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Chemical Analysis and Toxicological Evaluation of Methanolic Extract of Corn Silk on the Reproductive System of Japanese Quail Birds

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Abstract

Introduction: *This study was focused on investigating the chemical analysis and toxicological evaluation of the methanolic extract of corn silk using male quail birds.*

Material and methods: *Corn silk extract was obtained through a cold extraction method using methanol as solvent; later analysed for the mineral content and was characterized for various compounds using FTIR and GC-MS. The toxicological studies of the extracts were assessed using twenty-four quail birds divided into four experimental groups (1-4) of six birds each. Group 1 which served as control, received an oral doses of normal water, while others were given 250 mg/kg, 500 mg/kg and 750 mg/kg of extract daily, respectively. All bird was provided with unrestricted access to food and water throughout the period of study. They were sacrificed at the end of three weeks for toxicity assessment. The organ weights, the weight gain and others such as haematological and blood biochemical parameters were all evaluated.*

Results: *The extract was rich in potassium (180 mg/l). Total of 24 compounds belonging to phenolic, fatty acids, aldehydes and hydrocarbons were identified in the extracts. The weight gain of the birds of group 1 (control) was high (22.25±3.24g), and significantly different when compared with other groups (13.15g-13.95g). The organ weights did not differ considerably among the groups. Similarly, haematological and biochemical parameters showed no statistically significant variations across all treatment groups. Histopathological examination further revealed no observable lesions in the heart and brain tissues of birds in any of the groups. A severe congestion of the portal and intestitium was observed in the liver and the kidney of group 4, while no lesion was observed in the gland, glut and testes in all the groups of studies.*

Conclusion: *Corn silk is non-toxic at 250 mg/kg and 500 mg/kg of oral dose on quail birds. It is therefore safe for animal consumption as a food supplement at groups 2 and 3 levels.*

Keywords: *Corn silks, bioactive compounds, quail birds, reproductive system, toxicology, extract.*

INTRODUCTION

Medicinal plants have formed the foundation of traditional healthcare systems across different cultures for thousands of years, serving as primary sources of therapeutic agents for the prevention and management of numerous diseases [1]. These therapeutic effects of many traditional herbs are due to the presence of natural antioxidants, especially phenolic compounds [2]. In recent decades, this accumulated ethno-medicinal knowledge has attracted increasing scientific interest, particularly in the search for safer, affordable, and more accessible alternatives to synthetic drugs [2]. Evidence from scientific literature indicates that these medicinal plants may be administered in various forms, including extracts, decoctions, pastes, and as components of dietary supplements. They can be used either independently or in combination with conventional medical treatments to support and improve women's reproductive health [3].

Currently, over 1,200 plant species across the globe have been documented in traditional and ethno-medicinal practices for the management of various reproductive system disorders. A considerable proportion of these medicinal plants are now undergoing rigorous scientific evaluation to determine their therapeutic efficacy in conditions such as infertility, menstrual irregularities, erectile dysfunction, sexually transmitted infections, and other reproductive health complications [4]. In order to protect humans from oxidative stress and some other ailments, various herbs and plants are being utilised for their potential benefits in preserving health.

One of these herbs is corn silk (*Stigma maydis*).

Corn silk, the thread-like stigma of Zea mays, is generally regarded as an agricultural by-product generated during maize production [5]. Despite being treated as waste in large-scale cultivation, it has been utilised for centuries in traditional medicine and various dietary remedies. Moreover, available toxicological evaluations indicate that corn silk is relatively safe and exhibits low toxicity, and it is commonly marketed in the United States as a dietary supplement under regulations enforced by the United States Food and Drug Administration (FDA). It is widely employed in managing a variety of health conditions, particularly in countries like China, Turkey, the United States and France [4]. Corn silk is also traditionally recognised for its ability to lower blood sugar, reduce inflammation, promote urination, and alleviate swelling. Modern pharmacological studies have highlighted its therapeutic potential in addressing disorders such as diabetes, hypertension, and nephritis among others [5,6].

Corn silk threads may be steeped in boiling water to make tea. It has a variety of health benefits as it contains moderate amounts of iron, potassium, zinc, phosphorus, magnesium and calcium. A wide array of phytochemicals has been detected in corn silk, such as phenolic acids, flavonoids, carotenoids, tannins, sterols, volatile compounds, sugars, vitamins, minerals, polysaccharides, proteins, and peptides. These diverse bioactive constituents are linked to numerous promising pharmacological effects, including antioxidant, antimicrobial, anticancer, antihyperlipidemic, antihypertensive and antidiabetic activities [7,8]. which collectively make corn silk a valuable natural candidate for healthcare and therapeutic applications in modern research. Folk remedies show that corn silk has been used as an oral antidiabetic agent in China for decades, but the mechanism of its hypoglycaemic activity has not been elucidated [8]

Corn silk, previously used as tea because of its diuretic properties, might equally help in treating health conditions such as mumps or inflammation of the urinary bladder or urethra when combined with other herbs [4]. Polysaccharides extracted from corn silk, shown to be extensively biological activities, might be a potential drug for the prevention or treatment of kidney stones and could inhibit the crystal growth of CaOx [9,10]. Rich in bioactive compounds like phenols, flavonoids, tannins, saponins, and terpenes, corn silk is a valuable source of antimicrobial agents capable of limiting microbial infections, providing therapeutic benefits with strong antifungal activities. It demonstrates significant antimicrobial activity at low minimum inhibitory concentrations, highlighting its potential for developing new antimicrobial drugs and reducing antibiotic resistance. Therefore, corn silk deserves additional investigation to fully understand its health benefits [11]. Ethno-medicinal surveys have shown that corn silk herbal tea possesses a lot of medicinal potential. This study proposes that corn silk extracts might have significant therapeutic effects in addressing infertility and other reproductive health issues.

Therefore, it specifically investigates the chemical composition and toxicological impact of the methanolic corn silk extract on the reproductive system of Japanese quail.

MATERIALS AND METHODS

Samples collection

Corn silk (dried cut stigmata of *Zea mays*. L.) of a local maize variety were collected at a farm in Olorunda, Ibadan, Oyo State, Nigeria. The collected corn silk was air dried in a well-ventilated shaded place for 6-7 days at room temperature and afterward pulverised with an electric blender into powdery form. The powered corn silk obtained was kept in tight container for further analyses and experiments.

Preparation of methanolic extract of corn silk

600 g of the corn silk powder was soaked with 95% methanol (6.0 L) at room temperature in glass bottles for 7 days with constant shaking daily. It was then decanted and filtered using filter paper. Thereafter, extract was filtered using Whatman No. 1 filter paper and the resulting filtrate was concentrated using rotary evaporator to obtain a methanol extract (brownish-black paste) and the solvent recovered. The extract was stored in refrigerator until its use for chemical analysis.

Mineral determination

Mineral element such as calcium, iron, potassium, sodium, manganese, copper, zinc and magnesium were determined from the corn silk powder according to [12]. A flame photometry (Model, 405, Corning UK) was used to determine potassium and Sodium while a Perkin-Elmer 703 model 703 Atomic absorption spectrophotometer were used in the determination of other minerals. All analysis recorded in triplocate.

Fourier Transform Infrared Spectroscopy (FTIR)

The various functional groups that are found in the extracts and nature of bonds that exist in the extracts were distinguished by loading the extract into a System 2000, Perkin Elmer, Wellesley, MD, USA (FT-IR). The IR peak absorbance (cm^{-1}) was recorded in the range of 4000 cm^{-1} to 400 cm^{-1} [13].

Gas chromatography-mass spectrometry (GC-MS)

The corn silk methanolic extract was subjected for GCMS analysis following the method described in [13]. The analysis was carried out using an Agilent 7890A GC system that is connected to an Agilent 5975 c mass selective detector which is equipped with HP-5MS GC column [13,14]. The constituents were identified by comparing the mass spectra available in a MS database of National Institute Standard and Technology (NIST Ver. 8 and 11). Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The various spectrum obtained were matched with those of the compound already known and saved in NIST library where the names of the various identified components alongside with their structure and molecular weights were confirm authenticated [13,14].

Experimental birds

Twenty-four male Japanese quail birds (aged about 5 weeks, weighing between 90-130 g) were purchased from Foresight Hatcheries, Obasanjo Farm, Oluyole Estate in Ibadan, Nigeria. The birds were divided into four groups (1, 2, 3, 4) of 6 birds each with group 1 serving as control. Graded dose of the aqueous extract were administered separately to the birds in their various groups via oral cannula for 21 days. Group 1 received 250 mg/kg, group 2 collected 500 mg/kg while group 3 received 750 mg/kg of the extract. The birds were kept in the experimental animal house within the Department of Veterinary Physiology of the University of Ibadan, and they have access to feed and water *ad-libitum*.

Haematological examination

Three ml of each blood sample collected via cardiac puncture were stored in EDTA bottles at 10 °C. PCV, Hb, RBC and WBC counts were all evaluated using standard techniques as described by Dacie and Lewis, (2001). Other parameters including MCV and MCHC were equally obtained [15,16].

Blood biochemical analyses

A blood sample was drawn into a test tube and left for 30 minutes at room temperature to allow clot formation. The sample obtained after clotting was centrifuged for 10 minutes using a bench-top centrifuge at 3,000 rpm to ensure comprehensive and perfect separation of the serum from the clot. The resulting clear serum layer was cautiously withdrawn using a syringe and needle and reserved for further biochemical analysis. Parameters assessed included blood glucose, total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatinine, and urea, following the methods described by [16,17].

Histological studies

Small portions of the excised tissues were measured by weight and preserved in 10% formalin prior to fixation. The samples were then passed through graded xylene solutions for dehydration. Afterwards, they were embedded in paraffin wax, sectioned at approximately 5 µm thickness, and stained with hematoxylin and eosin (H&E). The prepared slides were subsequently examined under a light microscope to assess histopathological changes, [16,18].

Statistics

Data were expressed as the mean±S.D of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.20. Significant differences ($p < 0.05$) among the fractions were analyzed by Duncan 'triplicates range test.

RESULTS AND DISCUSSION

Mineral element

Mineral outline of corn silk extract, as displayed in Table 1, reveals the presence of essential elements including Ca, Mg, K, Na, Mn, Cu, and Zn. These metals are known to perform significant physiological functions in living organisms. Their concentrations varied within the extract, with potassium occurring in the greatest amount (180 mg/L) and copper in the smallest quantity (0.05 mg/L). The results suggest that corn silk extract is particularly rich in potassium and sodium as indicated by notable sodium content. This mineral is important for sustaining acid-base balance and supporting normal neural activity [19]. It also plays a fundamental role in cell signaling through the regulation of calcium ion transport within the cytoplasm [12]. In addition, potassium contributes to skeletal health by promoting bone density during growth and maintaining bone integrity in later stages of life. Manganese is involved in enhancing immune function, controlling blood sugar levels, and facilitating energy generation and cell development [20]. The zinc concentration was found to be 0.103 mg/L. Zinc is essential in controlling gene activity, modulating cellular proliferation, and functioning as an enzymatic cofactor in carbohydrate, protein, and nucleic acid metabolism. The concentration of Zinc obtained is quite low. Corn silk contains, therefore, an appreciable amount of nutritionally important minerals required for the proper growth and functioning of the body system. The ratio of sodium to potassium (Na^+/k^+) in the diet is more important predictor of hypertension than the amount of either one alone [21]. Corn silk extract contained therefore low concentrations of trace metals such as copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe), while exhibiting high levels of essential macronutrients including sodium (Na), potassium (K), and calcium (Ca), which may contribute to its potential health-promoting benefits.

FT-IR

Table 2 provides a detailed interpretation of the FTIR profile obtained from the methanol extract of corn silk, highlighting the principal absorption peaks and the functional groups they represent. The spectral pattern reflects the occurrence of several chemically active groups typically linked to secondary metabolites, including phenolics, flavonoids, tannins, and other plant-derived bioactive substances. A prominent broad band recorded near 3393.00 cm^{-1}, within the 3300-3400 cm^{-1} intervals, can be assigned to O-H stretching vibrations resulting from hydrogen bonding. This feature is characteristic of hydroxyl groups present in alcohols and phenolic structures. Such hydroxyl groups are well recognised for their contribution to the free radical scavenging and antioxidant behaviour of polyphenolic and flavonoid compounds. The absorption peak at 2947.56 cm^{-1}, situated in the 2920-2850 cm^{-1} region, corresponds to aliphatic C-H stretching vibrations. This signal points to the existence of long-chain hydrocarbons commonly associated with fatty acids and terpenoid constituents. A distinct band around 1641 cm^{-1} may be attributed either to C=C stretching within aromatic systems or to the amide I band, implying the presence of aromatic polyphenols and possibly protein-related materials. The strong band at 1412.47 cm^{-1} represents the stretching vibration of sulphates. These absorptions further support the occurrence of phenolic compounds and carboxyl-containing structures.

In addition, intense bands observed from 1261 to 1061.6 cm^{-1} are characteristic of (CO) and (COC) stretching vibrations. These features suggest the presence of ether groups, alcoholic functionalities, and glycosidic bonds, which are typical structural elements of numerous plant bioactive compounds [22].

The body and organs weights (g) of the experimental birds

The experimental birds' weights (g) and that of their organs were recorded in Table 3. A progressive increase in body weight was observed in all the birds at the end of each week. Group 1 showed the highest weight increase at the end of the first week, followed by group 2. At the end of the experiment, the birds in the group 1, group 2, group 3 and group 4 had average weights of 130.50 g, 128.75 g, 138.00 g and 137.75 g, respectively. Control showed the highest weight gain of 22.25 % followed by group 1 (250 mg/kg), which was 13.95 %. The closeness observed within the weights (13.95±2.60 to 13.15±3.43) of the experimental birds in groups 2, 3 and 4 suggests that this corn silk methanol extract was not toxic to the various bird under this study. A progressive increase in body weight was observed over the course of the treatment period, although the differences were not statistically significant. In a similar manner, a published 20-day investigation on alloxan-induced hyperglycemic mice found that administration of aqueous corn silk extract did not significantly affect body weight or feed consumption [23].

Gas chromatography-mass spectrometry (GC-MS)

They are Twenty-four bioactive compounds in the GC-MS chromatogram of corn silk methanol extract. These compounds were identified based on their retention time and molecular formula and presented in Table 4. The compounds identified included: 1,2,4-Benzenetriol, (Z)-9,12-octadecadienal, Hepta decanoic acid, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl4H-Pyran-4-one, Pentadecanoic acid, 2-methoxy-4-vinylphenol, Myristic acid, Pentadecanoic acid, 14-methylester, Pentadecanoic acid, 14-methylester, 2-methoxy-4-vinylphenol, Palmitic acid, 9,17-Octadecadienoic acid, methyl ester, (Z,Z)-9,12-Octadecanoic acid, Methyl stearate, Oleic acid, 9,17-Octadecadienoic acid, methyl ester. Palmitic acid is the most abundant peak area (21.17%), having the retention time of 22.92, and formula ($C_{16}H_{32}O_2$) is used as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, haemolytic inhibitor, and antiandrogenic [24]. 1,2,4-Benzenetriol, with the peak area (15.2 %) and formula ($C_6H_6O_3$) is used as a food additive and flavouring agent in industries. 2,3-Dihydro-3,5-Dihydroxy-6-Methyl4H-Pyran-4-one one with the peak area (13.84 %) having the retention time of 7.85, and formula ($C_6H_8O_4$) is used as an antifungal agent. Pentadecanoic acid, 14-methylester with the peak area of (9.00 %), having the retention time of 22.21 is used as a flavouring agents. (Z, Z)-9,12-Octadecanoic acid with the peak area (6.27 %) having the retention time of 26.45 is also used as antioxidant. 9,12-Octadecadienoic acid, methyl ester with the peak area (6.00 %) having the retention time of 25.48 is used as antioxidant.

The presence of these diverse organic compounds could explain the various biological activities (antimicrobial, anticancer, pain-relieving, liver-protective, and anti-inflammatory effects) that underpin their widespread application in traditional healthcare practices [24, 25, 26].

Haematological result

Evaluation of haematological indices in laboratory animals or birds is an important approach for determining the physiological and toxicological impact of xenobiotics on the body. PVC, WBC and other related blood indices analysis, provide valuable information for detecting and monitoring pathological conditions, including cancer, cardiovascular disorders, haemorrhagic abnormalities, and infectious diseases.White blood cells (WBCs) and their differentials, particularly lymphocytes, are important markers of the body's response to toxic agents and are vital components of the immune defense system [27]. Haematological analysis of corn silk methanolic extract is presented in Table 5. Group 3 exhibited the highest values of packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) counts (51.75%, 16.93 g/dL, and 4.21%, respectively), while those of the other groups were comparable. The absence of statistically significant differences in most haematological parameters following extract administration suggests that the treatment did not exert toxic effects on the blood. In particular, the non-significant changes observed in RBC and Hb concentrations across all groups may indicate that erythropoiesis remained stable and physiologically regulated. Since erythropoietin produced by the kidneys plays a central role in RBC production, the maintenance of comparable RBC and Hb values implies a balance between erythrocyte synthesis and destruction [28]. Moreover, the lack of significant alterations in RBC and Hb levels suggests that erythrocyte morphology, osmotic fragility, and haemoglobin incorporation into red cells were not adversely affected [27]. Consequently, the oxygen-carrying capacities of the blood and tissue oxygen delivery were likely preserved within normal physiological limits. Alterations in the liver and kidneys indices could serve as an important assessment tool in determining extract efficacy and toxicity in experimental animals [29]. White blood cells are essential for producing antibodies, thus preventing the entry of pathogens and strengthening the immune system of quails [30]. The values of white blood cells (14212.50-18112.50 $\times 10^3/\mu L$) were within the normal range 9-25($\times 10^6/\mu L$) cited by [18]. Lymphocytes, heterophilis, and monocytes values obtained in this study were comparable within the experimental groups. These findings further indicate that the birds in all experimental groups maintained normal haematological status and were clinically healthy. The non-significant variations recorded in these indices indicate that the extract did not induce microcytosis nor alter haemoglobin content per erythrocyte. This suggests that corn silk extract did not predispose the birds to anaemic conditions during the experimental period.

Overall, the findings demonstrate that dietary administration of corn silk at doses up to 750 mg/kg did not produce adverse haematological effects in the birds.

Biochemical

Evaluation of hepatic enzymes, including AST, ALT, and ALP, serves as an important approach for determining liver functional status [13]. The current study shows no statistically substantial variations in AST, ALT, ALP activities and other liver enzymes between each of the experimental rats (Table 6). Similarly, all assessed biochemical parameters remained comparable across the experimental groups. The findings indicate that administration of the methanolic extract of corn silk did not alter serum AST or ALT concentrations. These observations are consistent with reports by [31,32], who documented that dietary supplementation with avocado pit powder in rabbits and the use of *Dacryodes edulis* leaves in Gallus domesticus did not produce significant changes in serum AST or ALAT levels. This consistency may suggest that the methanolic extract of corn silk does not exert hepatotoxic effects. Regarding serum proteins, birds in group 2 recorded the highest total protein (4.45 g/L) and albumin (1.10 g/L) values, while globulin concentration was highest in group 4 (3.35 g/L) and lowest in group 1 (3.1 g/L). These variations were not statistically significant, and no meaningful differences were detected in the albumin-to-globulin (A/G) ratio among groups. Overall, the results indicate that corn silk did not produce adverse effects on serum biochemical indices at the administered doses. The liver is the central organ to detoxification and metabolic biotransformation processes. ALP, ALT, and AST are frequently employed as dependable markers of hepatic injury. In addition, creatinine is commonly used to assess renal function; elevated serum levels may signal kidney impairment. Increased serum creatinine and urea concentrations are often associated with compromised glomerular filtration and renal dysfunction [33]. The lack of significant alterations in AST, ALT, and ALP levels in this study further supports the conclusion that corn silk extract did not adversely affect the liver of the experimental birds.

Histopathological result

Histopathological analysis as presented in Table 7, revealed no significant pathological alterations in the liver kidneys, hearth, and brain of the experimental birds. Organ weight is an important index of physiological and pathological status in animals [12]. The testes and gland appeared to be normal with no visible lesions seen in them within all the experimental groups except for group 4 where immature germinal cells and cellular debris were found in the lumen of the seminiferous tubules were seen in the testes. Goblet cells population in the gut appears reduced in all treated groups, though it did not seem to confirm an adverse digestive challenge. Corn silk reduces the activity of mucus secretion (mucogenesis) of the gut. There can be a relationship between mucogenesis and corn silk properties. However, it should be noted that there was copious foamy light staining material found in the lumen of the glandular acini of the group 1 birds, while there was no observable lesion in the treated groups. Activities of cloacal gland is hormonal, and they are linked to the reproductive system. Since there was no regular pattern that was followed, the changes observed in the reproductive organ could not be attributed to the methanolic extract of corn silk given. It can be suggested that an investigation should be carried out on the cloacal gland and testes of the male birds to ascertain the effects of corn silk extract. It can also be proposed that the dosage should be limited to 500mg/kg.

Results:

Table 1: Result of mineral analysis of methanolic extract of corn silk

Elements	Corn silk (mg/l)
Ca	2.85
Mg	1.06
K	180.00
Na	15.00
Mn	0.05
Fe	0.11
Copper	0.078
Zinc	0.103
Na/K	0.083

Table 2: FTIR table for methanolic extract of corn silk

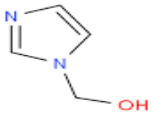
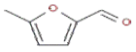
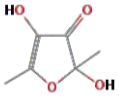
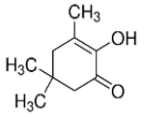
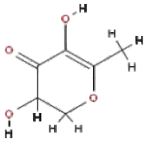
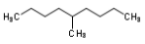
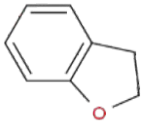
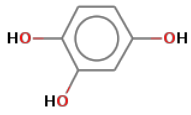
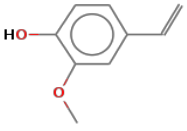
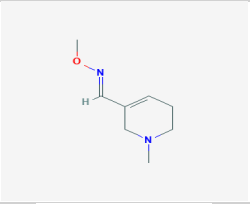
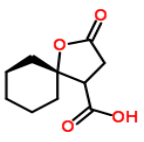
S/N	Frequency	Group	Appearance
1	3393.00	Alcohol (O-H) stretching	Strong, broad
2	2947.56	Alkane (C-H) stretching	Medium
3	2104.00	Alkyne (C≡C) stretching	Weak
4	1641.91	Alkene (C=C) stretching	Strong
5	1412.47	Sulphate (S=O) stretching	Strong
6	1261.00	Alkyl aryl ether (C-O) stretching	Strong
7	1061.60	(-C-O) stretching of Alcohol	Strong

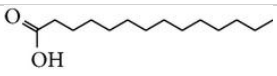
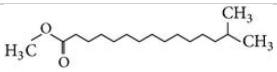
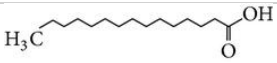
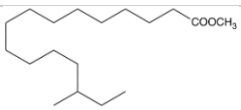
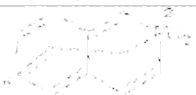
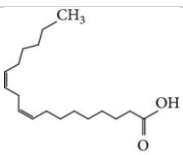
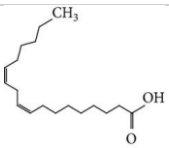
Table 3: Weight of birds' body and organs (g)

Parameters	Group 1	Group 2	Group 3	Group 4
Initial weight	104.25±11.18^{c}	113.00±1.63^{bc}	121.75±1.50^{ab}	123.50±5.20^{a}
Final weight	127.50±6.48^{c}	128.75±1.90^{bc}	138.00±3.83^{ab}	140.00±10.42^{a}
Weight gain	26.25±4.57^{a}	15.75±2.75^{b}	16.00±4.08^{b}	16.50±5.45^{b}
% weight gain	22.25±3.24^{a}	13.95±2.60^{b}	13.15±3.43^{b}	13.25±4.00^{b}
Organs				
Testes (Left and Right)	2.19±0.91^{a}	3.03±0.45^{a}	2.36±1.52^{a}	2.38±0.26^{a}
Liver	1.57±0.11^{a}	1.42±0.10^{a}	1.72±0.21^{a}	1.80±0.48^{a}
Heart	0.98±0.07^{a}	1.11±0.13^{a}	1.02±0.08^{a}	1.00±0.07^{a}
Kidney	0.57±0.15^{a}	0.58±0.19^{a}	0.49±0.07^{a}	0.60±0.34^{a}
Brain	0.48±0.12^{a}	0.41±0.07^{a}	0.45±0.06^{a}	0.48±0.15^{a}

Data are mean±S.D; Data with different letters are significantly different from each other

Table 4: Components of the methanolic extract of corn silk by GC-MS analysis

S/N	TP %	RT	MOL. FORMULAR	MOL. WT(g/mol)	NAME OF THE COMPOUND	CHEMICAL STRUCTURE	APPLICATIONS
1	2.29	4.00	$C_4H_6N_2O$	98.11	1H-imidazol-1-ylmethanol		Antifungal, antibacterial and anticancer.
2	1.57	4.36	$C_6H_6O_2$	110.11	5-Methylfurfuran		Food additives. Flavouring Agents
3	2.26	4.63	$C_6H_8O_4$	114.13	2,4-dihydroxy-2,5-dimethylfuran-3-one		No activity recorded.
4	1.51	6.85	$C_7H_{10}O_2$	126.15	2-hydroxy-3,5-diethylcyclopent-2-en-1-one		No activity recorded.
5	13.84	7.85	$C_6H_8O_4$	144.13	2,3-Dihydro-3,5-Dihydroxy-6-Methyl4H-Pyran-4-one		Antifungal activity.
6	1.76	8.77	$C_{10}H_{22}$	142.28	5-methyl-nonane		Antimicrobial and anti - inflammatory.
7	1.23	9.02	C_8H_8O	120.15	Coumaran		Antibacterial
8	15.12	9.37	$C_6H_6O_3$	126.11	1,2,4-Benzenetriol		Food additive and flavouring agent.*
9	2.08	10.79	$C_9H_{10}O_2$	150.18	2-methoxy-4-vinylphenol		Food additives and flavouring agents
10	1.87	14.76	$C_8H_{14}N_2O$	154.21	Milameline		No activity recorded.
11	0.77	15.18	$C_{10}H_{14}O_4$	198.22	Fumaric acid, cyclobutyl ethyl ester		Food preservatives and therapeutic uses. Manufacturing of perfumes and used for hair treatment*.

12	0.96	20.60	$C_4H_{28}O_2$	228.37	Myristic acid		Antioxidant, cancer preventive, nematicide,hypo-cholesterolemic, lubricant*.
13	2.03	21.93	$C_{15}H_{30}O_2$	242.40	Pentadecanoic acid		Rare fatty acid in nature, flavouring agent.
14	9.00	22.21	$C_{17}H_{34}O_2$	270.46	Pentadecanoic acid, 14-methylester		No activity recorded
15	0.93	22.92	$C_{18}H_{34}O_2$	266.47	Olealdehyde		Stearic acid is used with simple sugar as a hardener in making candies. It is used to produce dietary* supplements
16	21.17	22.92	$C_{16}H_{32}O_2$	256.43	Palmitic acid		Antioxidant, hypocholesterolemic, nematicide, pesticide,lubricant, haemolytic inhibitor*
17	0.85	24.04	$C_{18}H_{36}O_2$	284.48	14-methyl Palmitic Acid methyl ester		Antioxidant, Used as a natural additive in organ product*
18	1.10	24.50	C_9H_7P	160.15	Isophosphinoline		No activity recorded
19	2.41	24.86	$C_{17}H_{34}O_2$	270.45	Hepta decanoic acid		Lubricants and lubricant additives. Surface active agents*.
20	5.99	25.48	$C_{17}H_{30}O_2$	266.43	9,17-Octadecadienoic acid, methyl ester		Display antioxidant properties of some phytochemicals. Useful in cosmetic industries*.
21	2.49	25.61	$C_{18}H_{32}O$	264.45	(Z)-9,12-octadecadienal		Anti-inflammatory, hypocholesterolemic, cancer preventive, insectifuge,antiarthritic, hepatoprotective,, antihistaminic, antieczemic*.
22	0.76	25.73	$C_{18}H_{34}O_2$	282.47	Oleic acid		Diminishes threat of heart diseases, diabetes and high blood pressure*
23	1.77	26.19	$C_{19}H_{38}O_2$	298.51	Methyl stearate		Food additives Flavouring agents
24	6.27	26.45	$C_{18}H_{32}O_2$	280.45	(Z,Z)-9,12-Octadecanoic acid		Shows the antioxidant effect as polyphenols and natural phenols. It is used in cosmetic industries*.

RT: Retention Time TP: Total Percentage - Balasubramanian et al. (2018)*

Table 5: Results of Haematological analysis of blood samples of birds in all the groups

Parameter	Group 1	Group 2	Group 3	Groupn 4
PCV (%)	50.75±7.63^a	51.75±2.06^a	46.25±4.64^a	47.25±7.68^a
HB (mg/dl)	16.60±2.40^a	16.93±0.44^a	15.7±1.77^a	15.60±2.65^a
RBC (10^6/µl)	3.91±0.57^a	4.21±0.07^a	4.04±0.31^a	3.84±0.55^a
WBC(10^3/µl)	1567.00±2070.63^a	14400±3738.98^a	18112.50±1603.32^a	14212.50±3579.19^a
Platelets	135000±17088^{ab}	112500±14479.87^b	140750±12658.99^a	12750.00±13178.27^{ab}
Lymphocyte(%)	11719.63±1681.52^{ab}	10766.25±2822.83^{ab}	13615.25±1047.50^a	10305.50±2003.91^b
Monocyte (%)	517.25±249.71^a	370.50±35.45^a	542.38±153.69^a	494.13±121.15^a
Heterophil (%)	2766.25±452.89^a	2416.5±475.51^a	3319.38±710.76^a	2827.38±1434.76^a
Eonophil (%)	62787.50±30635.01^a	81675.00±50322.78^a	86700.00±22383.63^a	55862.50±13994.72^a
Basilophil (%)	4400.00±8800.00^a	3000.00±6000.00^a	4900.00±9800.00^a	2687.50±5375.00^a
MCV (fl)	12.97	12.29	11.45	12.30
MCH (%)	4.25	4.02	3.90	4.06
MCHC	32.71	32.71	33.95	33.02

Values are mean± standard deviation. PCV - packed cell volume (%), Hb – haemoglobin concentration (mg/dl), RBC – red blood cells (10^6/µl), WBC – white blood cells (10^3/µl) and platelets (cell/cu.mm). Values in the same row with the same superscript are not significantly different at (P>0.05)

Table 6: Result of Biochemical analysis of serum of birds in all the groups

Parameters	Group 1	Group 2	Group 3	Group 4
TP (g/L)	4.38±0.68[a]	4.18±0.28[a]	4.45±0.58[a]	4.40±0.67[a]
ALB (g/L)	1.03±0.59[a]	1.08±0.22[a]	1.10±0.38[a]	0.93±0.32[a]
GLOB (g/L)	3.35±0.44[a]	3.10 ±0.08[a]	3.35±0.27[a]	3.47±0.43[a]
GLU (g/L)	202.75±13.96[a]	205.25±4.86[a]	212.25±10.66[a]	200.25±11.90[a]
TRIG (g/L)	34.50±21.58[a]	38.75±12.77[a]	50.25±10.72[a]	37.25±17.54[a]
CHOL (g/L)	152.00±9.52[a]	146.00±7.83[a]	144.25±5.06[a]	147.00±6.88[a]
ALT (U/L)	26.75±7.5[a]	26.00±4.55[a]	28.25±4.03[a]	25.50±6.95[a]
AST (U/L)	142.00±17.34[a]	139.00±11.84[a]	139.00±11.84[a]	136.25±15.31[a]
ALP (U/L)	50.75±3.20[a]	51.25±7.27[a]	46.75±7.14[a]	52.00±6.06[a]
BUN (mg/dl)	12.77±3.20[a]	13.03±2.65[a]	14.48±2.16[a]	13.15±2.58[a]
CREATININE (mg/dl)	0.53±0.05[a]	0.48±0.05[a]	0.53±0.05[a]	0.53±0.05[a]
AG ratio	0.30±0.14 [a]	0.30±0.08 [a]	0.30±0.08 [a]	0.27±0.05[a]

Values are mean± standard deviation of triplicate results. A/G ratio – Albumin Globulin ratio, AST – aspartate aminotransferase (U/L), ALT – alanine aminotransferase (U/L), ALP – alkaline phosphate (U/L) and BUN – blood urea nitrogen (mg/dl). Values in the same row with the same superscript are not significantly different at (P>0.05)

Table 7: Result of histopathological analysis of organs

Organ	Group 1	Group 2	Group 3	Group 4
Heart	Lesions were not detected	lesions were not detected	No observable lesions detected	No observable lesions detected
Liver	lesions were not detected	There is a moderate to severe portal congestion.	There is a mild to moderate portal congestion. The periportal connective tissue is very prominent.	There is a very severe portal congestion
Brain	Lesions were not detected	No observable lesions detected	No observable lesions detected.	No observable lesions detected.
Kidney	Very mild interstitial congestion.	Lesions were not detected	No observable lesions detected.	There is a severe congestion of the interstitium.
Testes	Lesions were not detected	No observable lesions detected	Lesions were not detected	Immature germinal cells and cellular debris found in the lumen of the seminiferous tubules.
Gland	Copious foamy light staining material found in the lumen of the glandular acini.	Lesions were not detected	No observable lesions detected.	No visible lesions seen.
Gut	Lesions were not detected. A high density of goblet cells was within the epithelium.	Lesions were not detected. There is a high density of goblet cells within the epithelium.	Very scanty amount of goblet cells seen within the epithelium	No observable lesions detected. Very few goblet cells found in the epithelium

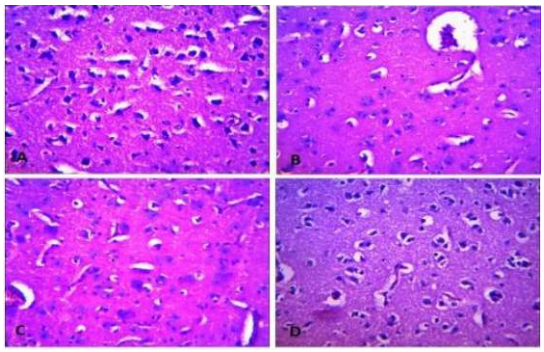


Fig. 1: Photomicrograph of the brain of birds (x550)

(a) Group 1 No observable lesion
(b) Group 2 No observable injury
(c) Group 3 No observable lesion
(d) Group 4 showing no visible lesion

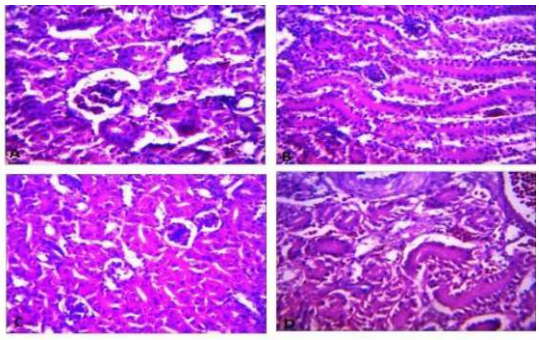


Fig. 2: Photomicrograph of the kidney of birds (x550)

(a) Group 1 showing a very mild interstitial congection
(b) Group 2 No visible lesion
(c) Group 3 No visible lesion
(d) Group 4 showing a very severe congestion of the interstitium (arrows)

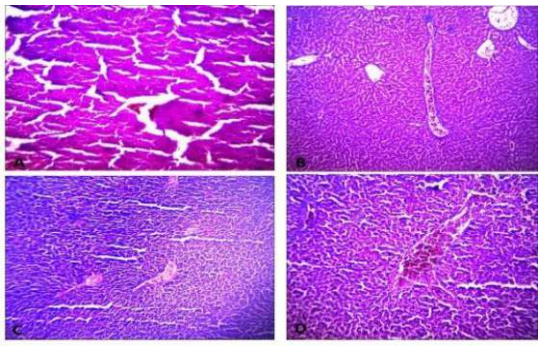


Fig. 3: Photomicrograph sections of the liver of Figure

(a) Group 1 no lesion was observed
(b) Group 2 A moderate to severe portal congestion
(c) Group 3 showing a mild to moderate portal congestion
(d) Group 4 showing no visible lesion (x400)

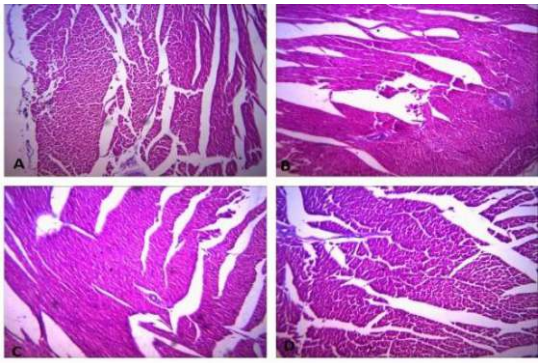


Fig. 4: Photomicrograph sections of the heart of birds in (x400) of birds in (x400)

(a) Group 1 showing no visible lesion
(b) Group 2 No lesion was observed
(c) Group 3 showing no visible lesion
(d). Group 4 showing a very severe portal congestion (x100)

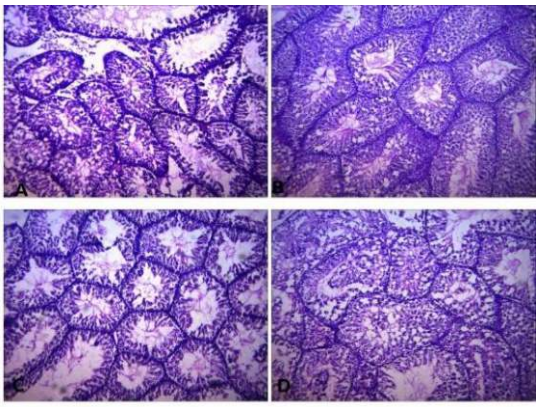


Fig. 5: Photomicrograph sections of the testes

(a) Group 1 with no observable lesion (×400).
(b) Group 2 with no observable lesion (×400).
(c) Group 3 showing a very scanty amount of changes (×400).
(d) Group 4 showing immature germinal cells, goblet cells within the epithelium, and cellular debris in the lumen of the seminiferous tubules (×400).

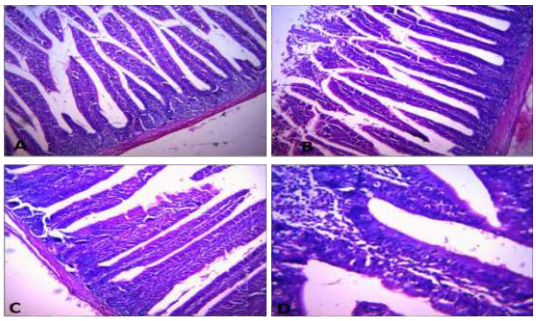


Fig.6: Photomicrograph of guts of birds in of birds in (x400) (x400)

(a) Group 1 with no observable lesion (×400).
(b) Group 2 with no observable lesion (×400).
(c) Group 3 showing a very scanty amount of changes (×400).
(d) Group 4 with no observable lesion (×400).

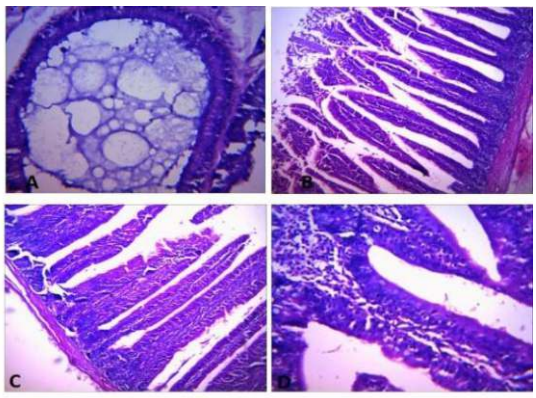


Fig. 7: Photomicrograph sections of the gland of birds in

(a) Group 1 showing copious foamy light staining material found in the lumen of the glandular acini
(b) Group 2 with no observable lesion
(c) Group 3 with no observable lesion
(d) Group 4 with no observable lesion (x400)

CONCLUSION

Corn silk methanolic extracts contain substantial amount of key macronutrients, together with Na, and K, that could make them potentially beneficial for the promotion of good health. The FT-IR spectra reflects the occurrence of several chemically active groups typically linked to secondary metabolites, including phenolics, flavonoids, tannins, and other plant-derived bioactive substances. Twenty-four bioactive compounds was found in the GC-MS chromatogram. The haematological and biochemical analyses of the bird organs showed no significant difference in the parameters. Histological assessment of the organs, which are kidney, brain, kidney, heart, liver, gut, cloacal gland and testes, tested in all groups showed no lesion attributed to the corn silk except for the testes in group 4, which showed immature germinal cells and cellular debris found in the lumen of the seminiferous tubules. Since no regular pattern was observed, it can be deduced that corn silk methanolic extracts don't show any toxicological effect on the reproductive system of the male birds at the tested levels of incorporation. In conclusion, it can be said that corn silk is non-toxic at 250 mg/kg and 500 mg/kg of oral dosing in birds. It is therefore safe for animal consumption as a food supplement at groups 2 and 3 levels.

Acknowledgement

Both the Chemistry Departments of the faculty of Science and the Veterinary Pathology Department of the Faculty of Veterinary of University of Ibadan were appreciated by the authors for using their facilities to accomplish this research work.

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