

Physical and Biochemical Studies on Excess Weight-Induced Rats Maintained on Mixed Spices-Supplemented Diet

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ABSTRACT

The research was conducted to assess some growth and biochemical changes in excess weight-induced male Wistar rats maintained on mixed spices-supplemented salty diet. The spices (onions, garlic, ginger and cloves) were separately pulverized and thereafter mixed in ratio of 1:2:2:2 respectively. Eight (8) Wistar rats (151.01 ± 1.12 g) were randomly divided into two groups (A and B) of four (4) rats each with nearly equal average weight. Three experimental diets, A, B and B* were formulated. Diet A was 100% starter's mash (control diet) while Diet B was 6% salt of starter's mash and B* was 2% mixed spices of diet B. The rats in groups A and B were maintained on their experimental diets (Diet A and Diet B respectively) *ad libitum* for four weeks. Diet B was then removed and replaced with diet B* and maintained on this diet *ad libitum* and the feeding trial continued for another three weeks. Growth, hematological and lipid profiles, and transaminase enzyme activities in the rats were determined. There was no significant variation ($p > 0.05$) in the feed intake between the group maintained on 6% salty diet (76.40 ± 3.20 g) and the control (82.93 ± 4.24 g). Feed conversion ratio (5.69 ± 1.13) was significantly lower while the average weekly weight gain (15.92 ± 2.39 g) was significantly higher in the group maintained on 6% salty diet when compared with the group on control diet. Supplementation of diet B with 2% mixed spices (Diet B*) caused significant ($p < 0.05$) decrease in weight gain (6.19 ± 2.01 g) and increase in feed conversion ratio (15.99 ± 4.23) in excess-weight induced rats. Excess-weight induced rats (Group B) maintained on 2% mixed spices of diet B had significantly ($P < 0.05$) higher PCV (41.33) and Hb (13.77), but there was no significant ($P > 0.05$) change in RBC (3.10), WBC (3.43) and Platelet (468.67) when compared with the control. Total cholesterol, low density lipoprotein, high density lipoprotein, and triglyceride were not significantly affected. The concentration of ALT was significantly ($p < 0.05$) higher in serum (36.67 ± 1.45 U/L) and liver (45.00 ± 2.65 U/L), but lower in heart (24.67 ± 1.76 U/L) and kidney (8.67 ± 0.33 U/L) of excess-weight induced rats (Group B) maintained on 2% mixed spices of diet B. AST however, increased ($p < 0.05$) in kidney (24.67 ± 1.764 U/L). Therefore, 2% supplementation of metabolic disease-causing diets with mixed spices (onions, garlic, ginger and cloves) may have positive effects on reversal of excess weight gain, lipid metabolism, blood formation and organ protection in subjects fed on the diets.

Keywords: Mixed-spices; Excess weight-induced; Biochemical; Hematology; Transaminase; Lipid.

INTRODUCTION

Overweight, which is excessive fat accumulation is a risk factor for so many non-communicable diseases such as cardiovascular diseases, diabetes mellitus and chronic respiratory diseases (WHO, 2011) and a major cause of premature death (Carmienke *et al.*, 2013). The reported increasing cases of overweight has been attributed to increase in consumption of more energy-dense, nutrient poor foods containing high levels of sugar and saturated fats, combined with reduced physical activity (WHO, 2003). Consumption of these types of food could lead to increased oxidative

stress which is thought to be the unifying factor in the progression of overweight related diseases (Delbosc *et al.*, 2005; Bloch-Damti and Bashan, 2005).

Numerous studies have confirmed the strong association between phytochemicals, antioxidant and fibre containing foods, and health (Khalesi *et al.*, 2015; Medina-Remón *et al.*, 2015). Spices such as ginger, clove, onion and garlic have been reported to contain antioxidants that are effective in scavenging free radicals. Lawal *et al.* (2015) reported syrup produced from the combination of onions,

garlic, tomatoes, ginger, water melon seeds, lemon and palm oil to have promising effect in the management of some of the traits of metabolic syndrome. This research was conducted to scientifically verify if supplementation of metabolic disease-causing diets with mixed spices (onions, garlic, ginger and cloves) could have effects on weight gain, lipid metabolism, blood formation and organ protection in subjects fed on the diets.

Materials and Methods

Pelletized starter's mash, product of Vital Feed, Jos, Nigeria was purchased from dealers in Dutsin-Ma Local Government. Fresh spices; ginger (*Zingiber officinale*), garlic (*Allium sativum*), onions (*Allium cepa*), clove (*Syzygium aromaticum*) were bought from Dutsin-Ma central market, Katsina state.

Experimental animals

Eight (8) male Wistar rats weighing were purchased from National Veterinary Research Institute, Vom, Plateau State. The rats were housed in standard wooden cages and allowed to acclimatize for one week before the commencement of the experiment.

Preparations of spices

Fresh bulbs of onions, garlic, ginger and cloves were carefully screened to remove spoilt ones. The spices (onions, garlic, ginger and cloves) were separately pulverized and thereafter mixed in ratio of 1:2:2:2 respectively. This was followed by thorough mixing to achieve homogeneity.

Formulation of experimental diet

The starter's mash (Vital feed, Jos) was mixed to form three experimental diets for two groups as follows:

Diet A: Diet based on 100% starter's mash (control diet)

Diet B: Diet based on 6% salt of starter's mash.

Diet B*: Diet based on 2% mixed spices of diet B.

These contents were mixed thoroughly to achieve homogeneity, pelleted and air dried for

six (6) hours after which they were packaged for use.

Experimental Design

The experiment was conducted in two stages;

Stage one

Induction of excess weight

The eight rats were divided into groups A and B with average weight of 151.01 ± 1.12 g. Each group contained four rats. Group A was given the control diet while Group B was given diet based on 6% salt of starter's mash (B) to induce excess weight. The rats were maintained on their respective diets and water *ad libitum* for four weeks.

Stage two

Determination of the effect of mixed spices in management of excess weight gain

Group A in stage one remained on diet A (control). In Group B (excess weight induced group), diet B (Diet based on 6% salt of starter's mash) was replaced with diet B* (2% mixed spices of diet B) and the feeding trial continued for another three weeks.

Determination of feed intake: To determine feed intake, the quantity of feed left over was subtracted from the quantity of feed served within 24- hour interval.

Feed conversion ratio: The weekly feed conversion ratio was calculated by dividing the mean of the feed consumed per week by the difference in weight per week.

Determination of body weight change: The rats were weighed at the commencement of the experiment using digital scale and thereafter on weekly basis to determine change in weight.

Animal sacrifice, collection of blood samples and preparation of tissue homogenate. After 49 days of feeding trial, the animals were weighed and sacrificed by anaesthetizing them in a jar containing cotton wool soaked in diethylether. Blood was obtained through the jugular veins in tubes containing EDTA as anticoagulant and in plain tubes. Serum was separated from the blood in plain

tubes by allowing the blood to clot for 3 hours. The clotted blood was spun in a bench top centrifuge at 1500 rpm for 15 minutes to obtain serum. The serum samples were thereafter separated into another set of plain sample tubes and kept in the refrigerator (Akanji and Ngaha, 1989). The sacrificed rats were dissected to remove the tissues of interest: liver, heart and kidney. The isolated tissues were weighed and a portion cut out, cut into very small pieces and then homogenized in ice-cold 0.25M sucrose solution (Akanji and Yakubu, 2000).

Determination of Haematological: Haematological parameters such haemoglobin (Hb), Packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells(WBC) and platelet were estimated using an automated haematology analyser, SYSMEX KX21-JAPAN.

Determination of biochemical parameters: Lipid profiles and transaminases (ALT and AST) activities were determined using commercially available Randox biochemical kits from Randox Laboratories Limited.

Statistical Analysis

Results were expressed as means ± SEM. Using statistical package program (SPSS 16.0 version), Student t-test was used to assess statistical significance where $p < 0.05$ was considered significant.

RESULTS

Feed consumption in rats maintained on 6% salty diet and in rats maintained on the control did not differ significantly ($p > 0.05$). Compared to the control however, rats fed on 6% salty diet had significantly ($P < 0.05$) lower feed conversion ratio but higher weight gain (Table 1).

Supplementation of 6% salty diet with mixed spices (diet B*) did not significantly ($p > 0.05$) change the food consumption pattern of excess

weight- induced rats (Group B), but significantly ($P < 0.05$) increased the feed conversion ratio and lowered the average weekly weight gain (Table 2).

Table 1: Feed consumption, feed conversion ratio and change in weight in rats fed on 6% salty diet (Induction of excess weight)

Parameters	Group A	Group B
Feed consumed (g)	76.40±3.20 ^a	82.93±4.24 ^a
Feed conversion ratio	14.10±6.76 ^a	5.69±1.13 ^b
Weight gained (g)	8.63±7.37 ^a	15.92±2.39 ^b

Results are means of 3 determinations per group ± SEM. Values along the row with the same superscript are not significantly different ($p > 0.05$); Group A: Rats maintained on 100% starters mash (control); Group B: Rats maintained on starter's mash containing 6% salt

Table 2: Feed consumption, feed conversion ratio and weight change in excess weight-induced rats fed on mixed spices-supplemented salty diet

Parameters	B	B*
Feed consumed (g)	79.34±3.20 ^a	82.07±3.09 ^a
Feed conversion ratio	4.57±0.35 ^a	15.99±4.23 ^b
Weight gained (g)	18.09±1.42 ^a	6.19±2.01 ^b

Results are means of 3 determinations per group ± SEM. Values along the row with the same superscript are not significantly different ($P > 0.05$), and are significantly different if the superscripts are different. Group B: Rats maintained on diet based on 6% salt of starter's mash (diet B) and water. Group B*: After four weeks of feeding on diet B by rats in Group B, diet B was replaced by diet based on 2% mixed spices of diet B and water

The plasma concentrations of platelet, MCV, MCH, MCHC, WBC and RBC in excess weight-induced rats fed on mixed spices supplemented-salty diet (diet B*) were not significantly different ($P > 0.05$) when compared to rats maintained on the control diet.

The result showed significant increase in PCV and HB in excess weight-induced rats fed on mixed spices supplemented-salty diet (Table 3). Compared to the control, rats fed on 2% mixed spices supplemented-salty diet showed no significant ($P < 0.05$) difference in lipid profile (Table 4).

Table 3: Hematological profiles of excess weight-induced rats fed mixed spices-supplemented salty diet

Parameters	Group A	Group B*
Platelet (1x10 ⁹ /l)	481.33 ± 8.67 ^a	468.67 ± 6.74 ^a
PCV (%)	33.67 ± 0.67 ^a	41.33 ± 2.03 ^b
HB (g/dl)	11.23 ± 0.23 ^a	13.77 ± 0.69 ^b
WBC (1x10 ⁹ /l)	3.53 ± 0.09 ^a	3.43 ± 0.15 ^a
RBC (1x10 ⁹ /l)	3.30 ± 0.12 ^a	3.10 ± 0.21 ^a
MCV (fl)	111.67 ± 5.67 ^a	133.33 ± 6.36 ^a
MCH (pg)	37.27 ± 1.87 ^a	44.57 ± 2.18 ^a
MCHC (g/dl)	33.27 ± 0.03 ^a	33.27 ± 0.09 ^a

Results are means of 3 determinations per group ± SEM
Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on 100% starters mash (control)

Group B*: After four weeks of feeding on diet B by rats in Group B, diet B was replaced by diet based on 2% mixed spices of diet B and water

RBC: red blood cells; HB: haemoglobin; PCV: Packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cells

Table 4: Lipid parameters of excess weight-induced rats fed mixed spices-supplemented salty diet

Parameters	Group A	Group B*
Total cholesterol(mmol/l)	2.6±0.00 ^a	2.63±0.33 ^a
Triglyceride (mmol/l)	1.00±0.00 ^a	1.01±0.58 ^a
HDL-C(mmol/l)	0.73±0.03 ^a	0.60±0.58 ^a
LDL-C (mmol/l)	1.48±0.33 ^a	1.50±0.23 ^a

Results are means of 3 determinations per group ± SEM
.Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on 100% starters mash (control)

Group B*: After four weeks of feeding on diet B by rats in Group B, diet B was replaced by diet based on 2% and water

The concentrations of serum and liver ALT were significantly (p < 0.05) higher in the excess weight-induced group maintained on 2% mixed spices-supplemented diet (Diet B*) as compared to the Control group while the concentrations in the kidney and heart were significantly (p < 0.05) lower (Table 5). The concentration of kidney AST was significantly (p

< 0.05) increased in the excess weight-induced group maintained on 2% mixed spices-supplemented diet as compared to the Control group. The concentrations in serum, liver and heart did not vary significantly (p > 0.05) (Table 6).

Table 5: ALT concentration(U/L) levels in excess weight-induced rats maintained on mixed spices supplemented diet

Organ	Group A	Group B*
Serum	34.33 ±5.48 ^a	36.67 ±1.45 ^b
Liver	42.33 ± 0.88 ^a	45.00 ±2.65 ^b
Kidney	13.33 ±2.91 ^a	8.67 ±0.33 ^b
Heart	28.00 ± 1.53 ^a	24.67 ±1.76 ^b

Results are means of 3 determinations per group ± SEM
.Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on diet based on 100% starters mash (control) and water.

Group B*: After four weeks of feeding on diet B by rats in Group B, diet B was replaced by diet based on 2% mixed spices of diet B and water

Table 6: AST concentration (U/L) levels in excess weight-induced rats maintained on mixed spices supplemented diet

Organ	Group A	Group B*
Serum	10.67± 0.33 ^a	10.33±0.67 ^a
Liver	12.00±1.00 ^a	11.93±0.67 ^a
Kidney	21.33± 0.33 ^a	24.67±1.764 ^b
Heart	14.00± 0.57 ^a	14.67±0.33 ^a

Results are means of 3 determinations per group ± SEM
.Values along the row with the same superscript are not significantly different (P>0.05), and are significantly different if the superscripts are different.

Group A: Rats maintained on 100% starters mash (control)

Group B*: After four weeks of feeding on diet B by rats in Group B, diet B was replaced by diet based on 2%

DISCUSSION

Feed consumption, feed conversion ratio and change in weight in rats fed on salty diet.

Although addition of salt has been reported to increase the palatability of many foods and encourage greater energy intake (Bolhuis *et al.*, 2012) by disrupting the satiety signal, the

insignificant increase in the food consumed by the rats on 6% salty diet could be due to high concentration of the salt. Key contents and physical properties of foods such as flavour, texture and taste affect satiety (Farran *et al.*, 2005). The significantly lower feed conversion rate in rats maintained on 6% salty diet indicates that 6% salty diet may cause a greater increase in weight as compared to the control. This is evidenced in the observed remarkably significant increase in weight of rats maintained on salty diets. Since feed consumption was not significantly different between the groups, the increase in weight could be attributed to the salt contents. This finding is in agreement with some studies that reported an association between sodium intake and adiposity measures, which are independent of energy intake (Yi *et al.*, 2015; Yoon, 2013; Larsen *et al.*, 2013). Although, the mechanism which may explain this relationship is not quite clear, findings from animal studies indicate that a diet high in sodium increases adipose tissue mass, and this is due to changes in insulin and glucose metabolism which favour fat accumulation (Fonseca *et al.*, 2007; Takada *et al.*, 2008). According to Mi-Kyung (2016), high sodium intake increases adiponectin level through the activation of adipose PPAR δ , and the enhanced adiponectin downregulates renal SGLT2. Therefore, long term consumption of salty diet could lead to obesity and metabolic syndromes such as diabetes.

Feed consumption rate, feed conversion ratio and change in weight in excess weight-induced rats fed on mixed spices supplemented-salty diet

The no effect of mixed spices supplementation on feed consumption indicates that the overall physical properties and key contents of the salty diet may not have been affected by supplementation with mixed spices. These properties such as palatability play significant roles in satiety and food consumption. Helene *et al.* (2010) reported that adding spices to the meals had only minor effects on total energy intake (kJ) and no effect on total food intake (g), water intake, hunger and satiety. The reduction in the weight of rats maintained on 2% mixed

spices of salty diet (Diet B*) could have been due to the pungent compounds in spices which have been reported to increase rate of metabolism and cause loss of weight (Jungbauer and Medjakovic, 2012). Aqueous extracts of clove (Broadhurst *et al.*, 2000) and ginger (Asdaq and Inamdar, 2011) have insulin-like biological activity and positive effect on weight reduction. Similarly, Kang *et al.* (2010) reported that 8 weeks' intake of an onion drink containing quercetin had anti-obesity effects in obese rats. It is evident from the finding in this work that these spices (onion, ginger, clove and garlic) could act synergistically to improve metabolic rate and decrease accumulation of excess weight.

Biochemical changes in excess weight-induced rats fed mixed spices-supplemented salty diet

Blood cells regulation to prevent excessive variation in their quality and quantity, according to (Guyton and Hall, 2004) is achieved via some specialized feedback mechanisms for the cells. The increase in packed cell volume (PCV) and haemoglobin concentrations in rats maintained on mixed spices supplemented salty diet suggests that bioactive contents of these spices such as alliin, allcin, eugenol could stimulate the erythropoetic synthesis of haemoglobin and increase the percentage red blood cells.

This is partly consistent with the finding by Enitan *et al.* (2012), who reported that PCV and haemoglobin were significantly increased in a dose-dependent manner in both onion and garlic treated groups when compared to the control.

The results of the lipid profiles are quite interesting since the problems of poor dieting and chunk foods bothers on lipid peroxidation and its concomitant effects (Muoio and Newgard, 2008). Lipid elevations, even if moderate, can result in severe risks. The insignificant difference could have been due to the ameliorating effects of the spices on the lipid parameters. Onion and garlic contain pungent sulfur compounds which according to

Lanzotti (2006) hinder among other things obesity and hypercholesterol. Ashraf *et al.* (2011) similarly reported that garlic reduces Total cholesterol and LDL cholesterol while increasing HDL to a greater amount in patients compared to the control. This may explain why garlic slows atherosclerotic plaque formation as reported by Budoff (2006). Ginger also has positive effect on lipid which include promoting cholesterol transformation into bile salts, promoting biliary excretion of excess cholesterol, improving blood viscosity by reducing platelet aggregation, and reducing inflammatory and oxidative damage to vascular endothelium by reducing thromboxane synthesis and favoring series 2 prostaglandins (Jillian, 2017). The mechanism for these effects is not very clear but allicin, an organosulfur compound found in some spices had been reported to inhibit HMG-CoA reductase in both rat hepatocytes and HepG2 cells (Gebhardt and Beck, 1996). The finding in this study indicates that dyslipidaemia which is always associated with poor dieting could be managed by supplementing the diets with spices mixed as shown.

Transaminases (ALT and AST) are predominantly found in the liver but are also localized within the cells of the kidney, heart, muscle and other organs. The significant increase in serum concentration of ALT in rats maintained on mixed spices supplemented salty diet could have been due to damage done to the kidney and heart since there was corresponding decrease in the activity of the enzyme in these organs. This indicates that salty diet and the consequent excess weight may have more deleterious effect on the kidney and heart rather than the liver. It is also probable that the inclusion of mixed spices restored the integrity of the liver from any damage that the salty diet might have caused. The inclusion of the mixed spices may have elicited increased synthesis of AST on the kidney as could be seen in the increased activities of AST in the kidney. The non leakage of this enzyme into the serum as was the case for ALT is not clear. It could however be due to ameliorating effect of the spices on the lipid bi-

layer of the organs. A serious limitation of this study is the fact that the damage/change caused by the salty diet on the studied parameters was not measured before supplementation with mixed spices. However, a large body of studies had established association of high salty diet with poor blood formation, dyslipidaemia and cytolysis (Ogihara *et al.*, 2002; Jian-Wei *et al.*, 2008; Lawal *et al.*, 2015).

CONCLUSION

It is our conclusion from the findings in this research that 2% supplementation of metabolic disease-causing diets with mixed spices (onions, garlic, ginger and cloves) has potential to decrease the average weekly weight gain, lipid metabolism, blood formation and organ protection in over-subjects fed on the diets.

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