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Biosafety assessment of Fig (*Ficus carica*) leaves, an alternative preservative agent

Olufemi Emmanuel Bankefa[1*], Ruth Adefolakemi Gabriel-Ajobiewe[1], Olubunmi Evelyn Ajayi[1], Simbiat Olufunke Ayilara-Akande[1], Seye Julius Oladeji[2], Gbenga Victor Kayejo[1]

[1]*Department of Microbiology, Federal University Oye-Ekiti, Ekiti-State, Nigeria.*
[2]*School of Food Science and Engineering, South China University of Technology, Guangzhou, China.*

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ABSTRACT

Due to the global disturbance of chemical resurgence in food, Fig leaves (*Ficus carica*) had been made to be applied as preservatives to extend the shelf life of perishable foods and to address chemical toxicity concern, despite limited information regarding its biosafety. Hence, the need to affirm the safety of this plant *in-vivo* was studied. Extracts of Fig leaves were mixed with basal diet and used as feed for one group of Wistar albino rats (treated group) while the second group was fed with sole basal medium to serve as control. The blood samples of both groups were taken before the rats were subjected to cervical dislocation. Haematological and histological parameters were studied to know the effects of the extracts and in order to justify the usage of plant extracts as preservatives for perishable foods. The haematological parameters of the treated group were higher than the control except for the insignificant changes in lymphocytes, basophils and eosinophils. Furthermore, the histological study revealed no sign of lesions in the all organs examined from both groups. The adoption of Fig leaf as preservatives has no contra-indication on the organs or blood parameters. Hence the global adoption as alternative preservative agents is undoubtedly recommended.

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1. Introduction

Ficus carica Linn known as Fig leaves, belongs to the plant family *Moraceae*. It is one of the exceptional *Ficus* specie widely spread in tropical and subtropical countries, which has edible fruits with high commercial value (1). The plant constitutes one of the largest genera in flowering plants (2) and has been adopted in folk medicine for treating various ailments such as irritation, gastric problems etc., (3). *F. carica* is an important source of minerals (such as calcium, vitamin K, manganese, potassium, magnesium, and copper), protein, vitamins, and carbohydrates (4) and the leaves (primary and processed form) have been harnessed for production of arrays of products such as liqueurs, spirits, jams, infusions etc.) (5).Mawa *et al*. (6) reported that the majority of the research works on the biological activities of *F. carica* leaves were mainly conducted on its crude extracts which have been shown to exhibit many biological activities such as anticancer, hepatoprotective, hypoglycemic, hypolipidemic and antimicrobial activities (7). The leaves and fruits of the plant are used in phytochemical studies and are rich in organic acids, phenolics and volatile compounds (8). Several reports on the antimicrobial properties (6,8) and the preservative efficacy of this plant have been documented (9).

*Corresponding author. Tel./Fax: +2348038736908
Email address : femibankefa@gmail.com

However, information regarding the biosafety of *F. carica* leaves is limited as majority of the research works on histological effect of the plant were conducted on induce-damaged liver (10) and heart of Albino rat (11), using chemicals such as tetrachloride and doxorubicin respectively.
In order to justify its promising preservative feature as an alternative to chemical preservatives whose resurgence has been implicated in food storage (12-14), there is need to investigate the safety of this plant (leaf). To the best of our knowledge, this is the first study reporting the *in-vivo* safety of *F. caraca* leaf extracts.

2. Materials and Methods

2.1. Study Location

This study was carried out at the State Specialist Hospital Akure, Ondo State, Nigeria and Department of Microbiology and Plant Science and Biotechnology of Federal University Oye-Ekiti, Ekiti State, Nigeria between May 2018 and March 2019.

2.2. Plant Material Collection

The leaves (fresh) of *F. carica* were obtained from the wild at Oye-Ekiti, Ekiti State, Nigeria. The leaves were identified and authenticated at the Herbarium section, Plant Science and Biotechnology Department of Federal University Oye-Ekiti, Ekiti State, Nigeria.

2.3. Preparation of Plant Materials and Formulated Feed

The preparation of plant materials (fresh leaves) was carried out simply by air drying the sample at room temperature (25^0C–27^0C), scrumbling mechanically to coarse powder and reducing to fine powder by means of an electrical blender (MarlexElectrolyne IS: 250). Formulated feed was prepared following the method of Attia *et al*. (14) by directly combining the basal feed diet and *F. carica* blended leaves in the ratio 1:1. The formulated feed was thereafter blended to achieve thorough mixing before administration.

2.4. Determination of body weights of Experimental Animals

The weights of the experimental animals were determined on the 1st, 7th and 14th day of feeding processes to know the differences between the treated and control rats upon feeding using calibrated weighing balance.

2.5. Determination of toxic effects of *Ficus carica* leaves on Wistar Albino rats

Two groups of six rats each were used to assess the toxic effects of *F. carica* on healthy albino rats. The first group was fed with formulated feed (60 grams of pulverized *F. carica* leaves and 60 grams of basal feed) and tagged "treated" while the second group was fed with basal diet feed only to serve as control. The treated and the control groups were fed for 14 days after their acclimatization and the rats were later sacrificed through cervical dislocation.

2.6. Haematological and Histological analyses

On the 14th day of the feeding process, blood samples were collected into the Ethylene-Diamine-Tetra-Acetic acid (EDTA) bottle and haematological studies were carried out on them at the Haematology Section of the State Specialist Hospital Akure, Ondo State, Nigeria. Full blood cell counts were conducted to ascertain the Red Blood Cell (RBC) and White Blood cell counts (WBC) (16). The Packed Cell Volume (PCV), the haemoglobin and other Red blood cell parameters were also quantified. After the sacrifice, the liver, heart and small intestine were also taken for histological investigation as described by Buncharoen *et al.* (17), on the 14th day of feeding processes.

2.7. Statistical analysis

This was done through the use of SPSS 15.0 for windows evaluation version by using ANOVA and DUNCAN'S Multiple Range Test for the estimation of means. The test value was tested at 95% confidence interval.

3. Results

3.1. Body weights of experimental animal

The body weights of the experimental animal were determined. Both treated and control rats recorded increase in weight, but at the 14th day of the feeding processes, the treated rats were observed to be significantly higher in body weight than the control rats (Table 1).

Table 1. Body weights of experimental animals (g)

Day (s)	Control	Treated
1	40.3 ±1.45[a]	39.4 ±2.31[a]
7	46.5 ±2.36[b]	45.2 ±2.01[b]
14	52.6 ±1.92[c]	54.3 ±2.46[c]

a, b and c are significantly different.

3.2 Haematological parameters of the experimental animal

The results of the haematological parameters of the experimental animal revealed highest total RBC and WBC counts of 9.28 $mm^3/\mu l$ and 205 K/µl in the treated group as compared to that of control group (7.93 $mm^3/\mu l$ and 166 K/µl) respectively (Figure. 1). In addition, the maximum PCV value (41%) and haemoglobin concentration (14.13g/dl) were recorded in the treated rats as compared to that of control rats (40.30% and 12.58g/dl) respectively (Figure. 2a). The findings of the WBC components revealed insignificant changes in the Basophil, Lymphocyte and Eosinophil levels of both treated and control rats. However, the Neutrophil and Monocyte levels of the treated rats (38.30% and 4.92%) were observed to be significantly higher than the values recorded for control rats (29.00% and 1.97%) respectively (Figure. 2b).

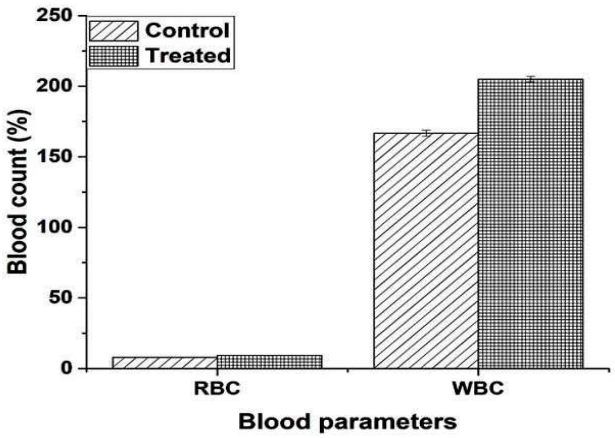

Figure 1. Blood parameters of the control and treated experimental animals. RBC (Red Blood Cell) ($mm^3/\mu L$), WBC (White Blood Cell) (K/µL). Three parallel tests were evaluated. Error bars represent deviations (n = 3). Statistically significant differences ($p<0.05$) were determined by student's t test.

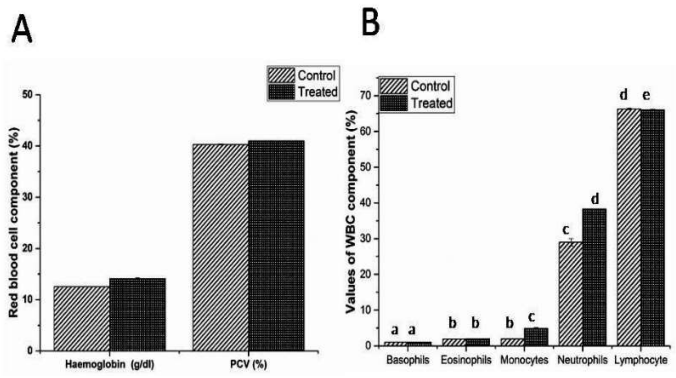

Figure 2. Full blood parameters of the treated and control rats. (A) The Red Blood Cell components. (B) The White Blood Cell components. Three parallel tests were evaluated. Error bars represent deviations (n=3). Statistically significant differences ($p<0.05$) were determined by student's t test.

3.3. Histological Examination

Organs were removed, grossly examined, processed and stained using haematoxylin-eosin before examination under the light microscope. Histopathological investigation revealed no lesions in the control and treated animals, and hence the organs were considered histologically normal (Figure. 3).

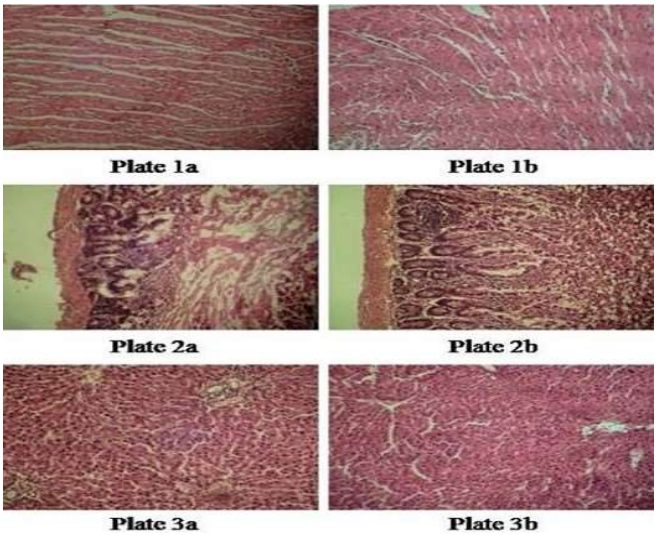

Figure 3. Photomicrograph of organs. Plate 1a: muscles of the heart for control rats. Plate 1b: muscles of the heart for treated rats. Plate 2a: Intestinal section of control rat. Plate 2b: Intestinal section of treated rat. Plate 3a: Liver section of control rat. Plate 3b: Liver section of treated rat. The organs were treated with normal saline and stained with H and E prior to microscopy view (x 400).

4. Discussion

Over the years, chemical preservatives are employed for controlling microbial growth and reducing food-borne disease (FBD) incidences, owing to their effectiveness against spoilage microbes; however, their resurgence has been implicated in food storage (12-14). Hence, there is a growing body of research on medicinal plants such as *F. carica* for eliminating foodborne pathogens as safe and natural remedies, thereby reducing the risk of FBD incidences while assuring consumers with safe food product (18). In addition, several reports on the ethno-medicinal uses of *F. carica* leaf extract as a remedy for diseases such as diabetes (19), haemorrhoids (20) among others and its preservative efficacy have been documented (9). Nevertheless, to recommend the preservative feature of this plant, there is a need to investigate it's *in vivo* safety and hence, the essence of conducting this study.

After the feeding processes, the treated rats fed on formulated feed were found to exhibit slight increase in body weight compared to the control rats fed on basal feed. This observation may be due to the enhancement of the nutritive quality of the formulated feed by *F. carica* leaf extracts. It has been reported that increase in body nitrogen could lead to increase in weight gain (21) and *F. carica* is an important source of amino acids in addition to other food components (4).The enhancement of the total red blood cell (RBC) and white blood cell counts (WBC) of the treated rats by the extracts as compared to the control rats may be attributed to the RBC- and WBC-enhancing abilities of the plant. Similar findings have also been reported in several studies (8,22-24). However, the finding of the total WBC count in the present study contradicts that of Tchombe and Louajri (25), who reported a decrease in the total WBC count of the experimental rats (tagged treated) compared to the control rats. These differences might be due to the conditions in which the plant is subjected to, which might have altered the composition of the plant parts.

The PCV and haemoglobin concentration of the treated rats were observed to be higher than that of control rats and this finding could be due to the capability of *F. carica* leaf extracts to improve the populations of RBC synthesized in the bone marrow while increasing the oxygen-carrying capacity of the full blood (22). These findings are in agreement with that of Nebedum *et al*. (22) and Odo *et al*. (23), who reported a time-dependent increase of packed cell volume (PCV) and haemoglobin concentration in rats exposed to *F. carica* extracts. These findings also elucidate that the leaf extract of *F. carica* is non-toxic as reduction in packed cell volume and haemoglobin concentrations implies toxicity of an extract to the red blood cells (23).

The observed beneficial effects of *F. carica* leaf extracts on the WBC components of the treated rats in the present study may be attributed to the immune-enhancing capability of *F. carica* extracts (8). The immune responses of *F. carica* leaf extracts have attributed to the immune activities of polysaccharide constituents of *F. carica* leaf (8). These findings corroborate to that of Ahmad *et al*. (24) who opined that *F. carica* leaves are superb natural folk blood promoters employed for devastating disorders/illnesses such as blood deficiency diseases and acute blood loss.

In agreement with our expectation, the histological analysis revealed no sign of lesion or abnormality in the hearts, small intestines and livers of the treated rats. The organs of the treated animals compared well with that of control. These observations may be attributed to the cardio-protective, laxative and hepato-protective abilities reported earlier in *F. carica* leaf by several studies (11,26-27). The cardio-protective activity reported in the plant has been attributed to its ability to decrease the elevated levels of cardiac enzymes due to the presence of antioxidant agents such as flavonoids, tannins, polyphenols and alkaloids (11). In the same vein, the laxative effect and the ability of the plant to improve digestive enzymes (26) may be attributed to its protective ability observed on the small intestines of the treated rats in the present study. Furthermore, *F. carica* has been found to have the ability to reduce the total serum bilirubin, alanine transferase, malondialdehyde equivalent, and the serum levels of aspartate amino transferase, which is an index of lipid peroxidation of the liver (27), and hence the protective activity of the plant observed on the livers of the treated rats.

Due to the absence of abnormality in the histological and haematological parameters of the treated rats, the plant could therefore be used in place of chemical preservatives for food preservation. However, further studies on other organs should be carried out to further ascertain its potential usage as food preservatives.

5. Conclusion

The adoption of fig leaves as preservatives has no contra-indication on the organs or blood parameters. In fact, the resurgence of this leaves may also pose health benefit(s) to consumers and hence, its global application as alternative to chemical preservatives is undoubtedly recommended.

Conflict of Interest

The authors declare they have no conflict of interest

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