ORIGINAL ARTICLE

An assessment of levels of inflammatory markers in Wistar rats fed with SAMPEA 20-T Cowpea Cultivars

Titus S.D[1]., Timothy H[1]., Baba S.G[2]. Leyoa A.A1., Moses O[1]., Daniel E.O[1]., Ojogbene E. B[1]
[1]Department of Biochemistry, Taraba State University, Jalingo Nigeria
[2]Department of Biology, College of Agriculture, Science and Technology, Jalingo Nigeria

ABSTRACT

This study investigates the impact of SAMPEA 20-T cowpea cultivar on inflammatory markers in Wistar rats. Wistar rats weighing 200–250g were assigned into four groups: a control group fed a normal diet and water, and three treatment groups fed 25%, 50%, and 100% SAMPEA 20T cowpea diets, respectively, for 14 days. Inflammatory markers, colon; histopathology C-reactive protein (CRP) and packed cell volume (PCV), were assessed to determine the effects of SAMPEA 20-T consumption. The is no significant differences in CRP levels across treatment groups, with mean CRP values of 1.23 mg/L in the 25% group to 1.42 mg/L in the 100% group, compared to 1.10 mg/L in the control group (p value 0.00). Similarly, PCV levels exhibited a decreasing trend from 45.00% in the control group to 36.50% in the 100% treatment group. Histopathological examination also, revealed no signs of inflammation or tissue damage in the colons of treated rats, with intact mucosal architecture and normal glandular arrangement across all groups. The findings suggest that SAMPEA 20-T consumption do not induce significant inflammatory responses and histopathological changes in Wistar rats. The study highlights the potential safety of SAMPEA 20-T as a dietary component and its non-inflammatory properties, contributing to its potential role in health management and nutrition.

Key words: SAMPEA 20-T, cowpea, inflammatory, C-reactive protein, packed cell volume, and colon histopathology.

*Corresponding Author

Titus stephen dio. Department of Biochemistry, Taraba State University, Jalingo Nigeria. titus.stephendio@gmail.com.

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INTRODUCTION

Inflammation is a key physiological response to infection [1], injury [2, 3], and exposures to allergens [3, 4], often marked by increased levels of specific biomarkers, such as C-reactive protein (CRP) [5, 6, 7]. Diet plays a pivotal role in modulating inflammatory processes [8, 9, 10, 11,12], with certain foods showing potency to reduce or exacerbate inflammation [13, 14, 15]. Cowpea (*Vigna unguiculata*), is a legume widely consumed in sub-Saharan Africa, is notable for its rich protein contents [16, 17, 18]. SAMPEA 20-T, a cowpea cultivar, has gained attention for its nutritional profile [19, 20, 21], but its impact on inflammatory markers remains underexplored.

This study aims to assess the inflammatory response in Wistar rats treated with SAMPEA 20-T cowpea cultivars by measuring levels of CRP, PCV as well as colon histopathology. Hence, elucidating the potential effects of SAMPEA 20-T on inflammatory markers.

MATERIAL AND METHOD

Sample Collection

SAMPEA 20-T was collected from Institute of Agricultural Research Zaria, Kaduna State and Wistar rats were collected from Department of Biochemistry, Modibbo Adama University, Yola.

Experimental Design

Wistar rats weighing 200–250g were used in the study with three animals in each group (nine in all). They were housed in a well-ventilated, ambient temperature environment and were fed for fourteen days. The experiment was carried out in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Determination of C-Reactive Protein (CRP)

The levels of C-reactive protein (CRP) in the blood sampls were estimated by immunoassay, according to the method of Kåpyaho *et al*. of 1989 and absorbance was measured at 415 nm [22]. Polystyrene tubes that were used are coated with 300 µL of anti-CRP 6404 (Prestige Antibodies® Merck, USA) by overnight incubation in 10 µg/mL phosphate-buffered saline (PBS).

Blocking was done with 0.5 g/L bovine serum albumin (Thermo Fisher Scientific Inc.) [22]. The coated tubes were incubated with 50 µL of CRP standards prepared in Tris buffer. Serum (50 µL) samples were also incubated in other tubes for 15 minutes at room temperature. After incubation, the tubes were washed twice with 25 mM Tris buffer (pH 7.4) containing 0.1% Tween-20, and once with PBS. Then, 300 µL of 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate solution (Merck, USA) was added to each tube for color development for 3 minutes after which, the reactions were stopped by adding about 200 µL of 1 mM sodium azide solution [22].

Determination of Histopathology of Colon

Colon harvested were preserved in 100% formalin and incubated. The tissues were fixed unto a slide and dehydrated through a series of alcohol solutions and clear the tissues with a clearing agent and also embed the tissues in paraffin wax to create blocks. The tissues slices were mounted onto clean glass slides and thereafter stained with hematoxylin and eosin (H&E) stain as described by Cardiff et al. (2014) [23]. The slides were examined under a light microscope for signs of inflammation, such as infiltration of immune cells, tissue damage, or changes in cellular morphology

Determination of packed cell volume (PCV)

Packed cell volume (PCV) was measured using hematocrit machine and reader. The PCV was calculated as the percentage of the total volume of the blood sample that is made up of red blood cells. It is calculated using the formula:

$$\text{PCV} = \frac{\text{Volume of Packed Red Blood Cells}}{\text{Total Volume of Blood Sample}} \times 100$$

Statistical analysis

Results were reported as mean ± standard deviation (SD). Also, one-way analysis of variance (ANOVA) was performed using SPSS software version 27.0 (SPSS Inc., Chicago, IL, USA). A *p*-value <.05 was considered significant.

RESULTS

Figure 1 is a photomicrograph of the transverse section of colon in control group. It shows normal mucosal (M), submucosal (SM), smooth muscle layer (MP), serosa (SE), surface epithelium (EP) and few goblet cells (GB.C) (H&E x100). While the transverse section of colon of 25% (w/w) of SAMPEA 20-T treatment group (Figure 2) also revealed intact continuous mucosal (M), with muscularis mucosa (MM), regularly arranged gland(G), muscularis propria (MP) and few goblet cells (black arrow). No inflammation cells are seen (H&E x100). For the group fed with 50% (w/w) of SAMPEA 20-T (Figure 3), showed surface epithelium (EP), mucosal (M), submucosal (SM), muscularis mucosa (MM), smooth muscle layer (MP), serosa (SE) and gland (G). while no inflammatory cells are seen (H&E x100). In the same vein, the group fed with 100% (w/w) of SAMPEA 20-T (Figure 4) showed muscularis propria (MP), mucosal (M), submucosal (SM), gland (G), surfaces epithelium (EP), lumen and numerous goblet cells (GB.C) with no inflammatory cells seen (H&E x100).

Table 2 present levels of CRP (mg/L) and PCV (%) for each group. The control group has a mean CRP level of 1.10±0.10 mg/L. As the proportion of SAMPEA 20-T increases (25%, 50% and 100% (w/w)), there is a trend of increasing mean CRP levels: 1.23±0.02 mg/L, 1.31±0.11 mg/L, and 1.420.10 mg/L, respectively. Although, there is a variability in CRP levels within each group, it is not statistically significant.

The result showed PCV levels (Table 2) in the control and treatment groups (25%, 50% and 100% (w/w) of SAMPEA 20-T) were; 45.0%, 37.50%, 36.00%, and 36.50%, respectively. However, there are variations in PCV levels within each group.

DISCUSSION

The findings from both the histopathological examination of colon tissues and the analysis of CRP levels and PCV levels in Wistar Rats treated with SAMPEA 20-T provide valuable insights into the potential physiological effects of consuming different proportions of this substance.

Across all treatment groups (25%, 50%, and 100% SAMPEA 20-T (w/w)), the histopathological examination revealed no signs of inflammation or tissue damage in the colon. The photomicrographs of the transverse colon sections showed intact mucosal architecture, normal glandular arrangement, and the presence of goblet cells, indicating the absence of pathological changes. In the control group as well, the histopathological features of the colon were similar to those observed in the treatment groups, further confirming the absence of inflammation or tissue damage. These findings suggest that consuming different proportions of SAMPEA 20-T did not induce significant histopathological changes in the colon tissue of the Wistar Rats.

The mean CRP levels increased with higher proportions of SAMPEA 20-T consumption (1.23 mg/L for 25%, 1.31 mg/L for 50%, and 1.42 mg/L for 100%). However, the variations in CRP levels within each group were not statistically significant, (F-value = 0.00). This suggests that the observed differences in CRP levels between control group and treatment groups may not be due to the effects of SAMPEA 20-T but could instead be attributed to random chance.

PCV levels showed a decreasing trend with higher proportions of SAMPEA 20-T consumption (37.50% for 25%, 36.00% for 50%, and 36.50% for 100%). However, similar to the CRP levels, the variations in PCV levels

within each group were not statistically significant, as indicated by the F-value of .00. This suggests that the observed differences in PCV levels between the control group and the treatment groups may not be because of SAMPEA 20-T but could instead be attributed to random chance.

These findings align with existing literature that emphasizes the importance of CRP as an early and sensitive marker of systemic inflammation. For example, Kimura et al. [24] observed elevated CRP levels in infants with food protein-induced enterocolitis syndrome (FPIES), suggesting CRP's utility in detecting intestinal inflammation. However, in this study, the CRP levels remained within ranges, supporting the notion that SAMPEA 20-T is unlikely to provoke inflammatory conditions such as FPIES-like responses. In another study, Yang et al. [25] reported no significant association between high-sensitivity CRP and allergic outcomes in adolescents, suggesting that not all dietary components or exposures trigger inflammation detectable by CRP, especially in the absence of underlying pathology. While, Zhao et al. [25] highlighted a case where CRP was the only detectable marker during early FPIES diagnosis, underscoring its sensitivity.

From a nutritional standpoint, the anti-inflammatory potential of plant-based foods has been documented. Esmaillzadeh et al. [27] reported that increased fruit and vegetable intake was associated with reduced CRP concentrations and lower risk of metabolic syndrome, while Qureshi et al. [28] found similar associations in children consuming higher amounts of grains and vegetables. Given that cowpea is a legume rich in fiber, antioxidants, and bioactive compounds, the lack of inflammatory response observed in our study may reflect these protective dietary attributes.

Additionally, PCV levels declined slightly with increasing SAMPEA 20-T proportion but, like CRP, the differences were not statistically significant. This suggests the cowpea diet did not impair hematological integrity. This finding complements the work of Egbi et al. [29], who observed that cowpea-based diets supplemented with fish meal and vitamin C improved hemoglobin concentration and reduced anemia risk in human subjects.

The absence of significant histological or biochemical changes in this study supports the safety of SAMPEA 20-T cowpea cultivar as a dietary component, with no evidence of systemic or localized inflammatory responses. The integration of inflammatory biomarker assessments such as CRP with histopathology provides a robust framework for evaluating dietary safety and potential immunological impact in future nutraceutical research.

CONCLUSION

The observation of no histopathological changes in colon tissue, combined with the absence of significant differences in CRP and PCV levels between the control and treatment groups, strongly indicates that varying proportions of SAMPEA 20-T are unlikely to adversely affect inflammatory markers or blood parameters in Wistar rats. This finding highlights the safety profile of SAMPEA 20-T and its potential for beneficial use without compromising health. However, it's essential to consider the limitations of the study, such as sample size, duration of exposure, and potential confounding factors, when interpreting these findings. Further research, including longer-term studies and additional mechanistic investigations, would be necessary to confirm these observations and understand the potential health implications of SAMPEA 20-T consumption comprehensively.

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TABLES AND FIGURES

Table 1: Experimental Design

Control Group	Normal diet + H2O
Treatment Group 1	100% SAMPEA 20-T (w/w) + H_2O
Treatment Group 2	50% SAMPEA 20-T (w/w) + H_2O
Treatment Group 3	25% SAMPEA 20-T (w/w) + H_2O

Table 2: Levels of C-Reactive Protein and Packed Cell Volume in Wistar Rats Treated with SAMPEA 20-T

Groups	C-reactive Proteins (mg/L)	Packed Cell Volume (PCV)
Normal Diet (Control Group)	1.10±0.10	45.00±1.41
25% (w/w) SAMPEA 20-T	1.23±0.02	37.50±3.35
50%(w/w) SAMPEA 20-T	1.31±0.11	36.00±1.41
100% (w/w) SAMPEA 20-T	1.42±0.10	36.50±3.12
	p=.00	

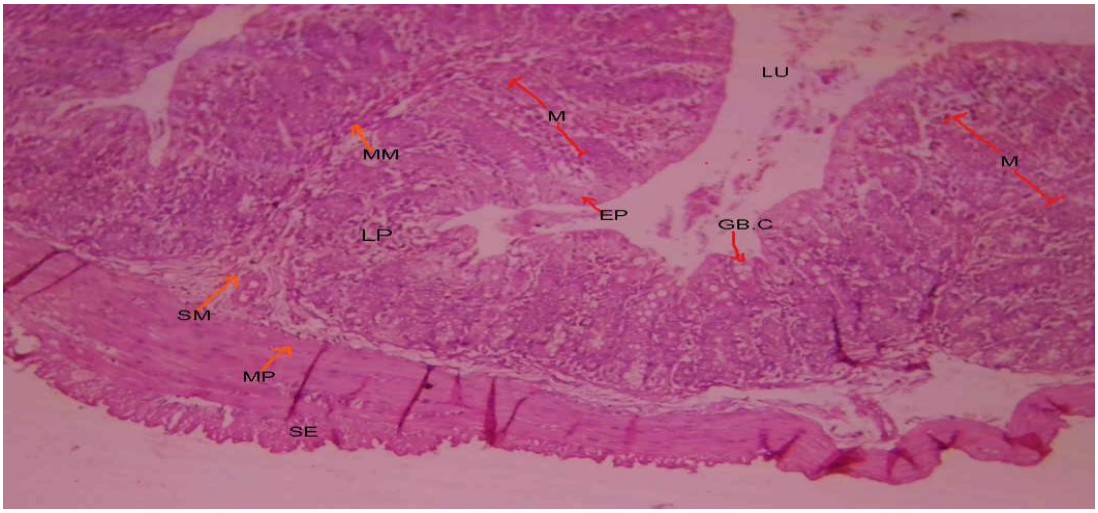

Figure 1: Photomicrograph of Transverse Section Colon of Control Group (Normal Diet).

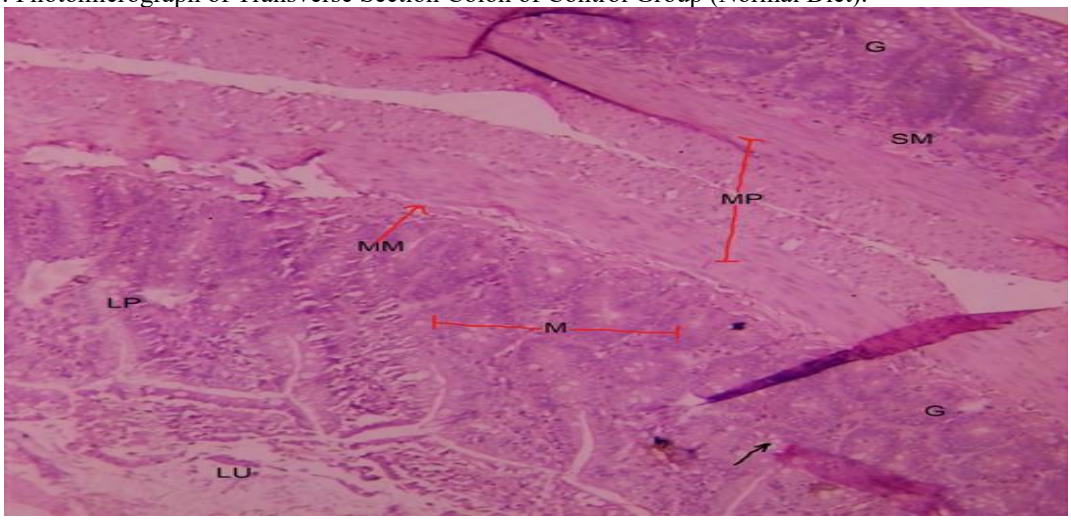

Figure 2: Photomicrograph of the Transverse Section of Colon of 25% (w/w) of SAMPEA 20-T Treatment Group

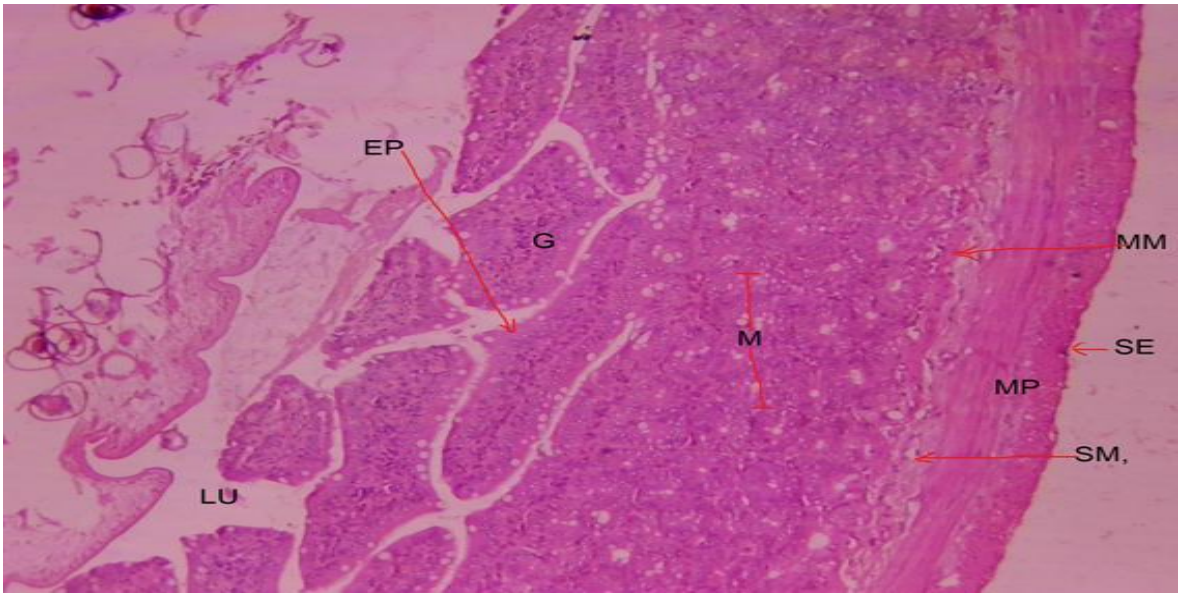

Figure: 3: Photomicrograph of the Transverse Section of Colon of 50% (w/w) of SAMPEA 20-T Treatment Group

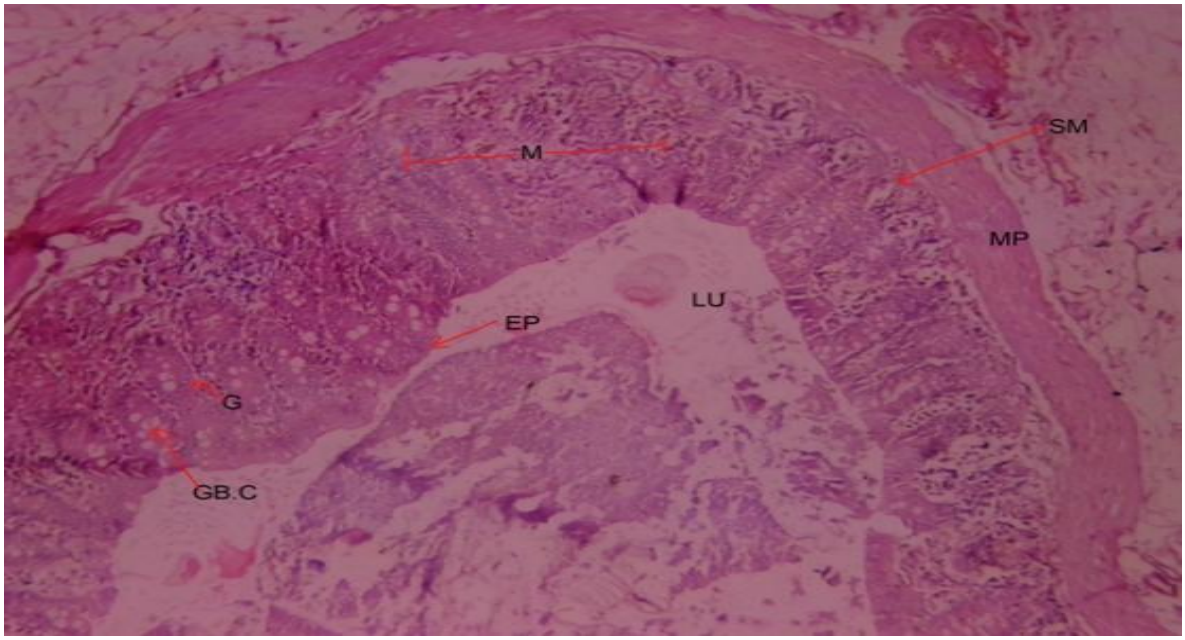

Figure 4: Photomicrograph of the Transverse Section of Colon of 100% (w/w) of SAMPEA 20-T Treatment Group