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# Studies on the Biochemical Indices of Guinea Pigs Fed with Mixed Spiced Diets.

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**Abstract:** In this study the biochemical effect of mixed spiced (*Piper guineense*, *Allium sativum*, and *Zingiber officinale*) diet (MSD) was investigated using Guinea Pigs as experimental animal. A total of forty (40) animals mean weight 1.202.63kg was divided into five to represent different experimental diet groups and the control, while feeding was done twice daily for a period of two months. Every two weeks, biochemical indices including protein, glucose, lipid profiles, and liver enzymes (aspartate and alanine transferases, and alkaline phosphatase) were analyzed. The result shows that the values of glucose, triacylglycerol, ($P \leq 05$) as compared with the control. However, the activities of liver enzymes increased though not significant ($p \leq 0.05$) throughout the period of the study. The implication of this result is that the MSD could be a very good nutritional therapy for such diseases like diabetes or atherosclerosis. The spices used are also relatively safe as the increases in the liver enzyme activities do not suggest liver toxicity.

**Key words:** Mixed spiced diet; Glucose, Lipids, Liver enzymes, Nutritional therapy.

## INTRODUCTION

The act of using spices as flavour enhancer in cooking is perhaps the differentiating factor between monotonous and dull food, and highly tasting and appetizing one. At times more than one spicing agents are used particularly in cases of special foods like during festivals. The use of spices whether exotic or indigenous has been based on their flavour enhancing ability without due regard or recognition of possible nutritional contribution. In

fact, some authors argue that spices do not contribute to nutrient content of foods (Purseglove *et al*, 1990), while others contend that they are invaluable sources of micronutrients (Ranjith and Relsbach, 1995, Nwachukwu, 2002).

Spices are also regarded to be generally safe based on the prolonged use without manifest health hazard. Toxicologically, prolonged use without any toxic manifestation is not sufficient proof of safety. There has to be scientific data to support the are *Zingiber officinale* (Ginger). *Piper guineense* (uziza) and *Allium sativum* (Garlic). Ginger is a slender perennial plant of the family zingberaceae, while Uziza is a climber of the family piperaceae (Purseglove *et al*, 1991). Garlic is a specie in the onion family, alliaceae. They all produce pungent aroma, and are found useful as flavouring agents in food, and as medicinal herbs. For instance, garlic has been shown to regulate blood glucose level and prevent complications of diabetes mellitus, and they prevents cancer due to the presence of allyl sulplur compounds like diallyl disulphide, and used as remedy against digestive disorders and fungal infections such as thrush (Alan *et al*, 1995; Sead *et al*, 2004). Also ginger has been found useful in the treatment of rheumatoid arthritis, ulcer, tumour, and cardiovascular diseases (Unriikrishman and Kuttah; 1998; Sulkawa and Yuasa, 1986).

Uziza has been found to be rich in minerals and vitamins, and useful in the treatment of bacterial infection, reduction of blood glucose and in arresting proliferation of cancer cells (Oniniwu and Ibeneme, 2002).

In this study we investigated the possible biochemical affect of these spices individually and in mixed form using guinea pigs as animal model. It is expected that the result will give insight to the possible mechanism of the supposed medicinal effects of these spices. It will also demonstrate possible toxic effect or safety of the individual spices or in mixed form.

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## MATERIALS AND METHODS

***Materials:***

The selected spices, *Allium sativum* (Garlic), *Piper guineense* (Uziza), and *Zingiber officinale* (Ginger) were purchased from Unuahia Central Market, Abia State, Nigeria, and were duly certified by a taxonomist at Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

***Preparation of Sample:***

The spices were sorted to select good, uninfected ones which were then sun dried to constant weight before grinding into powdered form using Arthur Thomas Laboratory Milling Machine. This powdered form was sieved with 1mm sieve and used to formulated the animal feed.

***Animal and Treatment:***

Forty guinea pigs, mean body weight 1.20±2.63 kg were bought from National Root Crops Research Institute, Umudike Umuahia, Abia State. They were group into five eight animal designated as A, B, C, D, and E to represent the control, and different diet groups as shown below. They were allowed to acclimatize for one week on normal animal feed (TOP feed, Nig. Ltd) before introducing experimental diet. Feeding was done twice daily while water was provided ad. Libitum to all the animal.

**Table 1:** Experimental Feeding Design.

| Groups | Normal feed (g) | Ginger (g) | Garlic (g) | Uziza (g) | Total composition |
|---|---|---|---|---|---|
| A | 100.00 | - | - | - | 100.00 |
| B | 90.00 | 10.00 | - | - | 100.00 |
| C | 90.00 | - | 10.00 | 10.00 | 100.00 |
| D | 90.00 | - | - | 10.00 | 100.00 |
| E(MSD) | 70.00 | 10.00 | 10.00 | 10.00 | 100.00 |

A = Control, B = Ginger alone, C = Garlic Alone, D = Uziza alone,

***Serum Preparation:***

Eighteen hours from the last feeding, once every two weeks, two animals from each diet group and control were sacrificed with the use of scapel. Sterilized dissecting sets were used to eviscerate the animals and blood collected through cardiac puncture into a test tube and allowed to stand for 30min. clotting time at room temperature before centrifuging at 600rpm for 30min. The supernatant was collected and used for analysis.

***Determination of Serum Glucose and Lipids:***

The method of Trindax (1995) is used to determine glucose content, while the method of Tietz *et al*, (1975) was used to determine the serum level of triacylyglycerol, cholesterol, and high density lipoprotein. Low density lipoprotein was determined according to the method of Curtis *et al*, (1995).

***Determination of Enzyme Activities:***

The serum activities of aspartate, and alanine amino transperases were assayed according the method of Reitman and Frankel, (1957), while the activity of alkaline phophatase was assayed according to the method of Moss *et al*, (1986).

***Results:***

In table, 2, the result shows that the serum glucose, (GLU), cholesterol, (CHO), Low density lipoprotein (LDL), and triacyglycerol (TG) generally decreased significantly ($P<0.05$), with diet group D, and C having the greatest reduction effect in serum glucose and lipid contents respectively. However, high density lipoprotein content generally increased in all the diet groups with diet group B exerting the greatest increase. Table 3 shows that the activities of AST, and ALP, significantly ($P<0.05$) increased in all the diet groups as compared with the control . Diet group C exerted the greatest increase in the activities. When the chronic effect of the spiced diet was investigated by extending the period of study to 8 weeks (Fig 1-8), similar results of decreases (GLU, CHO, TG, LDL,) and increases (HDL, AST and ALP) were sustained in all the diet groups throughout the period of study.

**Table 2:** Effect of Spiced Diet on Serum Glucose and Lipid Content (mg/ml).

| GROUPS | GLU | CHO | HDL | LDL | TG |
|---|---|---|---|---|---|
| A | 100.50+0.28 | 194.10+0.14 | 56.25+0.03 | 71.95+0.03 | 145.00+0.02 |
| B | 98.01*+0.01 | 190.10*+0.14 | 64.13*+0.04 | 61.11*+0.01 | 142.70*+0.14 |
| C | 96.77*+0.03 | 185.70*+0.14 | 64.49*+0.01 | 57.21*+0.03 | 140.80+0.42 |
| D | 98.63+0.01 | 189.20*+0.15 | 64.40*+0.04 | 61.08*+0.04 | 140.20+0.28 |
| E (MSD) | 98.95*+0.01 | 191.70*+0.08 | 64.31*+0.01 | 63.33*+0.03 | 140.90*+0.28 |

**Table 3:** Effect of SD on Serum Hepatic Enzyme Activities (1.U/L) Enzymes.

| CONTROL | AST | ALT | ALP |
|---|---|---|---|
| A | 11.50±0.03 | 11.50±0.03 | 34.63±0.07 |
| B | 20.50*±0.03 | 16.50*±0.01 | 37.54*±0.06 |
| C | 22.50*±0.03 | 15.50±0.01 | 37.54*±0.04 |
| D | 14.50*±0.03 | 15.50±0.03 | 37.05*±0.01 |
| MSD | 19.50*±0.06 | 15.50±0.04 | 37.70*±0.03 |

*Significantly different (p=0.05)
All determinations are 3+ SD

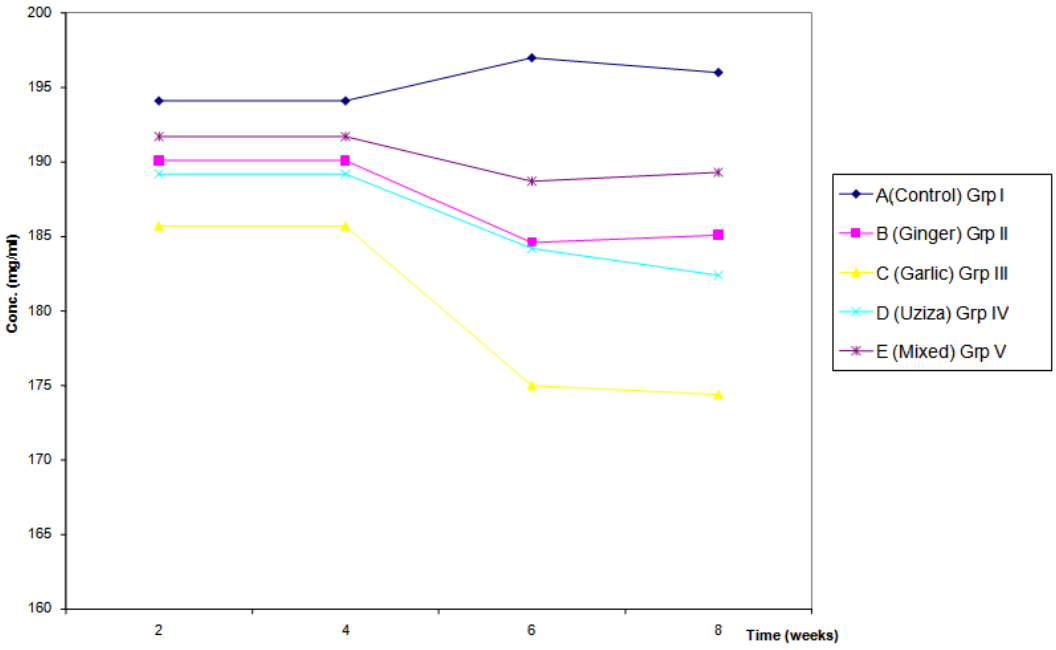


**Fig. 1:** Time dependent influence of SD on serum cholesterol (mg/ml).

***Discussion:***

In table 2, the serum glucose content decreased as compared with the control in all the diet groups with group C (Garlic alone) having the highest reduction effect. It is important that this hypoglycemic effect was sustained throughout the period of the study (Fig. 8). According to Bening *et al*, (2000), hypoglycemic effect may arise due to peripheral utilization of glucose, and also blockage of epinephrine action in glycogenolysis. Possibly these spices may have acted as biological response modifiers stimulating insulin secretion leading to increased utilization of glucose or dilated peripheral blood vessels enhancing glucose transport and utilization (Nwachukwu *et al*, 2007, Nwachukwu and Igwenyi, 2006).

There is no added advantage by using mixed spices, since diet group C had the highest reduction effect. Therefore in cases where there is management of diabetic condition, it is better to use individual spices rather than compounded ones. On the other hand, where traditionally function of the spices (enhance taste and palatability) is of priority, compounded spices are better.

The results of serum CHO, TG and LDL (Table 2) contents decreased, while that of HDL increased as compared with the control. Similarly, these decreases and increases were sustained throughout the period of the study. Interestingly, diet group C had the least reduction (CHO, TG) and highest increase (HDL) respectively. Jack (1995) reported that garlic reduced LDL and thus the risk of circulatory problem. These spices could have stimulated pancreatic bile secretions which play significant role in increased absorption of cholesterol and hence hypocholesterolemia, (Murray, *et al*, 1990).

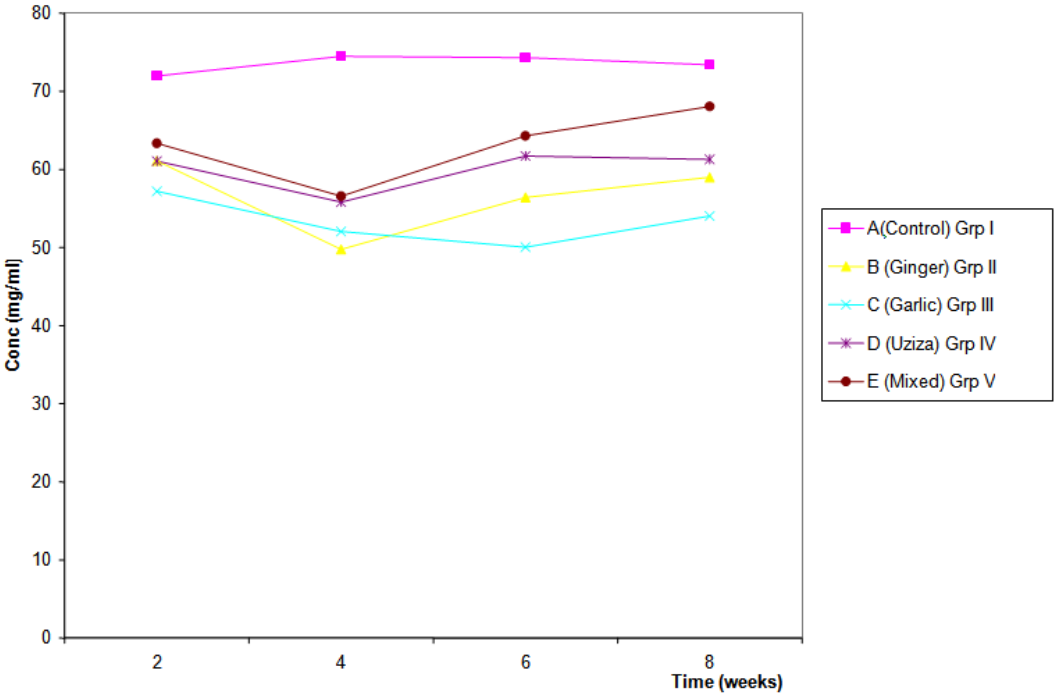


**Fig. 2:** Time dependent influence of SD on serum LDL (mg/ml).

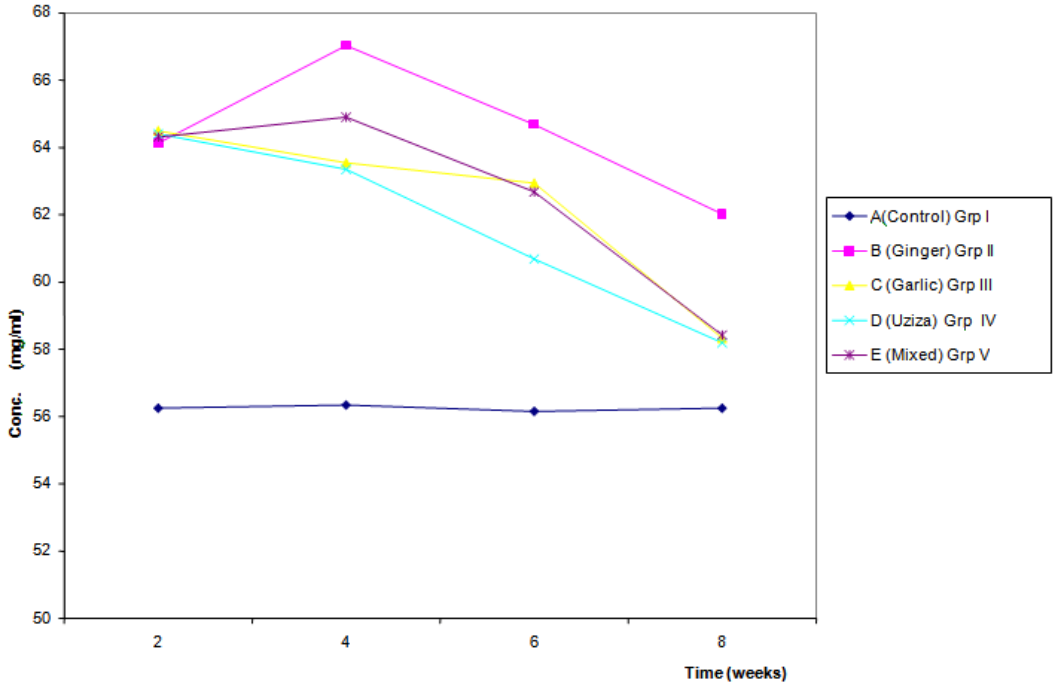


**Fig. 3:** Time dependent influence of SD on serum HDL mg/ml.

A recent theory of atherosclerosis recognizes that LDL content of the arterial intima sets the stage both for cholesterol build up and for cell responses to atherosclerosis,(Retention theory). Similarly, HDL can carry excess cholesterol from the intimal macrophages back across the endothelial cells into the blood stream, and back to the liver (Reverse cholesterol transport) where it is used for biosynthesis. Thus, atherosclerosis is simply a race between the balance of LDL bringing cholesterol and HDL removing it from the arterial intima,(Edward, 1991). This effect (lowering LDL, and increasing HDL) is clearly demonstrated, and it was shown that garlic alone is a better management option for coronary heart diseases as a result of hyperlipidemia rather than combination of spices.

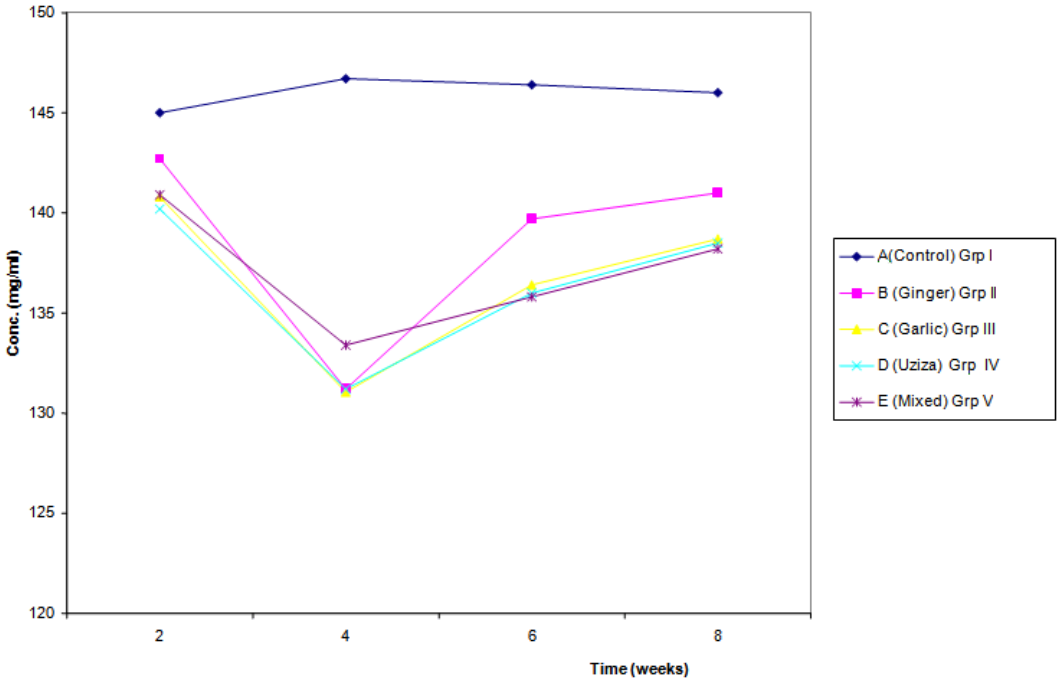


**Fig. 4:** Time dependent influence of SD on serum TG (mg/ml).

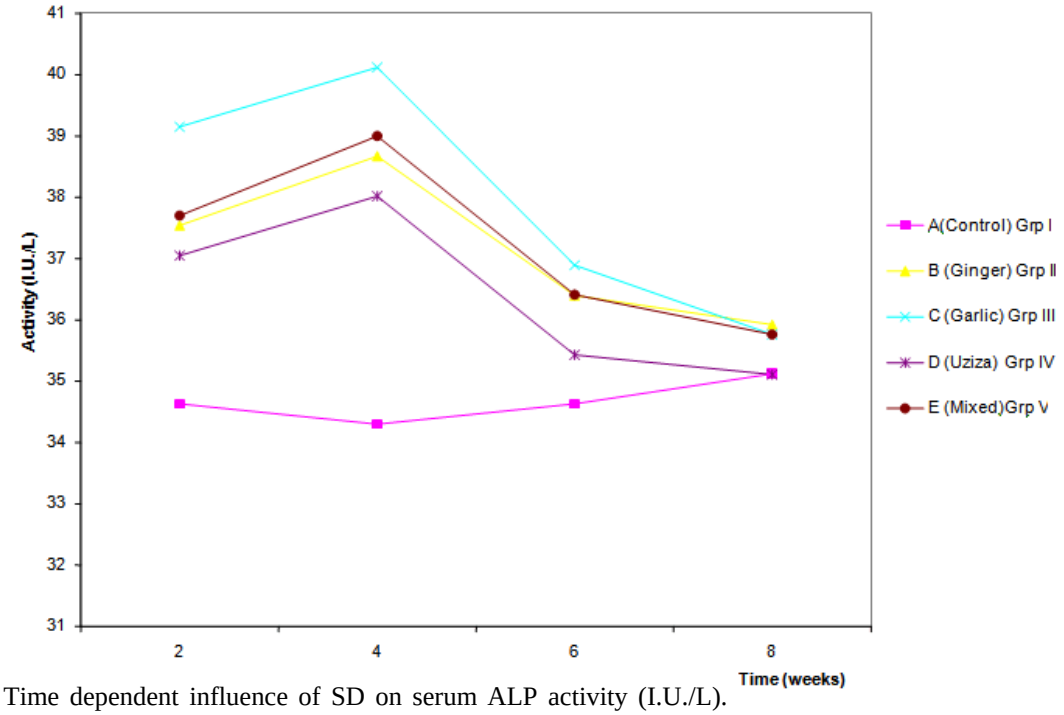


**Fig. 5:** Time dependent influence of SD on serum ALP activity (I.U./L).

Activities of AST, ALT, and ALP significantly increased in all the diet groups and throughout the study (fig 5-7) as compared with the control. Again, diet group C (garlic alone) had the highest increase in the enzyme activities. The liver integrity is commonly assessed by the activities of these enzymes.For instance, ALT levels are elevated in disorder that cause death of liver cells (viral hepatitis). In infectious hepatitis and other inflammatory conditions, AST and ALT levels may reach as high as 100 times upper limit (20-34i.u/L) though 20 to 50 fold elevations are usually encountered. In toxic hepatitis similar pattern of elevations of AST and ALT activities to that of infectious hepatitis are seen (William, *et al*, 1986).

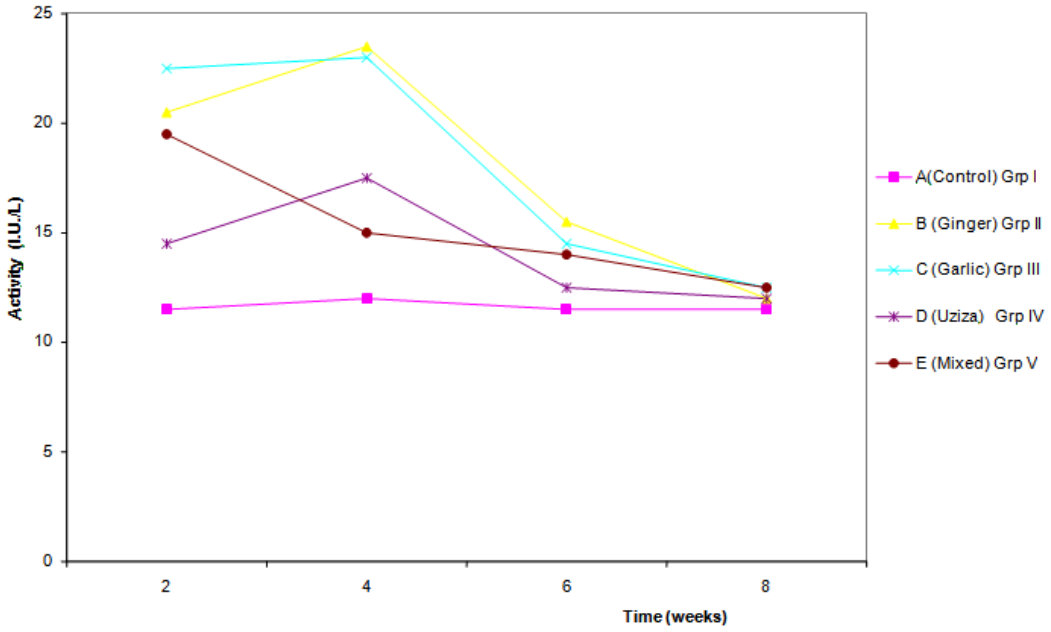


**Fig. 6:** Time dependent influence of SD on serum AST activity.

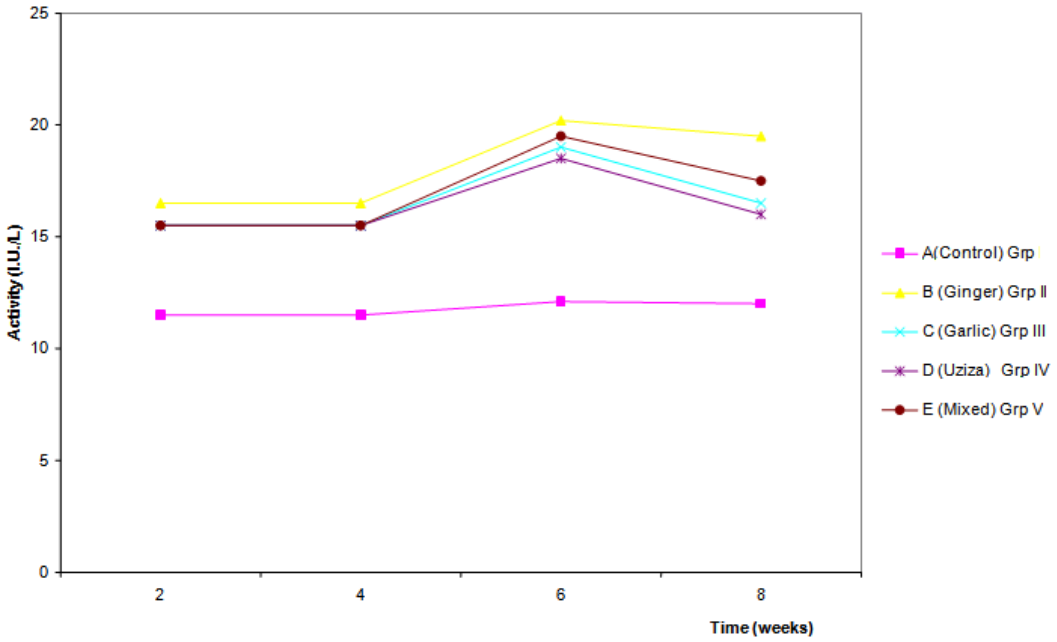


**Fig. 7:** Time dependent influence of SD on serum ALT avivity.

The elevation of ALP activities occurs in biliary obstruction and tends to be more pronounced in extrahepatic than in intrahepatic obstruction,(Moss *et al*, 1986). These elevations are not even upto twice the control values, and therefore may not be attributed to any toxic effect of the spices to the liver. It could have been probably due to normal metabolic response or activation of the enzymes by bioactive principles of the spices.

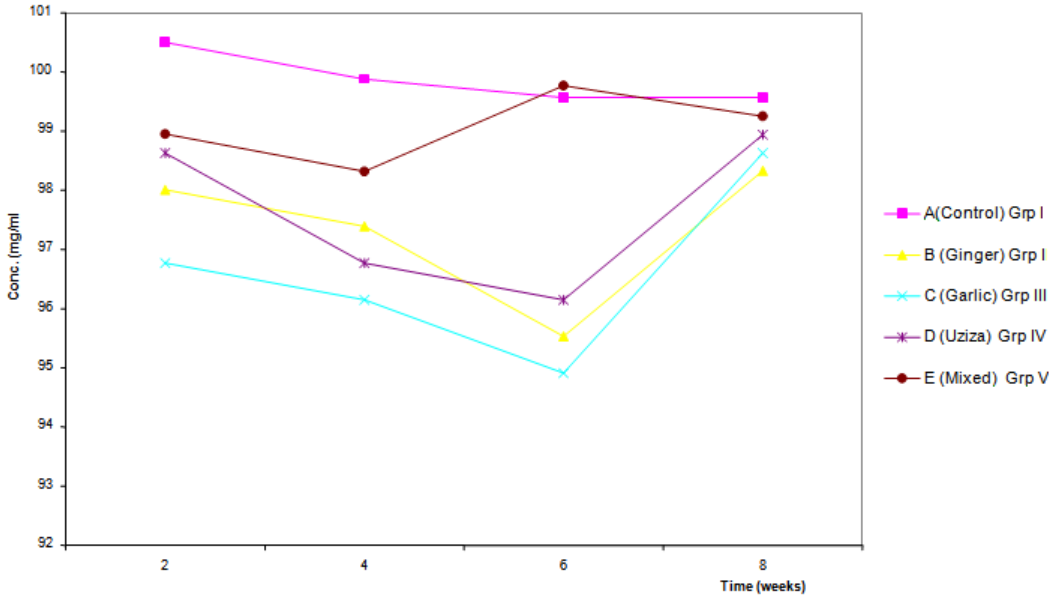


**Fig. 8:** Time dependent influence of SD on serum glucose content (mg/ml).

***Conclusion:***

The hypoglycemic and hypolipidemic effects of the spices individually and in combinations have been demonstrated with garlic alone possessing a greater effects. Medicinally, this is important in the treatment and management of certain diseases like diabetes, and coronary heart diseases. Secondly, the activities of liver enzymes (AST, ALT, and ALP) were elevated though not high enough to suggest liver toxicity. While those who are interested in the traditional use of the spices as taste enhancer may use compounds ones, there is no synergetic effect medicinally when they are combined.

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