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BIOCHEMICAL EFFECT OF *PIPER GUINENSE* DIET ON SOME ORGANS OF WISTAR ALBINO RAT

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ABSTRACT

The biochemical effect of *Piper guinense* diet on some organs; heart, kidney, serum, liver and brain of wistar albino rats was studied using glucose, protein, cholesterol and triacylglyceride content as biochemical indices. The activities of the following enzymes, Alanine transferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP) were also determined. The rats were divided into four different groups to represent control, low, medium and high dose of the *Piper guinense* "Uziza" diet. Feeding was done twice daily for seven days before the rats were sacrificed and the parameters determined. The result showed that both glucose and cholesterol levels increased over the control in all the organs and in all the groups. The activities of the enzymes ALP and ALT increased over the control, though the increases were not dose dependent. The activities of Alanine transferase (ALT) on the other hand increased over the control and the increase was found to be dose dependent. Also, the increase was statistically significant at $p<0.05$ level. The implication of the result is discussed.

KEY WORDS: Medicinal herb, liver enzymes; glucose; lipids; rats.

INTRODUCTION

Piper guinense (Uziza) is a slender perennial creeper with prominent nodes commonly found in high forest area. It belongs to the family piperacae with about ten genera and more than a thousand species. The leaves are ecliptic in shape with an aromatic smell when crushed. The fruits are in clusters and have a reddish-brown colour when ripe and turn black when dried. The chemical constituents include volatile and essential oil chevicine and piperine which volatilize at low temperature and are the main contributors to the flavouring and medical properties (Amosan and Okorie, 2002).

Piper guinense is one of the local spies commonly used in the flavouring of foods by most people in the Eastern part of Nigeria. The oil extract has been used as an insecticide and in folk medicine for the treatment of cough, intestinal diseases and rheumatism (Amosan and Okorie, 2002). Udoh et al., (2002) also found that the leaf and seed extracts possess a depolarizing neuromuscular blocking action in addition to other pharmacological properties.

The safety of most medicinal plants have been accepted on the basis of prolonged use without apparent hazard. There has to be scientific data to support the claim that these products are relatively safe. In this study, the biochemical effect of *piper guinense* enriched diet was determined in rats with a view to suggest the possible biochemical action of the active principle. Also the determination of the activities of the liver enzymes will be used as an index of liver integrity.

MATERIALS AND METHODS

MATERIALS

Dry leaves of "Uziza" were purchased at Eke Okigwe Market, Imo State, and were duly certified by a taxonomist. The leaves were sorted and the good one selected and ground into powdered form. This was stored in polythene bags at room temperature and later used to compound the feed for the rats.

FEED FORMULATION

The feed was formulated as shown below

GROUP	UZIZA LEAVES (g)	NORMAL RAT FEED (g)	TOTAL COMPOSITION (g)
Control	-	100	100
Group I	15	85	100
Group II	10	90	100
Group III	5	95	100

They were mixed, pelleted and fed to the animals twice daily for seven days. Each feeding therefore was made of 100g mixture of *Piper guinense* and normal feed as shown above. Only negligible quantity was left over.

ANIMALS AND TREATMENT

Sixteen healthy female rats, aged six weeks were separated into four groups of four rats each and housed in metal cages labeled A,B,C and Control. These represent high (15g), medium (10g), and low (15g) doses of the extract respectively and the control group received only water and normal rat feed. All the animals in these groups received water ad libitum.

PREPARATION OF SERUM

Eight hours after the last feeding, all the rats were sacrificed under light chloroform anesthesia. An incision was made transversely under the rib cage using a surgical blade and forceps. A 2.0ml syringe was used to collect blood into a centrifuge tube, and allowed to stand for 30 minutes clotting time. The tubes were centrifuged at 3000rpm for 5 minutes and the serum was collected and used for the analysis.

PREPARATION OF OTHER ORGANS

The kidney, brain and liver were carefully removed by dissection, washed in normal saline (30ml) and homogenized. The homogenates were stored at – 20^{0}C and used within 48 hours.

DETERMINATION OF PROTEIN, GLUCOSE, CHOLESTEROL AND TRIGLYCERIDE

Protein and cholesterol levels in the serum and the homogenate of other organs were determined by the method of Eaton (1986), glucose level by the method of Somogyi (1952), and triacylglycerol by the method of Gottfried and Rosenberg (1973).

ENZYME ASSAY

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined according to the method of Reitman and Frankel (1957) while Alkaline

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phosphatase (ALP) activity was determined using the method of King and King (1954) in the serum and the homogenate of other organs studied. Spectrophotometer (Sp 300: Optima) was used in all the analysis.

STATISTICAL ANALYSIS

The result are mean +S.D. of three (3) determinations. Variance of other results over the control was analyzed using single factor ANOVA. All differences were considered significant at $P \leq 0.05$.

RESULT

In table 3.1 and 3.2, the results show that the levels of glucose and cholesterol increased over the control in all the groups and for the doses. Similarly, the activities of the enzymes; ALT and also AST increased as compared to the control and in all the groups except for the value of AST in group I of the heart, which decreased (tables 3.3. and 3.4). The activities of ALP increased in all the groups, but decreased in all the groups of the brain and in group I of the liver and the serum.

TABLES:

Table 3.1: Glucose determination (mg/ml) in serum and organ homogenates

GROUPS	Serum	Brain	Kidney	Heart	Liver
CONTROL	38.0±1.5	54.0±0.5	20.0±2.4	29.0±2.0	145.0±0.0
GRP I	40.0±0.8	50.0±1.0	48.0±2.2	54.0±1.5	153.0±0.2
% Decrease /increase	5.2 ↑	7.4 ↓	140.0 ↑	86.2 ↑	5.5 ↑
GRP II	48.0±0.5	62.0±1.5	36.0±2.0	60.0±2.5	160.0±1.5
% Increase	26.3 ↑	14.8 ↑	80.0 ↑	106.0 ↑	10.3 ↑
GRP III	53.0±1.0	55.0±2.0	30.0±2.1	59.0±1.0	172.0±0.5
% Increase	39.41 ↑	1.8 ↑	50.0 ↑	103.4 ↑	18.6 ↑

Table 3.2: Determination of total cholesterol (mg/dl) in the serum and organ homogenate

GROUP	Serum	Brain	Kidney	Heart	Liver
CONTROL	90.0±0.5	200.0±1.0	14.0±0.1	10.0±2.1	143.0±1.0
GRP I	90.0±2.1	225.0±2.1	15.0±0.6	11.0±1.2	145.0±1.5
% Inc.	-	12.5 ↑	7.0 ↑	10.0 ↑	1.3 ↑
GRP II	160.0±1.2	300.0±2.5	27.0±0.2	18.0±1.5	158.0±0.5
% Inc.	77.7 ↑	50.0 ↑	92.0 ↑	80.0 ↑	10.4 ↑
GRP III	120.0±0.5	228.0±1.0	22.0±0.5	13.0±0.0	154.0±0.5
% Inc.	33.3 ↑	14.0 ↑	57.1 ↑	30.0 ↑	7.6 ↑

Mean +SD 3 Determinations

GRP = Group

% Inc = % Increase ↑

% Dec = % Decrease. ↓

Table 3.3: Activity of ALT (iu/L) in serum and organ homogenates

GROUP	SERUM	HEART	KIDNEY	LIVER	BRIAN
CONTROL	12.0±0.5	20.0±1.5	31.0±2.2	36.0±0.3	8.0±2.0
GRP I	15.0±0.5	32.0±1.8	36.0+2.5	40.0±0.5	10.0±2.1
% Inc.	25.0 ↑	60.0 ↑	16.1 ↑	11.1 ↑	25.0 ↑
GRP II	49.0±0.0	54.0±1.2	57.0±2.1	63.0±0.1	18.0±2.5
%Inc	308.3 ↑	170.0 ↑	83.9 ↑	75.0 ↑	125.0 ↑
GRP III	63.0±1.0	62.0±1.1	63.0±2.0	72.0±0.5	22.0±2.2
% Inc.	425.0 ↑	210.0 ↑	103.2 ↑	100.0 ↑	175.0 ↑

Table 3.4: Activity of AST (i.u/L) in the serum and organ homogenate

Group	Serum	Heart	Kidney	Liver	Brain
Control	8.0±0.2	23.0±2.5	13.0±1.5	23.0±1.5	4.0±1.0
Grp I	9.0±0.5	20.0±1.5	13.0±1.8	26.0±1.0	6.0±1.1
% Inc/Dec	12.5 ↑	13.0 ↓	-	13.0 ↑	50.0 ↑
Grp II	45.0±1.0	50.0±1.5	32.0±1.2	58.0±0.2	15.0±2.0
% Inc.	462.5 ↑	117.4 ↑	146.2 ↑	152.2 ↑	275.0 ↑
Grp III	14.0±0.3	38.0±1.0	32.0±1.1	42.0±0.2	9.0±1.5
% Inc.	175.0 ↑	65.2 ↑	146.2 ↑	82.6 ↑	125.0 ↑

All values are mean ± S.D. 3 Determinations

GRP = Group

% Inc = % Increase ↑

%Dec. = % Decrease ↓

DISCUSSION

The result shows that glucose level in all the organs increased over the control. The brain has the least increase, while the heart has the highest increase. The increases wee found to be significant at $P \leq 0.05$. Hyperglycemic effect could have been achieved through a depressive effect on the pancreas leading to low production of insulin or activation of vital enzymes like phosphofructose kinase, responsible for the synthesis of glucose. This spice therefore may not be ideal for diabetic individuals. The result also shows that the level of cholesterol in all the organs increased. The highest increase occurred with the medium concentration (10% group) of the sample, in the kidney, followed by the serum and the heart respectively; it was also found that the increase was dose dependent. This increase could have occurred due to synthesis of low density lipoprotein in the liver that transports cholesterol to the serum (Nelson and Cox, 2000). This probably explains why the increase in the serum is higher than that of the liver. Cholesterol is known to play an important role in the maintenance of membrane integrity. However, accumulation in the blood has been associated with atherosclerosis and hence coronary heart disease (Dawn et al., 1996).

The activities of aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) in all the organs when compared with the control increased. AST and ALT activities showed greater increase when compared with that of ALP. These increases were also found to be dose dependent, particularly for the ALP. Statistical analyses show that the increase were significant at $P \leq 0.05$. The increase in activities of these enzymes in the serum is important because it is an indication of cellular damage. According to Curthoy and Whatford (1995), they leak-out to the blood stream whenever there is hepatocellular damage. ALT activity inparticular is known to be highly elevated in cases of acute hepatitis, cirrhosis and toxicity of some drugs and chemicals. Very high levels suggest viral or severe drug induced hepatitis, or other hepatitis disease with extensive necrosis (Christein and Metzler, 1985). In the case of AST, elevations are associated with different types of liver diseases. For instance, very high elevation (greater than 20 times the normal value) may indicate acute viral hepatitis, drug induced hepatic injury or severe liver congestion; higher elevation (10 times greater than normal level) may indicate severe alcoholic cirrhosis, while low to moderate level (2-5 times greater than normal) may indicate metastatic hepatic tumors or fatty liver (Saskatchewan, 2000). Alkaline phosphatase activity on the other hand is generally measured to determine the integrity of the hepatobiliary system and flow of the bile into the small intestine. Elevation of ALP activity therefore is an indication of obstruction of the biliary system eg. obstructive jaundice.

SUMMARY AND CONCLUSION

The increases in activities of these enzymes in the serum were not as high as indicated above (not even upto 2 times the control value), except for the activity of ALT. Therefore there may not be any hepatocellular damage or injury in the use of *Piper guinense*.

Elevation of ALT activity alone may not be sufficient to predict liver toxicity.

Fortunately, as spice, it is utilized in little amount to achieve the traditional purpose of flavouring food. Also when it is used in folk medicinal it should be administered in low dose form (5% group).

The result also shows hyperglycemic and hypercholesterolemic effects which are disadvantageous to individual disposed to diabetic or cardiovascular diseases. This could be avoided by using small quantity.

TABLE 3.5: ACTIVITY OF ALP (I.U/L) IN SERUM AND ORGAN HOMOGENATES

GROUP	Serum	Heart	Kidney	Liver	Brain
Control	230.0±0.8	670.0±1.0	200.0±2.1	490.0±0.5	130.0±1.5
GRP I	220.0±1.5	85.0±1.5	172.0±2.1	463.0±0.5	85.0±1.5
% Inc/Dec.	4.4 ↓	41.7 ↑	14.0 ↑	5.5 ↓	6.2 ↓
GRP II	390.0±0.0	87.0±1.0	283.0±2.5	552.0±1.5	98.0±1.2
% Inc/Dec	26.1 ↑	45.0 ↑	42.5 ↑	12.7 ↑	24.6 ↑
GRP III	250.0±1.0	63.0±2.0	260.0±2.5	530.0±1.0	100.±1.0
% Inc/Dec	8.7 ↑	5.0 ↑	30.0 ↑	8.2 ↑	23.1 ↑

All values can ± S.D. 3 Determinations.

GRP = Group

% Inc = % Increase ↑

% Dec = % Decrease ↓

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