Haematologic and Gastric Histological Changes Associated with Administration of Ground Nutmeg (*Myristica fragrans)* Seed on Adult Male Albino Wistar Rats

ABSTRACT

The present study was designed to evaluate the effects of a daily administration of ground nutmeg seed at different doses on some hematological parameters. Histological changes in stomach triggered by ground nutmeg seed were also investigated. Thirty adult male albino wistar rats were divided into six equal groups, namely A,B,C,D,E and F; each group comprising of five rats. Group A served as the control and were administered only water and 100 g of rat feed; while 1 g, 5 g, 10 g, 15 g and 20 g/kg body weight, of the ground nutmeg *seed* mixed with 100 g of rat feed in each case, were given to groups B, C, D, E and F, respectively, for twenty six days. The weight measurement was done every seven days. The stomachs were harvested immediately after sacrifice, fixed in 10 % buffered formalin, and was later made to undergo Hematoxylin and Eosin staining method. Blood samples were collected via cardiac puncture. Data were expressed as Mean $\pm$ standard error of the Mean and subjected to one way analysis of variance. Significance differences between mean were assessed by student-New-Man-Keuls post hoc test. 95 % level of significance ($P<0.05$) was used for statistical analysis and Microsoft Excel 2010 package was used for graph and error bars. The control (group A) and the lowest dose-treated group (group B, given 1 g of ground nutmeg seed) had normal and intact histological structures of the stomach. The longitudinal gastric folds/ruggae, the surface mucous cells, mucous neck cells and parietal cells were not affected in Group C, given 5 g; but there was significant ($P<0.05$) degeneration of simple columnar epithelium and longitudinal folds. The result also showed significantly ($P<0.05$) degenerated simple columnar epithelium, longitudinal folds, coil pyloric glands and associated hypertrophy of the surface mucous cells and mucous neck cells in groups D, E and F, given, 10 g, 15 g, and 20 g, respectively; which were not dose dependent. There was significant ($P<0.05$) decrease of white blood cells and dose-dependent increase in blood sugar levels in the treated groups compared to the control. Ground nutmeg seed when consumed in large doses alters the histology of the stomach, slightly lowers the body's defense against infections and predisposes to diabetes. (Abstract is too lenghty reduce to about 250 words)

Key words: Blood, Nutmeg, Rat, stomach. Testes, Water.

INTRODUCTION

(Remove the image and transfer to materials and methods)

Figure 1. Nutmeg seed

Nutmeg (*Myristica fragrans)* is a brownish colored hard seed from a tropical evergreen tree botanically known as *Myristica fragrans* . It has a warm, spicy sweet flavor, and was regarded as one of the most valuable spices. It is in most cases ground into powder before use. Nutmeg is used in many dessert dishes, and also in savory recipes. Its culinary uses include spicy dishes like pies, custard, cookies spice cakes, cheese sauces, soups, egg and vegetables dishes. Butter known as nutmeg butter is usually gotten from the nut by expression. By the process of steam distillation, essential oil obtained from ground nutmeg is used widely in pharmaceutical industries. Nutmeg seed importance is innumerable; its uses cut across among others, oleochemical industries and in traditional medicine, were the oil is employed in managing disorders associated with the nervous and digestive systems. In fact, its use as a remedy for various ailments and improvement of health in general, dates back to the ancient times [1]. Nutmeg is reputed to play important roles in the removal of liver toxins, dissolving of kidney stones and relieve from infections. It also plays a crucial role by helping to increase blood circulation and appetite encouragement [2]. Its antioxidant properties is attributed to presence of phenolic compounds [3]; [4]; [5]; [6]; [7].

There is a demonstrated case of Immunoglobulin E reactivity, as well as reported case of allergy in nutmeg; limonene and eugenol, known chemical constituents of nutmeg are implicated as contact allergens [8]; [9]. Throughout their studies of this spice, researchers have found it to have a carminative effect. Carminatives are thought to relax the stomach muscles and are helpful in treating diarrhea, gas, nausea and indigestion [10].

Nutmeg is reputed to have a relatively narrow margin of safety; outcome of studies indicated that two to three teaspoons is the toxic dose of nutmeg when used as a spice [11]. There have been reported cases of acute psychosis and anticholinergic-like episodes associated with nutmeg consumption. The symptoms are characterized by cutaneous flushing, tachycardia, decreased salivation, GI symptoms (eg, nausea, vomiting, abdominal pain), fever, and CNS excitation with anxiety/fear [12]; [13]; [14]; [15]; [16].

STOMACH

The word Stomach has its origin from the ancient Greek word, στόμαχος, stomacho. It is part of the gastrointestinal tract. It is a hollow organ, muscular and dilated in structure. Its location is

between the oesophagus and the small intestine, both in humans and many other animals. In humans, the stomach walls comprised of mucosa, submucosa, muscularis externa, and serosa [17]; [18].

The stomach in humans is divided into four regions, each with its cells and functions that vary [19]. The cardia is the inlet to the stomach, where the contents of the oesophagus empty into the stomach. The fundus, a latin word, whose English equivalent is bottom, is located in the upper curved region. The body is the main, central region of the stomach. The pylorus, a Greek word, whose English equivalent is gatekeeper; is located at the lower region of the stomach, from which stomach contents empty into the duodenum [20]*;* [21].

Mucous secreting cells also referred to as goblet cells, Line the luminal surface of the stomach. Gastric pits and gastric glands are implicated in secretion of mucus and bicarbonate. Mucous neck cells produce mucin. Parietal cells, also known as oxyntic cells, secrete gastric acid. Chief cells also called, peptic or zymogenic cells, secrete pepsinogen, that digests protein [22].

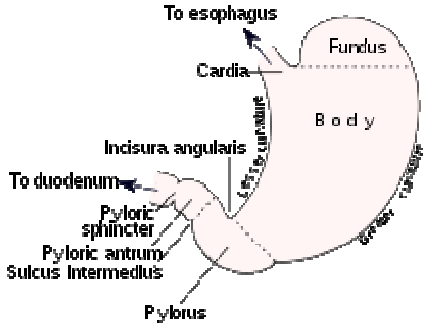

Figure 2. Gross anatomy of human stomach [23].

The rat stomach is located at the abdominal side of the diaphragm, and is connected to the esophagus at the gastroesophageal junction, and to the duodenum at the pyloric ring. The rat stomach is divided into the forestomach, also known as pars proventricularis, and glandular stomach, referred to as corpus or pars glandularis. The forestomach occupies about three fifths of the stomach area, while the glandular stomach is divided into the fundus and pylorus/antrum and communicates with the duodenum at the pyloric ring [24]; [25]. Heavily cornified squamous epithelium covers the forestomach. The mucosa of the glandular stomach is made up of rows of columnar cells that form numerous gastric pits which are based at the lamina muscularis mucosa and open into the lumen [26].

Substantially unproven scientifically are numerous assumed health benefits of nutmeg, just as seen or assumed in other herbs and spices. Moreover, a few of the scientifically substantiated research done were on the nutmeg oil and the mace. There is paucity of data based on scientific protocol on research carried out using numerable varying ranges of dosage of ground nutmeg seed; which is the major form in which nutmeg is consumed as a spice, hence, the need to carry out this research work titled Haematological and Gastric Histological Changes Associated with Administration of Ground Nutmeg Seed on Adult Male Albino Wistar Rats; employing Routine laboratory method involving H and E staining technique and light microscopy.

MATERIALS AND METHODS

Nutmeg seed was obtained from Itam market, Itu Local Government Area of Akwa Ibom State, Nigeria. It was identified by a plant taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria and a voucher specimen deposited at the herbarium of the same department. Herbarium No: Oke, UUH 3726 (Malaysia). The Nutmeg seeds were ground into powder at the Department of Pharmacognosy and Natural Medicines, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria. Out of the total gram of ground nutmeg seed obtained, a given gram was subjected phytochemical analysis to determine its phytochemical properties. The method of Odebiyi and Sofowora [27] was employed in the analysis.
(Place fig 1 image here)

Thirty adult male albino wister rats weighing between 180 g to 200 g were used for the study. The rats were purchased from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria, and were fed standard growers mash feed, produced by grand cereals limited, Nigeria The rats were divided into six equal groups, namely A,B,C,D,E and F. Group A served as the control and were administered only distilled water and 100 g of rat feed; while groups B,C,D,E and F, the treatment groups, were administered varying doses (1 g, 5 g, 10 g, 15 g and 20 g/kg body weight, respectively) of the ground nutmeg seed mixed with 100 g of rat feed, respectively, for 26 days.

The animals were weighed on the 26th day of the experiment and anaesthetized using light ether. The stomach were harvested and fixed immediately in 10% buffered formalin for routine histological techniques, while the blood samples were collected by cardiac puncture in EDTA bottle and plain bottle for hematological studies. The gastric tissues were stained using Hematoxylin and Eosin; Drury and Wallington, method [28].

This research received a go-ahead-order to be carried out, from the appropriate Research and Ethics Committee. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

STATISTICAL ANALYSIS

Data were analyzed using descriptive statistical tool, Primer, version 3.0, and were expressed as mean ± standard error of the mean (M±SEM) and subjected to one way analysis of variance. Significant difference between means was assessed by Student - Newman-Keuls post hoc test. 95% level of significance (P = 0.05) was used for the statistical analysis. Microsoft excel 2010 package was used for graphs and error bars.
RESULT

Following the conduction of phytochemical analysis on ground nutmeg seed, to determine the active ingredients present, the various observations and inferences shown in table 1, below, were made.

Table 1. Result of Phytochemical Analysis of Ground Nutmeg Seed

Test	Observation	Inference
Alkaloids	Red orange colour precipitate observed	++
Flavonoids	Orange colour precipitate observed	+++

Tannins		Bluish – black colouration observed	++
Saponnins		Persistent frothing observed	++
Cardiac Glycosides			
(1)	Lieberman	A green ring observed	+
(2)	Salkowski test	A brownish-red ring observed	+
(3)	Keller killiani test	Brown ring observed	+

Key:
+ = slightly present
+ + = moderately present
+ + + = heavily (strongly) present

There was increase in body weight of the control group (group A), which is directly proportional to the number weeks involved in the research. However, in the test groups, B to F, the weight did not increase proportionally with the number of weeks the rats were fed; instead, there was fluctuating increase and decrease in their body weight, as shown in table 2.

Table 2. Comparison of Changes in Body Weight of Albino Rats

Groups	Week 1	Week 2	Week 3	Week 4
A	167.50±17.69	185.25±22.73	193.75±23.93*	205.50±26.38*
B	155.00±8.79	164.00±7.84	169.20±9.00	159.60±7.46*
C	192.40±13.92*	201.60±15.44*	191.00±16.97	198.60±18.85
D	148.20±7.13*	154.80±9.77*	153.40±10.77*	160.60±12.70
E	174.25±22.50	185.00±25.09	185.50±24.39	179.67±26.09
F	182.80±11.49	188.40±12.14	178.40±13.57	175.60±13.95
	P = 0.293	P = 0.411	P = 0.591	P = 0.432

Values are expressed as mean ± standard error of mean (M±SEM). 95% level of significance (P = 0.05)

Key:
A = rats given distilled water (Control).
B = rats given 1g of nutmeg/ kg bw/day mixed with 100g of feed.
C = rats given 5 g of nutmeg/ kg bw/day mixed with 100g of feed.
D = rats given 10 g of nutmeg/ kg bw/day mixed with 100g of feed.
E = rats given 15 g of nutmeg/ kg bw/day mixed with 100g of feed.
F = rat given 20 g of nutmeg/ kg bw/day mixed with 100g of feed.

In table 3, the outcome of full blood count is shown.

Table 3. HAEMATOLOGIC RESULT

FULL BLOOD COUNT	DIFFERENTIALS

Group	PCV (%)	Hb (g/dl)	WBC (l)	RBC (l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	N (%)	L (%)	M (%)	E (%)
A	43	14	15.6	8.7	49	16	32	32	60	05	03
B	38	12	10.3	8.0	47	15	31	30	61	04	04
C	45	14.3	11.0	9.1	49	16	32	20	67	07	06
D	47	13.9	9.6	9.1	51	14	28	40	56	02	02
E	45	14.5	12.2	9.0	50	16	32	30	60	05	05
F	41	14.3	12.2	8.7	47	17	35	40	50	04	06

KEYS

PCV - Packed Cell Volume
Hb - Haemoglobin
WBC - White Blood Cell
RBC - Red Blood Cell
MCV - Mean Corpus
MCH - Mean Corpuscular Heamoglobin
MCHC - Mean Corpuscularm Haemoglobin Concentration
N - Neutrophils
L - Lymphocytes
M - Monocytes
E - Eosinophils

Dose dependent marked elevation in blood sugar across the groups, B to F were observed compared to the control group. This was clearly shown in table 4.

Table 4. Blood Sugar

Group	Blood sugar (mmol/l)
A	3.8
B	5.0
C	5.2
D	5.8
E	6.0
F	6.1

The outcome of histopathological analysis point to the fact that nutmeg consumption at higher doses above 10 g/kg bw per day is toxic to the testes. These are depicted succinctly in figures 3 to 14.

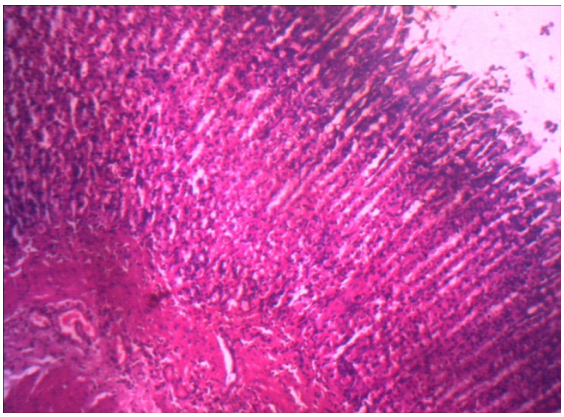

Figure 3. Group A (Control). (H&E method, X100).

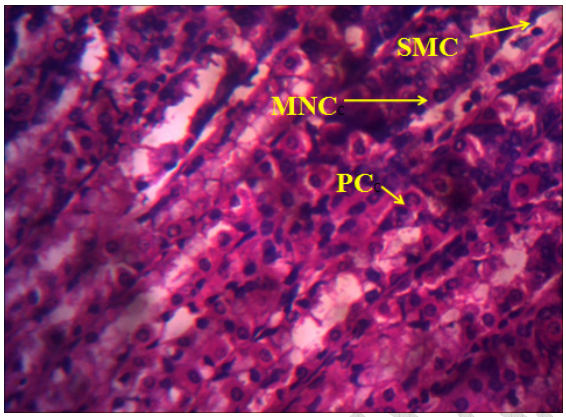

Figure 4. Group A (Control). (H&E method, X400).

Histologic section of the gastric mucosa (cardia, fundus and body regions) of rat given distilled water. Section revealed intact, longitudinal gastric folds/ruggae with intact surface mucous cells SMC, mucous neck cells MNC and parietal cells PC.

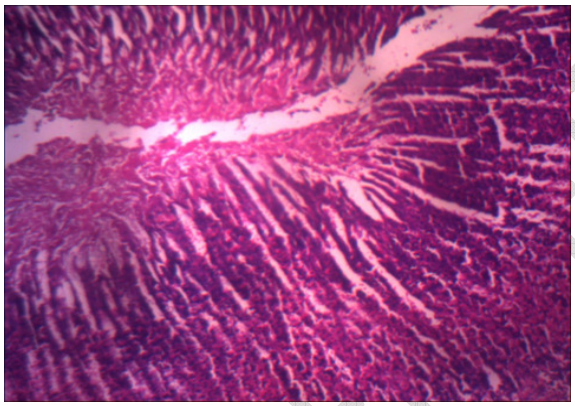

Figure.5. Group B. (H&E method, X100).

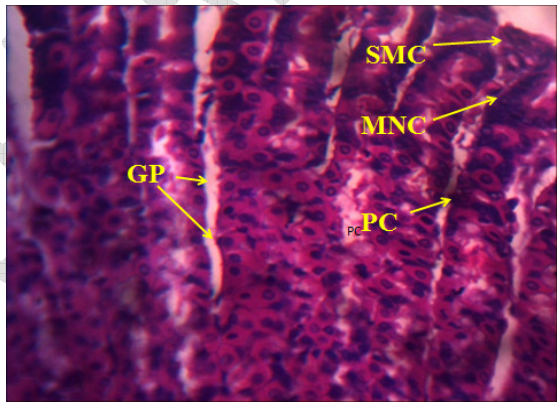

Figure 6. Group B. (H&E method, X400).

Histologic section of the gastric mucosa (cardia, fundus and body regions) of rat given 1g of nutmeg/ kg bw /day mixed with 100g of feed. Section revealed intact, longitudinal gastric folds/ruggae with intact surface mucous cells SMC, mucous neck cells MNC and parietal cells PC. Inference: not affected by 1 g of nutmeg.

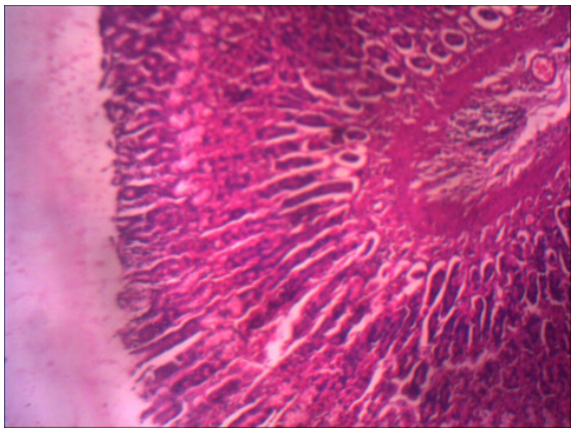

Figure 7. Group C. (H&E method, X100).

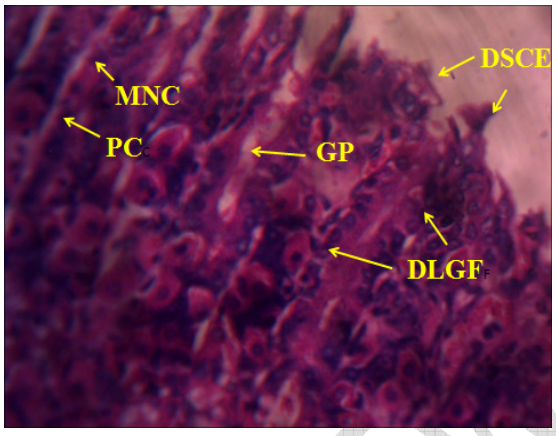

Figure 8. Group C. (H&E method, X400).

Histologic section of the gastric mucosa (Cardia, fundus and body regions) of rat given 5g of nutmeg/ kg bw /day mixed with 100g of feed. Section revealed intact, longitudinal gastric folds/ruggae with intact surface mucous cells SMC, mucous neck cells MNC and parietal cells PC in some part and degenerating simple columnar epithelium DSCE and degenerating longitudinal folds DLGF in another area. Inference: moderately affected by 5 g of nutmeg.

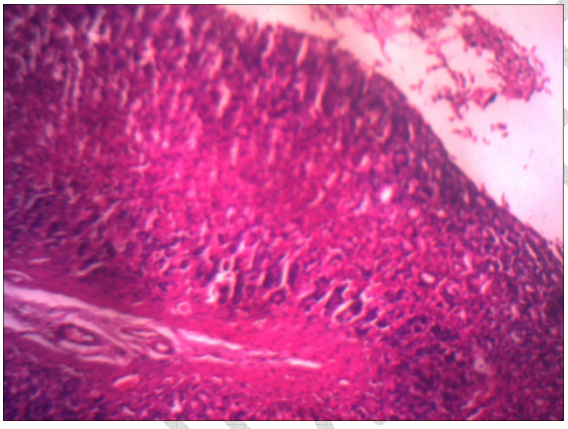

Figure 9. Group D. (H&E method, X100).

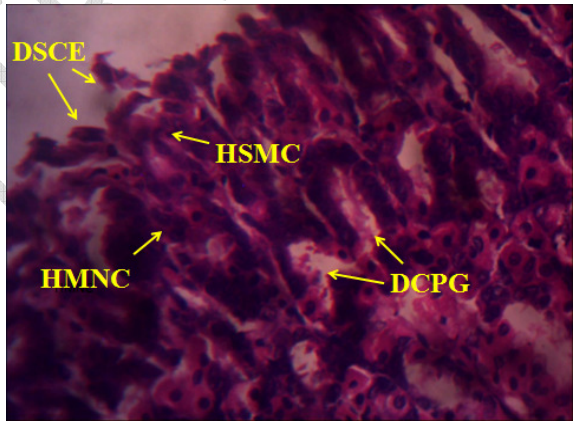

Figure 10. Group D. (H&E method, X400).

Histologic section of the gastric mucosa (Pyloric region) of rat given 10 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed degenerating simple columnar epithelium DSCE, degenerating longitudinal folds DLGF associated with hypertrophy of the surface mucous cells HSMC and hypertrophy of mucous neck cells HMNC. There is also degeneration of the coil pyloric glands DCPG. Inference: severely affected by 10 g of nutmeg.

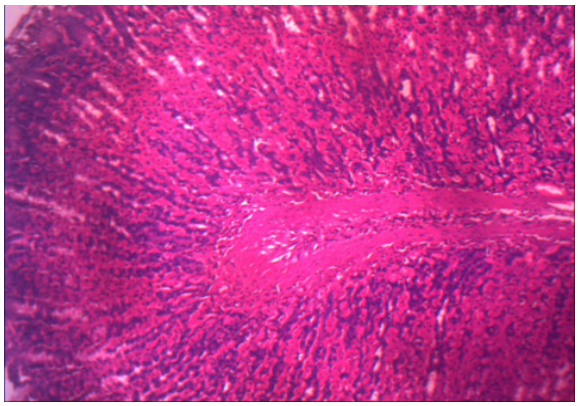

Figure 11. Group E. (H&E method, X100).

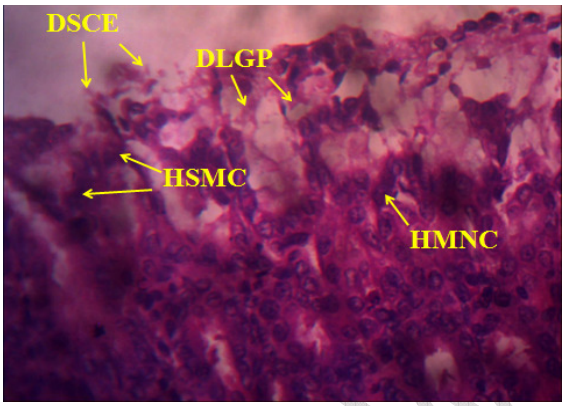

Figure 12. Group E. (H&E method, X400).

Histologic section of the gastric mucosa (fundus and body region) of rat given 15 g of nutmeg/ kg bw /day mixed with 100g of feed. Section revealed degenerating simple columnar epithelium DSCE, degenerating longitudinal gastric folds DLGF associated with hypertrophy of the surface mucous cells HSMC and hypertrophy of mucous neck cells HMNC. Inference: severely affected by 15 g of nutmeg.

Figure 13. Group F. (H&E method, X100).

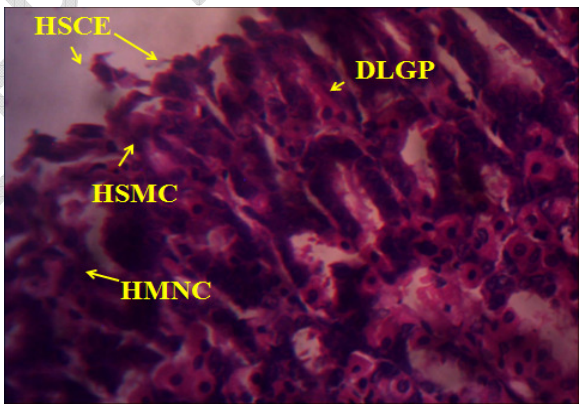

Figure 14. Group F. (H&E method, X400).

Histologic section of the gastric mucosa (Pyloric region) of rat given 20 g of nutmeg/ kg bw /day mixed with 100g of feed. Section revealed hypertrophy of simple columnar epithelium HSCE, degenerating longitudinal folds DLGF associated with hypertrophy of the surface mucous cells HSMC and hypertrophy of mucous neck cells HMNC. Inference: severely affected by 20 g of nutmeg.

Discussion

Understanding of pathologic processes associated with toxicity, drugs and chemical compounds safety profile are some of the insights normally made available by hematology. In addition,

information gotten from microscopic examinations, biochemical assay, urine analysis and clinical signs are complemented by hematology [29]. According to Johnson-Delaney [30], rat hematologic reference ranges are: RBC 6.76-9.75 x 10^6/mm^3 ,PCV 37.6-50.6%, WBC 6.6-12.6 x 10^3/mm^3 , Hemoglobin 11.5-16.1 g/dL, Neutrophils 1.77-3.38 x 10^3/mm^3 , Lymphocytes 4.78-9.12 x 10^3/mm^3 , Eosinophils 0.03-0.08 x 10^3/mm^3 , Monocytes 0.01-0.04 x 10^3/mm^3 Basopnils 0.00-0.03 x 10^3/mm^3 Platelets 150-460 x 10^3/mL. Information emanating from rat's basic biological data, by [31], as reported in Pass and Freeth 1993, stated as follows: Red blood cell count 7-10 x 106cells/µl, Hemoglobin 11-19 gm/dl (dl = deciliter), Packed cell volume 40.5-54%, Leukocyte count Total 9(6-18) x 103/µl, Neutrophils 14-20%, Lymphocytes 69-86%, Monocytes 1-6%, Eosinophils 1-4%, Basophils Rare, Platelets 500-1,000 x 103/µl. A.D., Stammers [32], in his study "the blood count and temperature in normal rats" conveyed the following information concerning RBC count: 7.4, 7.5, 8.0, 8.4, 10.0, all in 1 case; 8.6, and 9.0, in 2 cases; 8.8, 9.1, 9.6, 9.8, and 9.9, in 3 cases; 8.7, 9.3, and 9.5, in 4 cases; 9.2, and 9.4, in 6 cases; 8.9, in 7 cases. Subtle observation, perusal and comparison of the figures above with the result of our own hematology, as stated in table 3, indicates no provable contradictions and point to the fact that ours fall within the hematologic ranges of rat; thus, can serve as a premise to warrant the valid conclusion that ground nutmeg seed administered within the ranges 1 g to 20 g has no negative effect on full blood count.

As regards effects of ground nutmeg seed on blood sugar level, our findings suggest dose dependent marked elevation in blood sugar level across the groups, B to F, compared to the control group; as was clearly shown in table 4. The study results are not consistent with previous reports of activity of nutmeg against diabetes. In their experiments, Shyni *et al*., [33] indicated that nutmeg can improve the body's response to insulin by activating a protein regulated by insulin which transports glucose. Knowledge of nutmeg's traditional use led pharmacists in Pune and Sagar, India, to study nutmeg scientifically. Their controlled tests on rats showed that extracts of the spice: significantly decreased blood glucose levels; improved the lipid profile in the blood, stimulated the beta-cells of the pancreas to release insulin; improved body and organ (liver and pancreas) [34].

The outcome of our histopathological analysis point to the fact that nutmeg consumption at higher doses above 10 g/kg bw per day is toxic to the stomach. Our histological findings are consistent with that of Greger [35], which stated that "Large dosage (about 30 grams a day) of nutmeg can be toxic, producing disorientation, double vision and convulsions".

There are no provable contradictions of our work with that of Adjene and Igbigbi [36], who concluded that "high dose and chronic administration of nutmeg to adult Wistar rats caused varying degrees of distortion in the lining and glandular epithelial cells of the stomach. Adjene and Igbigbi, also found and concluded that "neoplastic changes, proliferation, hyperplasia and atrophic changes were also observed in the stomach with a high dose of nutmeg. Therefore, it is likely that function of the stomach may be adversely affected by high dose of nutmeg". However, there are discrepancies in the method of preparation of the ground nutmeg seed, in the duration of administration and in the quantity of ground nutmeg seed administered in grams per kilogram body weight.

RECOMMENDATION AND CONCLUSION

More research is needed to confirm these findings. There have to be definitive answers to these issues, otherwise, it is difficult to belief the role of nutmeg on blood glucose control; because, much of the insulin-like activity in action attributed to nutmeg is anecdotal.

CONSENT
It is not applicable.

ETHICAL APPROVAL
All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

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