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Chemical Analysis and Short-term Toxicological Evaluation of *Garcinia mangostana* Seed Residue in Albino Rats

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Abstract
The short-term toxicological evaluation of defatted *Garcinia mangostana* seed residue in rat feed has been investigated in order to determine its suitability as an additive in feed supplement. Proximate analysis of the *G. mangostana* seed residue (GMSR) showed that it had a high carbohydrate and low protein values: 71.02±0.79 % and 8.09±0.21 % respectively. *In vivo* experiment with albino rats fed with feed that had its wheat constituent totally replaced by GMSR lasted for six weeks. The albino rats appeared to suffer no toxicological effect and weekly monitoring of the rats showed good physical appearance. The rats in the test group displayed fairly similar body weight gain when compared with those from the control group. There was no significant difference between the haematological and histopathological results obtained for both the experimental and control groups. GMSR seemed to be a good replacement for wheat in rat feed.

Key words: Diet, *Garcinia mangostana*, proximate composition, total replacement, wheat.

1. Introduction

Garcinia mangostana (mangosteen) is native to Southeast Asia, Australia, Africa and Polynesia and has been used as herbal medicine to treat infections (Phongaichit *et al.*, 2006). In Indonesia, the indigenous communities have been using *G. mangostana* plant in different ways for the treatment of various infectious diseases. However, increasing consumption of *G. mangostana* in recent years, overexploitation and over-cutting of original plants in Indonesia has caused depletion of the natural resources. Mangosteen has become recently popular as an alternative medicinal product. *G. mangostana* is presumed to have combination of appealing subjective characteristics such as taste, fragrance and visual qualities, nutrient richness, antioxidant strength (Moongkarndi *et al.*, 2004) and a potential impact for lowering the risk of human diseases (Pedraza-Chaverri *et al*., 2008). The pericarp have been used as a traditional medicine for the treatment of diarrhoea, skin infections and chronic wounds in South East Asia for many years and are nature's most abundant sources of chemical substances such as xanthones (Pedraza-Chaverri *et al*., 2008; Suksamrarn *et al*., 2006) isoflavones, tannins and flavonoids (Yu *et al.*, 2007) extracted from the rind which possess numerous bio-active properties. The safety of *G. mangostana* pericarp extract has been shown by an oral toxicity study in rats. Recent studies has shown the effect of diethylnitrosoamine (DEN) on the bio-chemical profile of the liver tissue of rat and also the protective effect of the pericarp extract of *G. mangostana* against DEN induced hepatocellular carcinoma by using Fourier Transform Infra Red (FTIR) and auto fluorescence spectroscopy (Manoj *et al*., 1999; Margarat and Jagadeesan, 2000; Rigas and Wong, 1992; Vishnu *et al.*, 2010).
Investigations on the effects of crude saponins and condensed tannins in mangosteen peel on rumen microorganisms and fermentation, microbial protein synthesis and nutrients digestibility in some animals has also been carried out. Fungal endophytes from leaves and small branches of *G. mangostana* have been isolated and identified **(**Radji *et al.*, 2011). In this study, the effect of total replacement of wheat with defatted *G. mangostana* seeds in rat feed is being looked into.

1.1 Materials and methods

1.1.1 Plant material

Garcinia mangostana fruits used for this work were collected in the month of February, 2011 from the Botanical Garden of the University of Ibadan, Oyo State, Nigeria. The seeds were removed from the fruits, washed with water and left to air dry for two days at room temperature.

1.1.2 Sample preparation

The seed husks were decorticated manually to give the kernels of *Garcinia mangostana* which were ground using a domestic grinder to give the grits. The crude oil was extracted with a soxhlet (40–60 ^{o}C) extractor using normal hexane as solvent. The oil obtained, after distilling off the hexane, was stored in a labeled flask. The defatted seed flour was desolventized and kept at room temperature for 48 hours. They were then packed in transparent plastic bucket prior to further analysis.

1.1.3 Proximate analysis of defatted seeds and compounded feeds

The moisture content of the seed residue and the compounded feed was determined by drying a representative 2 g sample in an oven with air circulation at 105 ^{o}C (Ajayi, 2009). Nitrogen content was estimated by the kjedhal method (El-Adawy and Taha, 2001). Crude protein was calculated by multiplying the evaluated N by 6.25. Crude fat and ash of the seed residue and the compounded feeds were analyzed according to AOAC (1990). Carbohydrate was content was determined by the [100 (protein + ash + crude fat + crude fibre + moisture content)] (Ajayi *et al.*, 2007).

1.1.4 Feed compounding

A basal diet was formulated to meet the entire nutrient requirement for young albino rats averaging 100 %. The diets were prepared according to the procedure described by Souza *et al*. (2007) with slight modification. The ingredients used for the control diet were 2800.00 g of maize, 1274.70 g of soy bean, 231.00 g of calcium, 55.30 g of salt, 994.00 g of groundnut cake, 495.60 g of palm kernel cake, 495.60 g of wheat, 495.60 g of corn bran and 158.20 g of oyster shell while 495.60 g of GMSR totally replaced wheat in the experimental diet (Table 1). The ingredients were weighed to be 7000.00 g for each diet respectively.

1.1.5 Animal, diets and feeding

The weight of the 8 weeks old albino rats (n = 14) used in this work ranged from 114.29± 9.76 to115.71±19.88 g. The rats were divided into 2 groups of A and B for control and experimental group respectively. During the 6 weeks of the experiment, the rats were fed the compounded diets as shown in Table 1. All rats were fed *ad libitum*. They had unrestricted access to drinking water. The feed intake and body gain were monitored daily weekly following the method described by Leontowicz *et al*. (2007).

1.1.6 Haematological analysis

At the end of the feeding period of six weeks, the rats were fasted over night. Haematological analyses were carried out in about 3 ml of rat blood collected into EDTA bottles through ocular puncture. The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC), white blood cell (WBC), differential WBC counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined and calculated respectively using the standard technique as described by Jain (1986).

1.1.7 Tissue pathology

Histological analyses of the heart, liver, kidney, lungs intestine, spleen and brain samples were carried out. Small portions of these tissues already harvested and stored in formalin were fixed and put through series of dehydration in graded concentration of xylene. They were embedded in wax, sectioned at 5 µ and transferred to clean glass slides. The thin sections were stained with haemotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes following the method outlined by Jain (1986). These tissues were observed for gross lesions.

1.1.8 Statistical analysis

Differences between groups were tested by two-way ANOVA and Duncan test. The p values of <0.05 were considered significant.

1.2 Results and discussion

1.2.1 Proximate composition

The proximate composition of *G. mangostana* seed residue is shown in Table 2. The moisture content of 10.47±1.12 % is high when compared to groundnut (Onyeike and Acheru, 2002), *Treculia africana* and *Artocarpus heterophyllus* (Ajayi, 2008), but quite low when compared to raw *Pentaclethra macrophylla* (Kingsley,

1995). The ash content is 1.80±0.02 % and it is closely similar to that of *Opuntia stricta* seeds (Monia *et al*., 2005) but lower than those obtained for *Artocarpus camansi* (Adeleke and Abiodun, 2010) and African *Cucurbita pepo* (Younis *et al*., 2000). The total carbohydrate value of 77.51±0.79 % suggests *G. mangostana* seed residue as useful supplement in compounding animal feed. The protein content (8.10±0.22 %) is higher than 6.57 % reported for *G. mangostana* (Ajayi *et al*., 2007).

After compounding the feed, diet samples were analysed once more to ascertain their chemical composition. It was observed that there was an increase in the crude protein, ash and crude fat contents in the diet when compared to the pre diet compounding proximate values. Also, a decrease was noticed in the moisture content while the crude fibre content almost remained the same. Interestingly, moisture, crude protein and crude fat contents were not significantly different in both diets labeled groups A and B. It was only ash, crude fibre and carbohydrate contents that differed in the two diets.

1.2.2 Feed intake and body weight changes

The feed intake and resultant body weight changes of test and control rats are shown on Tables 3. There was a gradual increase in the quantity of feed consumed by rats in the two groups till the fourth week when there was a decrease assumed to be from cold weather though the normal pattern of feed intake continued afterwards. From Table 3, it could be seen clearly that the experimental diet was consumed more than the control diet. All the rats in both groups recorded steady increment in their body weight throughout the experimental period and no significant differences were noted within and across the groups.

1.2.3 Physical appearance

The physical appearance of the rats was normal throughout the six weeks of the experiment (Table 4). The eyes and the mouth of the animals used in their respective groups appeared normal throughout the period of the study. Both the test group and control group hairs were normal and there was no offensive odour perceived in the two groups.

1.2.4 Organ weight

The weight of the seven organs collected for tissue pathology did not differ significantly from each other in both groups (Table 5). An average kidney weight of 0.83±0.26 g was noted for control rats while 0.75±0.27 g was noted for the test rats. Vishnu *et al*. (2010) had similar report for pericarp extract of *G. mangostana*.

1.2.5 Haematological analysis

Presented on Table 6 are the haematological parameters of both the test and control rats. There was significant difference in the platelet count. All the other haematological parameters did not differ significantly from each other in the two groups. Thus, indicating that the diet compounded with *Garcinia mangostana* seed residue had no adverse effect on the blood of the rats under study as it compared favourably with the indices obtained for the diet compounded with wheat. The haematological values obtained from this study are similar to what was reported in the toxicity study of *Garcinia mangostana* pericarp extract in rats (Vishnu *et al*., 2010).

1.2.7 Hitopathological analysis

There was moderate locally extensive neuronal degeneration and necrosis, multiple micro cavities and swollen endothelial cells, circumscribed focus of neuronal degeneration and possible gliosis in the brain of the test and control rats but it was noticed that some in group B had no visible lesion. Moderate proliferative thickening of the interalveolar septae and presence of basophilic materials trapped within a fibrin meshwork within a blood vessel was observed in the lungs of rats in group A but those in group B had mild thickening of the alveolar interstitum with extensive accumulation of neutrophils, a few lymphocytes, plasma cells and formation of germinal centres within the bronchial-associated lymphoid tissue. Widespread foamy appearance of the cytoplasm, slight dissociation and individualization of the hepatocytes were observed in all the livers but some liver tissues in group A had no visible lesion. Slight thinning of the hepatic cords was also observed in group A rats. Surprisingly, intraluminal parasite (likely to be a flatworm), mild to severe loss of villi and hypercellular appearance of a few villi were seen in group B but moderate villi stunting with necrotic surface epithelial cells were also observed in group A. Clearly, no visible lesions were observed in the heart, spleen and kidney of rats fed with both diets.

Conclusion

In the light of the result obtained from the total replacement of wheat with *Garcinia mangostana* seed residue in albino rat feed, it is probable that *Garcinia mangostana* seed residue could successfully be used to totally replace wheat in rat diet with other supplements. This will reduce the competition on wheat as most people on diet due to one health condition or the other base their staple food on wheat especially the diabetic patients and aged people.

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Table 1. Composition of the diets

Ingredients	Amount (%)	Group A (g)	Group B (g)
Maize	40.00	2800.00	2800.00
Soy bean	18.21	1274.70	1274.70
Bone	3.30	231.00	231.00
Salt	0.79	55.30	55.30
Groundnut cake	14.20	994.00	994.00
Palm kernel cake	7.08	495.60	495.60
Corn bran	7.08	495.60	495.60
Wheat/GMSR*	7.08	495.60	495.60
Oyster shell	2.26	158.20	158.20

**Garcinia mangostana* seed residue; A= Control; B= Experimental.

Table 2. Proximate composition of *G. mangostana* seed residue and the compounded diets

Parameters (%)*	GMSR	Compounded rat diets	
		Group A	Group B
Moisture content	10.47±1.12	9.26±0.57[a]	9.23±0.92[a]
Ash content	1.80±0.02	9.73±0.01[a]	9.01±0.01[b]
Crude protein	8.10±0.22	22.11±0.31[a]	21.89±0.00[a]
Crude fat	2.13±0.04	7.22±0.05[a]	7.07±0.04[a]
Total carbohydrate	77.71±0.79	51.72±0.43[a]	52.79±0.02[b]

*Values are expressed as mean ± SD; Means in the same row having the same letter are not significantly different ($P < 0.05$).

Table 3. Weekly feed consumption by rats and their body weight (g)

Week*	Feed consumed		Body weight	
	Group A	Group B	Group A	Group B
0			114.29±9.76	115.71±19.88
1	715	730	120.71±9.32	116.43±14.92
2	750	765	124.17±3.76	125.00±14.72
3	770	865	129.17±12.42	129.29±15.39
4	700	840	143.33±9.83	139.29±16.69
5	790	900	146.67±5.16	146.43±16.00
6	835	950	148.33±7.53	151.43±14.64

*Values are expressed as mean ± SD; A= Control; B= Experimental.

Table 4. The weekly physical appearances of the control and experimental rats

Week	Eyes		Mouth		Hairs	
	Group A	Group B	Group A	Group B	Group A	Group B
1	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++
3	+++	+++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++	+++
5	+++	+++	+++	+++	+++	+++
6	+++	+++	+++	+++	+++	+++

+++= normal.

Table 5. Weight of organs (g) of control and experimental rats

Tissue*	Group A	Group B
Kidney	0.75±0.27[a]	0.83±0.26[a]
Liver	4.25±0.69[a]	4.33±0.88[a]
Lungs	1.00±0.00[a]	1.00±0.00[a]
Heart	0.50±0.00[a]	0.50±0.00[a]
Spleen	0.50±0.00[a]	0.50±0.00[a]
Intestine	1.33±0.26[a]	1.67±0.26[a]
Brain	1.42±0.20[a]	1.50±0.00[a]

*Values are expressed as mean±SD; Means in the same row with the same superscripts are not significantly different at P< 0.05.

Table 6. Result of haematological analysis of control and experimental rats

Parameter	Group A	Group B
PCV (%)	42.17±2.64[a]	41.33±2.25[a]
RBC (10^6/µl)	6.99±0.43[a]	6.88±0.40 [a]
Hb (mg/dl)	13.88±0.89[a]	13.57±1.16[a]
MCV (fl)	60.32±1.40[a]	60.13±0.46[a]
MCHC (%)	32.93±0.68[a]	32.78±1.17[a]
WBC (10^3/µl)	5791.67±2218.43[a]	5658.33±2079.76[a]
Lymphocyte (%)	69.33±5.54[a]	57.50±13.59[a]
Neutrophis (%)	28.17±4.96[a]	41.33±13.91[a]
Eosmophis (%)	0.00±0.00[a]	0.17±0.41[a]
Monocytes (%)	2.17±2.14[a]	1.00±0.89[a]
Platelets (cells/cu.mm)	154666.67±26590.73[a]	132285.71±16819.77[b]

Values are expressed as mean±SD; Values in the same row with different superscripts are significantly different at P< 0.05.