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The Use of Bioassay (Albino Rat) in Screen Toxicity in Complementary Weaning Diets Prepared from Cereal-Based Meal and Vegetable Protein.

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ABSTRACT

The present study investigated the use of bioassay in screen toxicity in formulated weaning diets. Thirty (30) male rats (Rattus novergicus) were procured from farm house in college of Agriculture, Abubakar Tafawa Balewa University Bauchi, (ATBU). They were acclimatized for 10 days in well ventilated cage and fed with Vital fed (control) and inspected on daily basis for appearance and any signs of toxicity being developed. Then, rats were randomly grouped into six groups (five animals each) and separately housed after adaptation period. Groups were provided with formulated diets and water offered. Thereafter, rats in all groups were anaesthetized and sacrificed on the 28days, some vital organs were harvested for histological, biochemical and lipid analysis were also evaluated. Biochemical and histological parameters were not significantly $p \leq 0.05$ different among the treatment and controls. However, the mean values obtained from lipid profiles were ranged: total cholesterol (3.24%-2.66%), triglyceride (1.60%-1.38%), HDLC (1.40%-0.88%), LDLC (1.00%-0.84%) respectively. The control feed has higher lipid contents than formulated diets; this may lead to obesity, high risk factor for developing other chronic diseases including T2D, CVD, osteoarthritis, certain cancers and neurodegenerative diseases. The results revealed that no significant pathological changes were observed in liver, kidney, heart and spleen of rats fed with formulated diets. In addition, there were no signs of toxicity and death recorded within the study period. These findings suggest that product may be safe and useful as an Immune adjuvant.

Keywords: Bioassay, Albino Rat, Toxicity, Weaning, Screen

1. INTRODUCTION

The use of various underexploited food materials in production of weaning food is on the increase, probably due to growth in human population with resultant hike in prices of foods. Toxicological implications of such products and their phytoconstituents on consumer's health are rarely investigated (Saad, B., Azaizeh, H., Abu Hijleh, G. and Said, O. (2006)).

Interactions between constituents in the food materials during processing and their adverse effect cannot be ruled out. Thus, it becomes imperative to ascertain the level of risk before launching such food product into market. One way of achieving this is through animal study. The use of small mammals such as rats whose body physiology and nutrient requirements closely resemble that of humans have been widely documented (Ani, A.O., Onweluzo, J.C. and Asogwa, I.S., 2012; Princewill-Ogbonna, I.L., Abagha, O.J., and Ijioma, S.N., 2015). Toxicology of infant weaning diets from underutilized materials like maize, soy milk, coconut milk, and carrot and date powder can be examined this way.

The transition from exclusive breast feeding and/or infant formula feeding to complementary food referred to as complementary feeding, typically covers the period from 6 - 24 months of age and is a very vulnerable period (WHO, 1998). This is because after 6 months of age, the contribution from human milk becomes progressively insufficient as a unique nutrient source in relation to the optimal requirements for growth and thus, a greater demand is place on the complementary food-part of the diet. It is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children less than five years of age worldwide and affecting subsequent optimal development (WHO 1998; Ikujenlola and Fashakin, 2005).In most developing countries, complementary/weaning diets are derived mainly from local staples such as cereals and tubers, with animal proteins used as supplements.

However, since animal proteins are expensive, attempts have been made to identify alternative sources of protein especially from plants (Ihekoronye and Ngoddy 1985; Metwalli *et al*., 2011). In Nigeria, many attempts to produce weaning food which are quite rich in protein and other nutrient by combination of cereals with various sources of rich protein from legumes have been reported (Ozumba *et al*., 2002; Okafor *et al*., 2008; Okafor and Ozumba, 2006). However, it is therefore desirable to study ways and means of developing less costly nutritious complementary food with adequate necessary nutrients to feed the young growing child and to help avoid protein and energy malnutrition that may be within the reach of the wider population. Since most commercially available weaning foods are expensive and not affordable by low income mothers, the problem of malnutrition in infants can be solved by the introduction of nutritious complementary food. In addition, the family diets to which some infants are weaned are also low in nutritional value.

Many investigators (Akinrele and Edward, 1971; Oyenuga, V.A, 1968 ;Akinyele, A.J,1995.) have reported that the majority of traditional foods are low in protein and that other nutrients are lost because of poor processing. Bulk is a major problem of the traditional West African weaning foods (Naismith, D.J, 1973;Eka and Edijala, 1972). For adults and older children, it is usually possible to achieve an adequate intake of protein and energy by increasing the daily intake of starchy foods of low nutrient density.

This is not possible for infants and small children because of the small size of their stomachs and the large volume of traditional foods that must be ingested to cover energy needs (Eka, O.U 1982). However, the use of foods of high nutrient density and frequent feeding schedules can help provide adequate food for in developing countries predisposes them to various infectious diseases, such as diarrhea, whooping cough, and acute respiratory infections (Serimshaw, N.S, *et al*., 1968). Solving the problems associated with weaning foods in Nigeria may involve improving the quality of traditional weaning foods, ensuring household food security, providing nutrition education, and improving the income-generating capacity of women.

Traditional weaning foods might be improved by combining them with locally available foods that complement each other in such a way that the new pattern of amino acids created by this combination is similar to that recommended for infants (Uwaegbuta and Nnanyelujo, D.O, 1987). Growth and activity. The problems inherent in the traditional weaning foods and feeding practices predispose the infant to malnutrition, growth retardation, infection, and high mortality. Studies have shown that one major cause of malnutrition during the weaning period is the low quality of weaning food given to the child when breast milk is no longer meeting its nutrient requirements (Akinyele and Omolola, 1986; Guro, A.T, *et al*., 1987). Protein–energy malnutrition is common among infants and children in the lower socioeconomic groups of developing countries.

As a result of socioeconomic factors, taboos, and ignorance, people from low-income groups seldom give high-quality weaning food to their children (Nnanyelugo, D.O 1985; Uwaegbute, A.C,1982; Brown, T.E and Pollit, T.E, 1996).Severe protein-energy malnutrition results in growth retardation and poor cognitive development (Ivanovic, D.M, *et al*., 2002; Ivanovic, D.M, *et al*., 2000; Leiva, B, *et al*., 1968). Development of functional food from maize, soy milk, coconut milk, carrot and date powder may alleviate the scourge of degenerative diseases such as overweight, obesity, and diabetes especially in the developing countries, where the cost of medication is quite unaffordable for many sufferers. However, knowledge of safety or otherwise of such product should be established as sparse information about this is available. The aim of the present study is to evaluate the effect of bioassay (experimental animal) in screen toxicity in formulated weaning food.

2. MATERIALS AND METHODS

2.1 Source of raw materials

Yellow variety of maize, local rice, coconut, Soybean, carrot and date palm used for this study were purchased from a local market in Bauchi State (Muda- Lawal).

2.2 Samples Preparation

2.2.1 Processing of malted maize flour

The method described by Ikujenlola and Fashakin, (2005) was used in the malting of the maize grain. The maize grain (1kg) with seed coat was weighed on the weighing balance. The grains were poured into a stainless steel tray and were winnowed to remove chaff and foreign particles. The grains were washed using clean portable water to remove other foreign particles Such as dirt, dust and contaminants. The grains were then soaked in portable water (steeping) in the ratio of 1:3 W/V (grain: water) at room temperature in a clean stainless steel bucket for 24 hours and was then drained. The grains after steeping were allowed to air rest for 1 hour. The grains was put into a jute sack with water been sprinkled intermittently on them. They were left to germinate at room temperature while spraying with clean portable water at the interval of 12hours. The malted maize was dried using sun drying. The grains were milled using attrition mill, sieved and packed into cellophane bag for further analysis.

2.2.2 Processing of malted local rice flour.

Processing of malted rice – Sorted clean grains of rice weighing 1000 g were steeped in water (1:3 w/v, grain: water) for 4 h. The steeped grains were then transferred to a wide container with cotton wool to allow for germination at room temperature (30°C) for 3 days. The washed germinated grains were dried in the oven at 35°C for a total of about 10 to 12 h. The grains were then cleaned of sprouts and hulls by hand rubbing and winnowing, after which they were dried in a forced-air oven at 50°C to a uniform colour.

The dried grains were ground to fine flour and passed through a 0.5 mm sieve (Elemo *et al.,* 2011).

2.2.3 Processing of soy milk.
Soybean seeds (1Kg) were cleaned by removing stones and foreign materials. The grains were then washed with clean portable water in order to remove dirt, dust, and other foreign particles. The cleaned soybean grains were then boiled for 30 minutes and were drained. After draining, the soybean grains were washed with clean portable water and were steeped by soaking in clean portable water for 24 hours with 6 hourly change of water. The test of the soybean grains were removed and washed again with clean portable water. The grains were then dried, milled and sieved. They were then packaged for further analysis, (Nyagaya 2008).

2.2.4 Processing of coconut milk.
Processing of coconut milk – The method described by Victor and Aniekpeno, (2016)was employed in preparing the coconut milk. The coconut was dehusked, cracked to separate the meat from the shell while the coconut water was poured into a container and stored for further use. The brown skin of the coconut meat was removed and the meat thoroughly washed and grated using manual grater. The grated coconut meat was mixed in a ratio of 1:1 with a solution containing 75% distilled water and 25% coconut water and allowed to stand in a water bath at stipulated temperatures and time . The slurry was then pressed and filtered through cheese cloth to remove the solid residue and recover the milk. The milk was pasteurized at 90°C for 30 min and allowed to assume room temperature (37◦C).

2.2.5 Preparation of carrot powder
Carrot tubers were prepared using the method described by Mohammed and Hussein (1994). The carrots were trimmed, scrapped, washed and cut into 1cn cube and thoroughly mixed. The carrot cubes were blanched in a water bath (Precision stainless steel, model- 184) at 70°C for 20mins with solution of 2% glycerol, 1% calcium chloride and 0.1% sodium metal bisulphate which were dissolved in distilled water (to prevent loss of carotenoid). Immediately after blanching, the carrot were soaked in distilled water contain ice cubes for 0◦C for 15mins to prevent further cooking. The blanched carrot cubes were placed in a stainless pan and dried in oven at 72◦C for 48hrs.After drying, the cubes removed and blend with food processor (Cuisinart, Smart powder Duet (R) BFP-703). The powder was later dried in food dehydrator at temperature of 70◦C for 15mins. It was dried until moisture content below 0.34%. The powdered carrot was sieved and package in cellophane bag for subsequent used.

2.2.5 Preparation of date powder
The dried date fruits were sorted, graded, cleaning and opened to remove the seeds. The dates were pouring in the stainless pan and dried in the cabinet drier at temperature of 72°C for 48hrs. .After drying, the dates were removed and blend with food processor (Cuisinart, Smart powder Duet (R) BFP-703). The powder was later dried in food dehydrator at temperature of 70◦C for 15mins. It was dried until moisture content below 0.34%. The powdered carrot was sieved and package in cellophane bag for subsequent used.

2.2.6 Preparation of formulated foods
2.2.6.1 Formulation of blended weaning diet: The malted maize, rice flour were mixed with dried powder of carrot together with date powder and soy milk was mixed together into slurry in ratio 80%:20%, 75%:25%, 60%:40% and 60%:40% respectively. The slurries were boiled at temperature of 100°C for 3-4mins in sauce pan and transfer into individual serving bowls were labeled and preserved at temperature of 4^{0}C for further evaluation.

Table1: Formulation table of blend diet prepared from maize, rice, soy and coconut milk.

Ingredient	ABC	CDE	EFG	GHI
Maize flour	80g	00g	60	00
Rice flour	00g	75g	00	60
Coconut milk	20ml	00ml	40ml	00ml
Soy milk	00ml	25ml	00ml	40ml
Carrot powder	5g	5g	5g	5g
Date powder	5g	5g	5g	5g

Key:
Control: 100% (Normal pellet diets purchase from vital feed Ltd.)
ABC: 80% (Maize flour) and 20% (Coconut milk).
CDE: 75% (Rice flour) and 25% (soy milk).
EFG: 60% (Maize flour) and 40% (coconut milk).
GHI: 40% (Rice flour) and 60% (soy milk).

2.3 Animals and treatment

Total number of thirty (30) male albino rats (*Rattus novergicus*) weighing between (100 and 200 g), obtained from the animal house in college of Agriculture, Abubakar Tafawa Balewa University Bauchi, were used. They were acclimatized for 10 days to well ventilated room at temperature 30 ± 4°C and relative humidity of 60%. They were housed in standard cages, fed *ad libitum* with standard rat feed (Vital Feeds Ltd., Kaduna, Nigeria) and clean water. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animal (pub. No. 58-23, Revised 1985) as reported by Akah, J.A, *et al*.,(2009). The study was conducted at veterinary medicine Department, Abubakar Tafawa Balewa University, main campus Gubi.

2.4. Animal handling

Rats were randomly grouped into six groups (five animals each) and separately housed after adaptation period. Groups were provided with formulated diets and water offered to them *adlibitum* (Akande, T.O, *et al*, 2014; Ani, A.O, *et al*., 2012). All animals were inspected daily for appearance of signs of toxicity and possible deaths. The same level of hygiene was maintained throughout the 28 days experimental period. This duration was based on the clinical trial conducted by Ismail, M.M, *et al*., (1998) in similar study. Thereafter, rats in all groups were anaesthetized and sacrificed on the 28days, the animals were thereafter quickly dissected and the liver, heart, spleen and kidneys harvested for histopathological assessment.

2.5 Biochemical profile

Some biochemical parameters (nitrogen retention (NR), protein efficiency ratio (PER), biological value (BV), true digestibility (TD), true digestibility (TD)), were determined according to the method described by AOAC, 2000.

2.5.1 Nitrogen retention

The nitrogen retained in the experimental animal was calculated as the algebraic difference between the foods and sum of both the fecal and urinary nitrogen for the collection period by kjeldhal method (AOAC, 2000)

NR=Ni – (FN + UN)
Where;
NR= Nitrogen retained;
Ni = Nitrogen intake in foods;
FN = fecal nitrogen
UN = Urinary nitrogen

2.5.2 Protein efficiency ratio (PER)

Protein efficiency ratio (PER) was calculated according to the method described by AOAC,(2000) as follows:

PER = weight gain/protein consumed

2.5.3 Biological value (BV)

Biological value (BV) was calculated according to the method described by AOAC, (2000):

Bv = (Ni – NF2) + (NU1 –NU2) X 100 / Ni – (NF1 –NF2)

Where,
Ni = Nitrogen intake of animals that were fed test food
NF1 = Nitrogen excreted in the faeces of animal that were fed test food
NF2 = Nitrogen excreted in the faeces of animals that were fed protein – free food
NU1 = nitrogen excreted in the urine of animals that were fed test food
NU2 = nitrogen excreted in the urine of animals that were fed protein – free food

2.5.4 True digestibility (TD)

True digestibility (TD) was determined according to the method described by AOAC, (2000)
TD = [(Nutrient in – Nutrient out) / (Nutrient in)] x 100
Net protein utilization (NPU)
Net protein utilization (NPU) was determined according to the method described by AOAC, (2000)
NPU = BV X TD/100

2.6 Histopathological analysis

Four rats from group (I–VI) were sacrificed through vascular dislocation, liver and Kidney was removed from the animals. They were weighed and subjected to Histopathological analysis (toxicity signs) after fixation in slides using the method of Adesiji, (1999).

2.7 Lipid profile analysis

On the last day of the experiment period, all rats were starved for about 3 hours and weighed. The rat was sacrificed and the blood was collected into bottles. The blood sample were analyzed by using cardio check cholesterol analyzer, strip method was used to determine total cholesterol.

High Density Lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol. Triglyceride (TG) cholesterol .Tissues were carefully removed from the sacrificed animals using a pair of gloves, dissecting set and collected in 15 ml 0.25 N sucrose and then homogenized . 1g each of tissue (liver, kidney, heart and spleen) was weighed and homogenized in ice-cold 10ml trios' buffer (pH 7.8)7. The homogenates were centrifuged at 2000 rpm for 10 minutes. The supernatant was carefully decanted into specimen bottles, kept frozen overnight to ensure maximum release of the enzymes in the tissue cells (Oyedemi *et. al.,* 2011).

2.7 Statistical analysis of data

All tests were replicated and data obtained were statistically analyzed using a one-way analysis of variance (ANOVA) and means were separated by Duncan's Multiple Range Test (DMRT) using the Statistical package for social science (SPSS) IBM VERSION 21.0 package. Significance was accepted at .05 probability level.

3. RESULT AND DISCUSSION

Table 2: Biochemical profile of rats fed with formulated diets.

Parameters	Control	ABC	CDE	EFG	GHI
Nitrogen retention (NR %)	1.24^{a}	0.64^{b}	0.66^{d}	0.82^{a}	1.04^{c}
Protein efficiency ratio (PER %)	4.29^{c}	2.76^{d}	2.84^{a}	3.18^{d}	3.20^{c}
Biological value (BV/%)	50.42^{a}	42.22^{c}	44.28^{b}	46.56^{d}	48.04^{b}
True digestibility (TD/%)	69.88^{a}	44.01^{b}	46.28^{d}	50.24^{c}	54.30^{a}
Net protein utilization (NPU/%)	52.38^{a}	42.00^{c}	45.24^{d}	46.08^{a}	48.22

Means with the same superscript in the same column are not significantly different ($P \leq 0.5$).

KEY:
Control: 100% (Normal pellet diets purchase from vital feed Ltd.)
ABC: 80% (Maize flour) and 20% (Coconut milk).
CDE: 75% (Rice flour) and 25% (soy milk).
EFG: 60% (Maize flour) and 40% (coconut milk).
GHI: 40% (Rice flour) and 60% (soy milk).

Investigation carried out on biochemical profile of rat fed with formulated diets, parameters in this study were not significantly $p \leq .05$ different among the treatment and were consistent with those of controls (Table 2). Similarly, the above result shows that Nitrogen retention (NR %) ranges from (1.24-0.64). Meanwhile, there was significant different ($p>0.05$) in protein efficient ratio of control followed by formulated diets GHI. The biological value of control was higher than the formulated diets. There was increase in true digestibility (TD %) of control () followed GHI. However, net protein utilization (NPU %) varies from (52.38-42.00). This result was in line with findings of Kazeem, K. and Gibson, L., (2018).

Table 3: Effect of formulated diets consumption on organ of rats

Parameters	Control	ABC	CDE	EFG	GHI
Kidney	0.74[c]	0.60[c]	0.62[a]	0.64[a]	0.66[b]
Liver	3.85[b]	3.68[a]	3.77[c]	3.80[d]	3.82[a]
Heart	0.60[a]	0.58[a]	0.58[c]	0.56[a]	0.56[b]
Spleen	0.64[a]	0.58[b]	0.58[a]	0.60[a]	0.62[b]

Mean values with the same superscript in a row are not significantly different (P< 0.05)

KEY:
Control: 100% (Normal pellet diets purchase from vital feed Ltd.)
ABC: 80% (Maize flour) and 20% (Coconut milk).
CDE: 75% (Rice flour) and 25% (soy milk).
EFG: 60% (Maize flour) and 40% (coconut milk).
GHI: 40% (Rice flour) and 60% (soy milk).

The result of pathological test assessed on liver, kidney, heart and spleen are presented in table 3.

There were no significant different in ($p<0.05$) pathological changes in organ of rats fed with formulated diets at different proportion, especially at EFG and GHI (60%:40% and 40%:60%) blended of maize flour. coconut milk and rice flour.soy milk. The liver showed no form of necrosis or lesions and any histopathological alterations and distortions. Similar trend was observed for the kidney histology. There was no sign of glomerular shrinking nor hemorrhage, necrosis, glomerular cells shrinking, and interstitial pneumonia. The heart dissection showed no trace of atherosclerotic lesions as result of higher level of cereals-based protein in the diets.

Whole cereal grains have been considered heart-healthy due to their high concentrations of fibre such as β-glucan (found in oats and barley), arabinoxylan, cellulose and pectins; however, protein from cereal grains has also been shown to have beneficial effects on the heart. A foxtail millet protein hydrolysate fed to spontaneously hypertensive rats (SHR) was effective in significantly reducing blood pressure and lowering the ACE activity and angiotensin II levels compared to the controls (Chen,J., *et al*., 2017). Rice protein hydrolysate was observed to have a protective effect on cardiomyocytes H9C2 against hydrogen peroxide-induced proliferation suppression and apoptosis (Yang,T. *et al*., 2012). A risk factor associated with the pathogenesis of atherosclerosis is high levels of LDL-cholesterol, which results in an increase in oxidative products of LDL (oxLDL) that bind to scavenger receptors on macrophages, promoting the formation of foam cells, which contribute to the development of atherosclerotic fatty streak lesions (Burris,R.L., *et al*., 2010).

However, Fermented rice bran (FRB) has also been evaluated for heart-healthy properties. Fermentation is a cost-effective method often used to produce antihypertensive peptides derived from food proteins. FRB has been shown to possess ACE inhibitory activity in vivo. Additionally, FRB has not only exhibited antihypertensive effects in stroke-prone SHRs, but has also been able to improve glucose metabolism as well as liver TC and TG levels (Alauddin, M.,*et al*., 2016). There is a possibility that non peptide compounds in FRB contribute to the antihypertensive effects. For this reason, the isolation of functional peptides in FRB and human clinical trials evaluating their effects are necessary.

Also, splenomegaly (spleen enlargement) was not observed from the blood cells of the rats. The changes in splenic function and morphology have been implicated in the pathogenesis of diabetes and obesity-related cardiovascular disease and kidney disease (Unruh,D., *et al*., 2015, Gotoh, K., *et al*., 2012).

Altunkaynak, B.Z. et al., (2018), reported that, feeding mice with a diet high in fat and addition of sugar for 12- weeks led to obesity, high blood sugar, elevated inflammatory markers and spleens that were 50% larger than those of mice fed on a standard diets. Meanwhile, spleen weight was reduced through exercise and treatment with genistein. It is isoflavone plants compound found in certain food such as soy beans, lupins and fava beans.

Table 4: Lipid profile of formulated diets consumed by rats.

Parameter	Control	ABC	CDE	EFG	GHI
Total cholesterol (TC %)	3.24^{a}	2.66^{a}	2.68^{c}	2.80^{d}	2.80^{a}
Triglyceride (TG %)	1.60^{a}	1.38^{c}	1.40^{b}	1.50^{c}	1.50^{a}
HDLC (%)	1.40^{c}	0.88^{b}	0.90^{c}	0.98^{a}	1.00^{b}
LDLC (%)	1.00^{d}	0.84^{a}	0.86^{b}	0.88^{a}	0.94^{c}

Mean values with the same superscript in a row are not significantly different ($P< 0.05$).

KEY:
Control: 100% (Normal pellet diets purchase from vital feed Ltd.)
ABC: 80% (Maize flour) and 20% (Coconut milk).
CDE: 75% (Rice flour) and 25% (soy milk).
EFG: 60% (Maize flour) and 40% (coconut milk).
GHI: 40% (Rice flour) and 60% (soy milk).

Table 4 shows the lipid profile of formulated diets consumed by rats were presented has followed; Total cholesterol, (3.24-2.66%), Triglyceride, (1.60-1.38%) HDLC, (1.40-0.88%) and LDLC, (1.00-0.84%) respectively. The experimental rats that were fed with sample ABC showed minimal triglyceride and also LDLC. There was significant different ($p>0.05$) in control sample when compare with all the lipid parameters, discrepancies may be due to high level of fat content in Vital feed meals. Therefore, this may lead to obesity, high risk factor for developing other chronic diseases including T2D, CVD, osteoarthritis, certain cancers and neurodegenerative diseases.

Cereal grains have also been reported to be effective against hypercholesterolemia. Rice protein has been proven effective in modifying TG metabolism and improving lipid homeostasis. Rice protein is thought to decrease the hepatic secretion of TGs and cholesterol by up regulating lipolysis, down regulating lipogenesis and interfering with very-low-density lipoprotein (VLDL) synthesis and secretion, lowering lipid accumulation, as shown in normal Wistar rats (Yang, L., *et al*., 2012). Rice protein was also shown to increase the activity of mRNA levels of cholesterol 7α hydroxylase and decrease the activity and gene expression of acyl-CoA cholesterol acyltransferase in hypercholesterolemic rats (Yang, L., *et al*., 2013). Ronis et al., 2010, reported that the consumption of RPI offered protective effects against problems associated with a high-fat Western diet such as insulin resistance, hypercholesterolemia and steatosis in Sprague Dawley rats.

The authors also found RPI to inhibit expression of hepatic genes involved in fatty acid synthesis (Ronis, M.J., *et al*., 2010). Another study determined that rice protein was able to lower serum and hepatic TC as well as hepatic total lipid levels in Sprague Dawley rats by increasing fecal TC and bile acid excretion (Um, M.Y. *et al*., 2013). Rice protein was shown to increase antioxidative capacity in normal adult male Wistar rats by increasing activities of superoxide dismutase and catalase, as well as stimulation of glutathione synthesis. This resulted in significantly reduced plasma TC levels. As previously mentioned, oxidative stress is an important factor in hypercholesterolemia.

Therefore, the increased antioxidative response should prevent oxidative damage to lipids and proteins, contributing to the lipid lowering effects exerted by rice protein (Cai, J., *et al*., 2014). Additionally, a single clinical trial reported that the administration of a daily dose of 10 g of rice endosperm protein for four weeks was able to increase HDL-C levels and reduce serum uric acid levels in adult male subjects with metabolic syndrome risk factors. However, no changes in TGs or LDL-C were observed (Hosojima, M., *et al*., 2016). Additional clinical trials are needed to evaluate the antioxidative and hypocholesterolemic effects of rice protein.

4. CONCLUSION

Biochemical profile (Nitrogen retention (NR %), Protein Efficiency Ratio (PER %), Biological Value (BV %), True Digestibility (TD %) and Net Protein Utilization (NPU %)), pathological test (Kidney, liver, heart and spleen) and lipid profile (Total Cholesterol (TC %), Triglyceride (TG %), HDCL % and LDLC %) parameters of the animals were within normal range and compared very well with those in control groups, with no signs of toxicity, thereby suggesting the safety of the snack in animals. It can therefore be safely recommended for clinical trials in human.

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