enzymes studied is not surprising in view of the short feeding period.

CONCLUSION

The activities of acid and alkaline phosphatase was found to be reduced in bones (femur and tibia) of animals fed with diet supplemented with 5% peroxidized soyabean oil. This was found to agree with the work of Yoshika and coworkers (13), who reported that rat liver enzymes thiokinase and succinyl dehydrogenase had low activities when oxidized fats were fed. The activities of these enzymes were higher in 15% peroxidized soyabean oil when compared to the control. There was no significant difference between the activities of the enzymes in the bones of the animals when compared with the control group (P>0.05)

The enzymes assayed are marker enzymes and the intake of the peroxidized soyabean-oil in the diets have indicated little or no effect on the activities of the enzymes studied. This is in actual agreement with the view of miller and long (1) who reported no significant change in survival, enzymatic and morphological changes of laboratory animals under similar condition.

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