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Ethnobotanical Relevance of Spice Plants [*Parkia biglobossa* and *Gongronema latifolium*]: Indices for Conservation and Domestication

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ABSTRACT

The need to conserve and domesticate extinction-threatened spice plants such as *P. biglobosa* and *G. latifolium* has prompted the search for their ethno-botanical importance. Sixty-four healthy matured male albino rats weighing between 120-180 grams were used in the present study. Four experimental groups of eight rats each per extract were used for the study in 2x4 factorial experimental layouts using completely randomized design. Group 1 rats served as control and received 1ml of normal saline and normal chow. Rats in group 2 received 200 mg/kg BW while rats in groups 3 and 4 received 300mg/kg BW and 400 mg/kg BW of each extract, respectively for 60 days through oral gavage. Rats were sacrificed using chloroform anaesthesia. Sperm, hormonal and biochemical parameters were estimated. Results showed that sperm parameters and testicular weight were significantly affected ($P < 0.05$), especially at higher doses while epididymal weight was not significantly affected. Reproductive hormones were enhanced by the administration of both spice extracts at dose of 200mg/kg but increasing dose caused significant reduction. Our results also revealed that the spice extracts significantly affected the albumin level, total protein, ALT, AST and ALP while creatinine level showed no significance ($P > 0.05$). Generally, extract from *P. biglobosa* seems to exert more effects on the parameters than that of *G. latifolium*. Succinctly, our results are explicit indication that *P. biglobosa* and *G. latifolium* extracts might be used to enhance sperm quantity and quality, reproductive hormones and liver enzymes production, especially at lower doses of 200mg/kg. There might be disruption of biosynthetic pathways administering the extracts at higher doses.

KEY WORDS: *P. biglobossa*, *G. latifolium*, sperm profile, reproductive hormones, liver enzymes, conservation/domestication.

INTRODUCTION

One of the biggest challenges facing Africa as a continent is the obvious neglect of our forest resources. Approximately, 4 percent of Nigeria's rainforest disappears everyday [1]. This forest would have served as reservoirs for pharmaceutical/therapeutic precursors, nutriceutical, industrial raw materials, etc. Rather than explore and exploit them, there are abandoned for "exotic" materials produced unfortunately from the same raw materials from the forest. According to [2] forest resources directly contribute up to 80% of the livelihoods of the people in countries living in extreme poverty. This neglect obviously has led to the extinction of these useful resources. This undoubtedly, paints a bleak picture of the future in terms of industrial development and food security in the country. Some of these extinction-threatened and endangered plant species are the spices, which are either dried or fresh seeds, fruits, roots, barks or vegetables used in nutritionally insignificant quantities as food additive for flavouring, colouring or as preservatives.

Interestingly, some of these spice plants that have attracted much research attentions in recent times are *Parkia biglobosa, Gongronema latifolium, Ocimum gratssium, Ocimum basilicum, Monodora myristica*, etc. This resurgent interest and paradigm shift fundamentally stems from the fact that they are extinction-threatened, which obviously demands an urgent and pragmatic efforts towards their conservation and domestication.

According to [3], *Parkia biglobosa* is an important multipurpose tree due to the increasing recognition of its contribution to fulfill basic needs of people such as food and wood production, supply of timber, firewood, pulp and fibre through fodder, gum, drugs, dyes, food security and conservation of natural resources [3,5,6]. The roots, barks, leaves, stems, flowers, fruits and seeds are used as therapeutic agents against diarrhoea, ulcers, pneumonia, burns, coughs, and jaundice [3]. The pulp contains higher cellulose and sucrose but less ascorbic acid than the cotyledons. The pulp also contains simple sugars except maltose [7]. The seeds of *P. biglobosa* on fermentation are used in cooking stew and soup. The sweet yellow pulp contains 60% sugar when ripe and the seeds contain 30% protein as well as vitamins and minerals [3].

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Gongronema latifolium on the other hand, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [8,9,]. It has been reported by various researchers to contain essential oils, saponins and pregnanes among others [10,11,12]. According to [9,13,14] aqueous and ethanolic extracts of *G. latifolium* had hypoglycemic, hypolipidemic and antioxidative properties while [12] reported its anti-inflammatory properties. [15] reported a general significant decrease ($P > 0.001$) in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ – glutamyl transferase (AST, ALT, ALP and GGT) activities of diabetic test groups when compared with the corresponding non-diabetic groups after *G. latifolium* leaf extract treatment.

As interesting and thrilling these developments might seem, there are paucity of information regarding the effects of these spice plants on sperm quality assessment, liver enzyme assay and hormonal studies using animal models. It is therefore on this premise that this research is mounted. The findings of this present research will be an added guide to their conservation and domestication.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *G. latifolium* and fermented seeds of *P. biglobosa* were sun-dried and ground into powder with electric blender (Model: 4250 Braun, Germany). They were soaked in 98% absolute alcohol for two weeks. They were then filtered and the filtrates concentrated *in vacuo* using a rotary evaporator (Labo rota 3000 Resona, Edwards's model). Thereafter each extract was transferred into a round bottom flask and allowed to evaporate completely under pressure at 50˚C. Each extract was stored in a refrigerator until required. The concentration of the extract was gravimetrically determined.

Experimental animals

Sixty-four healthy matured male Albino weighing between 120- 180 grams were used in the present study. They were housed in cages under standard laboratory conditions of temperature range of 25-29˚C and 12h light/dark cycle throughout the experimental periods. The rats were left to acclimatize for two weeks with water and feed *ad libitum*. Four experimental groups of eight rats each per extract were used for the study in 2x4 factorial experimental layouts using completely randomized design. Group 1 rats served as control and received 1ml of normal saline and normal chow. Rats in group 2 received 200 mg/kg BW while rats in groups 3 and 4 received 300mg/kg BW and 400 mg/kg BW of each extract, respectively for 60 days through oral gavage. Blood samples from the rats in the different treatment groups and controls were collected through cardiac puncture after chloroform anaesthesia. This was then used for the analyses.

Hormonal assay

The blood samples were spun at 2500 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25˚C. Serum samples were assayed for levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone/ interstitial cell stimulating hormone (LH/ICSH), estrogen (estradiol) and prolactin using the Microwell enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA) [16].

Biochemical assays

These assays were adapted from [15]. The analysis for ALP activity in the diluted sample was determined by an optimized and standardized colorimetric (Randox kit) method according to the recommendation of the German Society of Clinical Chemists (GSCC). The absorbance was measured using Optima Spectrophotometer SP-300 (Optima Inc. Chicago, USA). Alanine and aspartate aminotransferases' activities were also determined using analytical kits obtained from Randox.

Sperm quality analysis

Estimation of sperm count

This was carried out according to the method of [17]. The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymes in a glass petridish containing physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. The sperm cells were counted by cytometry. Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

Evaluation of sperm motility

The sperm cell suspension was diluted in 2ml of physiological saline and dropped on glass slides. This was viewed under light microscope as to determine the motile and non – motile sperm cells by their movement [18].

Sperm viability determination

This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin – nigrosin stain and air dried smears were prepared on glass slides for each samples according to [19]. The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin – nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

Sperm head abnormality test

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to [20].

RESULTS

Effect of extracts administration on sperm profile of rats

Result on the sperm profile of rats treated with *P. biglobosa* and *G. latifolium* crude extracts showed that there were significant differences ($P < 0.05$) in all the parameters studied except on the epididymal weight that showed no significant differences ($P > 0.05$) (Table 1). Our result revealed no significant differences between rats in the control and those treated with 200mg/kg and 300mg/kg of *P. biglobosa* extract and 300mg/k of *G. latifolium* extract, respectively. Generally, the administration of the two spice plants to rats caused significant reduction in sperm count. Sperm viability was also significantly reduced, especially at higher doses while there was no significant difference across the treatment levels, except when *P. biglobosa* was administered to rats at 400mg/kg concentration. Abnormal sperm morphology was significantly increased as the concentration of both extracts increased. However, there was no significant difference between the abnormal sperm morphology of rats treated with 300mg/kg *P. biglobosa* extracts and 400mg/kg of *G. latifolium* extract. Testicular weight was significantly reduced with increasing dosage, which was more pronounced on rats administered with 300mg/kg and 400mg/kg of *P. biglobosa*.

Table 1 : Effect of *P. biglobosa* (Dawa dawa) and *G. latifolium* (Utazi) extracts on sperm quality in albino rats

Parameters	*P. biglobossa* (mg/kg)				*G. latifolium* (mg/kg)			
	0	200	300	400	0	200	300	400
Sperm count	6.68c±0.12	7.19c±0.12	6.81c±0.16	5.44a±0.21	6.68c±0.12	6.51b±0.08	6.76c±0.26	6.02b±0.29
Sperm viability	81.94b±3.0	82.08b±2.89	78.24a±2.52	74.11a±1.23	81.94b±3.0	87.04b±2.76	84.12b±2.90	71.99a±0.50
Sperm motility	81.18b±4.86	82.58b±4.02	75.47b±4.33	61.11a±2.57	81.18b±4.86	77.25b±4.53	79.21b±3.94	76.82b±3.54
Sperm morphology	5.22a±0.67	6.31a±0.34	11.08bc±0.73	13.63cd±1.04	5.22a±0.67	8.68b±0.36	10.54b±0.43	11.76bc±0.78
Weight of testes	1.09d±0.07	0.74b±0.04	0.57a±0.02	0.62a±0.03	1.09d±0.07	0.9b±0.04	0.93bc±0.07	0.82b±0.08
Weight of epididymis	0.35a±0.02	0.33a±0.02	0.36a±0.02	0.29a±0.02	0.35a±0.02	0.37a±0.02	0.34a±0.01	0.32a±0.02

* Means followed with the same superscript indicate no significant difference ($P > 0.05$) from each other

Effect of extracts administration on hormonal profile of rats

Result obtained as presented in Table 2 showed the effect s of *P. biglobosa* and *G. latifolium* extracts on the reproductive hormones of male albino rats. The testosterone level was significantly ($P < 0.05$) affected by spices, which was dose-dependent. It revealed that treating rats with P. biglobosa extract at 400mg/kg resulted to a significant reduction. This was the same trend with luteinizing hormone. Follicle stimulating hormone increased when the rats were administered with 200mg/kg of both spice plants but increasing the concentration to 300 and 400mg/kg, respectively caused resulted to a steady reduction. It was also observed that the administration of the crude extracts from the two plants significantly reduced the level of prolactin and estradiol in a dose-dependent fashion.

Effect of extracts administration on biochemical parameters of rats

Result on some biochemical parameters showed that there were significant differences ($P < 0.05$) in the levels of albumin, total protein and the liver enzymes – ALT, AST and ALP while the creatinine level in the serum

showed no significant differences (P > 0.05) after treating with extracts of *P. biglobosa* and *G. latifolium*. The level albumin in the serum increased when 200mg/kg and 300mg/kg of *P. biglobosa* extract were administered to rats but declined at 400mg/kg, which was also the trend when rats were treated with *G. latifolium* extract. Conversely, the protein level increased with increasing dose. For the liver enzymes, the general trend observed revealed that at higher doses, the enzymes declined while lower doses enhanced them, the spice plant notwithstanding (Figures 1-6).

Table 2: Effect of *P. biglobosa* (Dawa dawa) and *G. latifolium* (Utazi) extracts on hormonal profile of albino rats

Parameters	*P. biglobosa* (mg/kg)				*G. latifolium* (mg/kg)			
	0	200	300	400	0	200	300	400
Testosterone	5.48c±0.01	6.52f±0.14	5.91d±0.15	4.22a±0.03	5.48c±0.01	5.94d±0.01	6.20de±0.01	4.87b±0.01
Luteinizing hormone	8.00c±0.04	8.38d±0.05	8.91e±0.04	5.64a±0.10	8.00c±0.04	8.08c±0.05	8.52d±0.07	7.13b±0.06
Follicle stimulating hormone	7.94c±0.05	7.97c±0.02	7.12b±0.02	5.15a±0.02	7.94c±0.05	8.16d±0.06	7.15b±0.07	5.27a±0.07
Prolactin	3.86e±0.1	3.22bc±0.1	3.05b±0.05	3.01b±0.03	3.86e±0.10	3.32cd±0.05	3.03b±0.05	2.82a±0.07
Estradiol	5.78f±0.10	5.11cd±0.14	4.89±0.04	4.54±0.03	5.78±0.10	85.20±0.06	4.99±0.03	4.78±0.06

*mean followed with the same superscript along each horizontal array indicates no significant difference (P > 0.05) from each other.

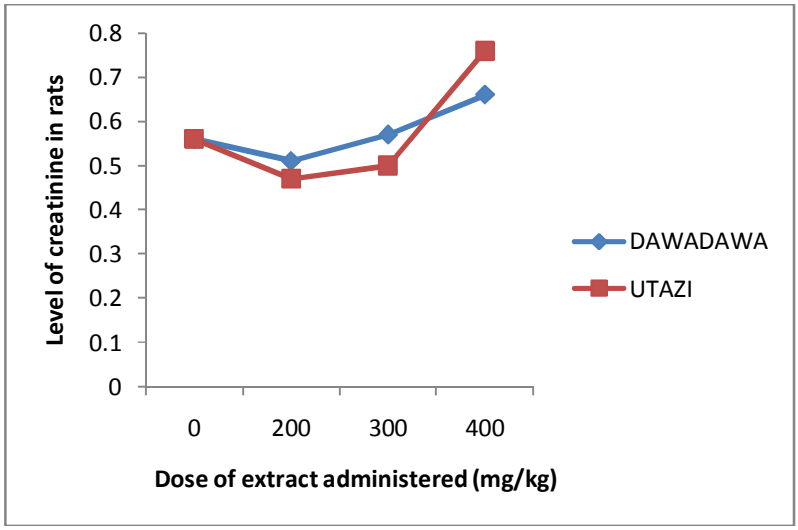


Fig., 1: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on creatinine level in rats after treatment.

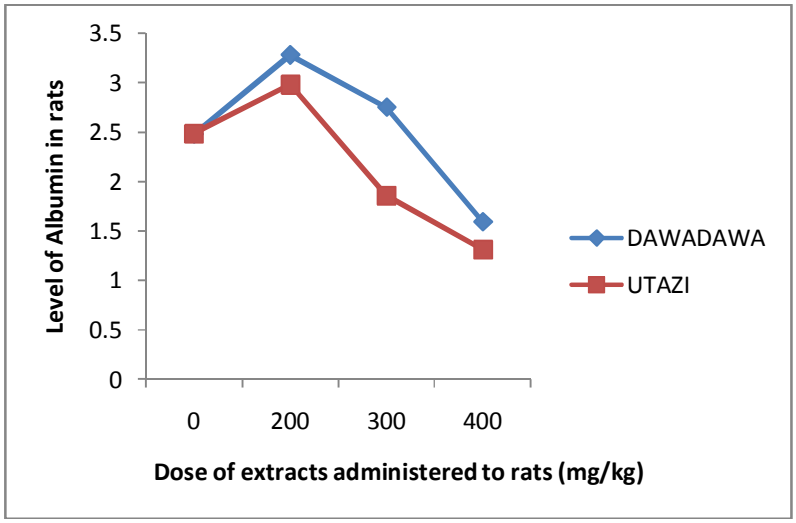
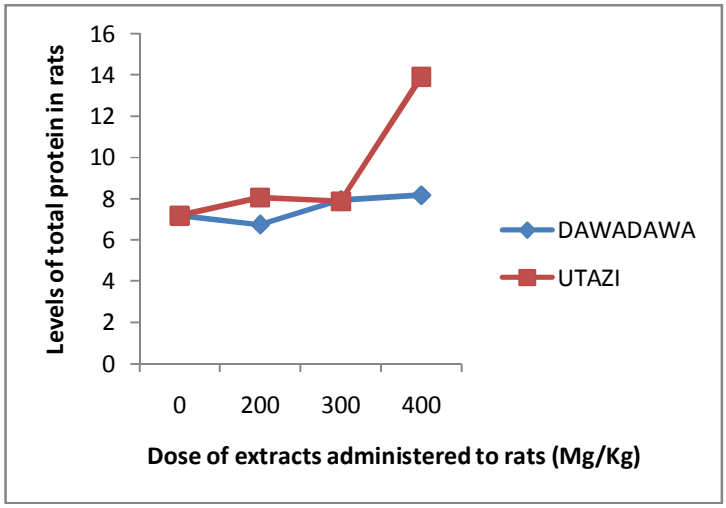


Fig., 2: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on albumin level in rats after treatment.



ig., 3: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on total protein level in rats after treatment.

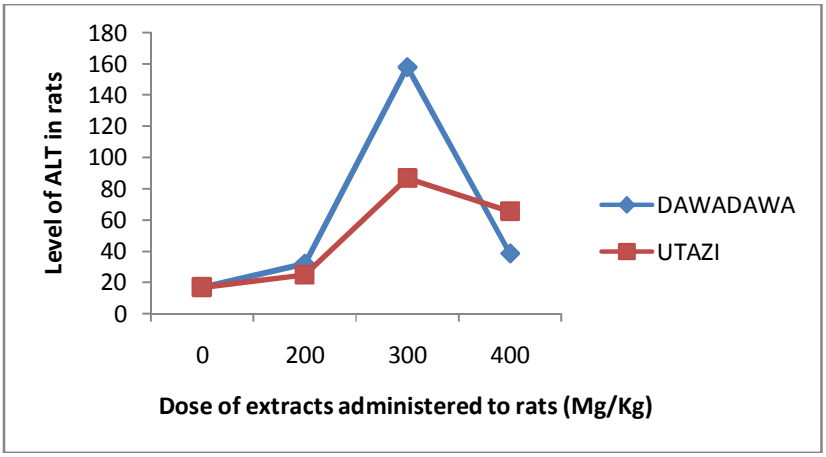


Fig., 4: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on alanine aminotransferase (ALT) level in rats after treatment.

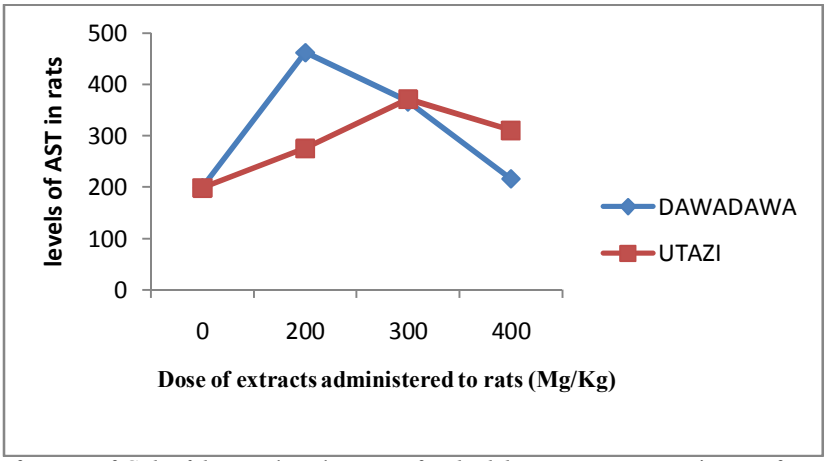


Fig., 5: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on aspartate aminotransferase (AST) level in rats after treatment.

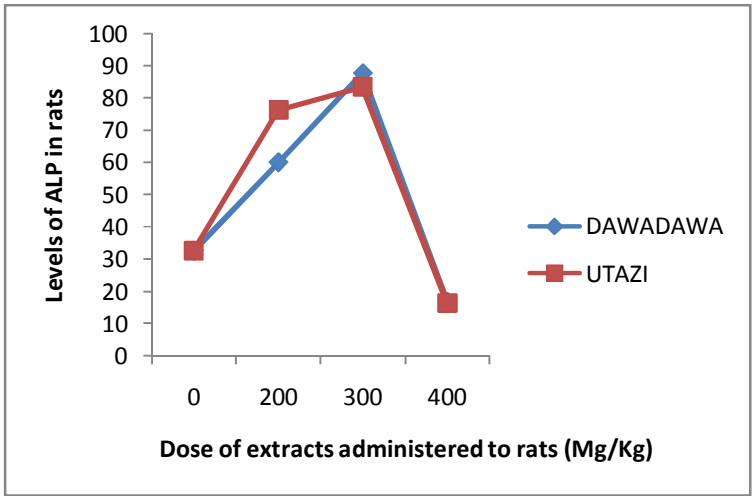


Fig., 6: Effects of leaf extract of *G. latifolium* and seed extract of *P. biglobosa* on alkaline phosphatase (ALP) level in rats after treatment.

DISCUSSION

Given that forest resources directly contributed up to 80% of the livelihood of the people in countries living in extreme poverty [2], it would leave nobody in doubt about the extent of care and prudent management that ought to be employed for maximum benefit. [21] in realization of this fact suggested the conservation and domestication through tissue culture and further genetic manipulations/modifications of these plants of pharmaceutical/ therapeutic, nutriceutical and industrial import to forestall their extinction.

Our result on sperm quantity and quality assessment showed that there were significant reduction ($P < 0.05$) in the sperm count, sperm viability, sperm motility and weight of testis with increase percentage of sperm head abnormality, especially at higher doses. This reduction suggests that these spice plants are efficacious in disrupting spermatogenic processes and pathways. Additionally, the observed increase in percentage of sperm head abnormality and subsequent reduction in sperm count may have resulted from the alteration in the epididymal environment as was reported by [22]. In a similar development, [20] asserted that this increase is an indication of the increase in the rate of induced mutation on the sperm cells at the level of spermatogenesis. According to [21,23, 24], distortion in the fertility of male mammals are directly correlated with the distortion in spermatogenesis. This probably suggests that *P. biglobosa* and *G. latifolium* extracts might impair fertility if caution is not exercised by consumer.

It is obvious that from the result (Table 1) that the effects of the two spice plants differed as *P. biglobosa* had more severe effect on the sperm quantity and quality. Undoubtedly, these plants are endowed with a variety of phytochemicals that are thought to act on a variety of targets by various modes and mechanisms [25, 26, 27]. It is also probable that the seeds of *P. biglobosa* might still contain anti-nutritive factors after fermentation, which might account for the differential. Thus going by this therefore, the differential effects are justifiable. Cellular alterations and distortions of the testicular cells maligned spermatogenesis [21,28] with its resultant effect on sperm count. It is clearer to assert that the reduction in the sperm count as suggested by our result could be attributed to these distortions of the cellular machinery underlying the processes of spermatogenesis.

According to [29] chemical action during the spermatogonial phase will probably have greater effect on sperm output than the action during the spermatid formation phase. It is possible that the bioactive compounds repository in these spice plants might have exerted the effects at the spermatogonial phase of spermatogenesis. The reduction in sperm motility might not be unconnected to the phytochemicals that might have caused immobilization/weakening effects on the sperm cells and may also have permeated the blood-testis barrier, especially on rats treated with 400mg/kg BW of *P. biglobosa*.

Though our result on the hormonal profile of rats after treating with *P. biglobosa* and *G. latifolium* indicates that they can be used to boost hormonal levels, especially when administered at doses of 200mg/kg and 300mg/kg, respectively, however, increasing the treatment dose more than the aforementioned might lead to adverse effects on the hormone production. The effects of the extracts on the sperm parameters are directly proportional to their effects on the level of the hormones. There exist a synergy between serum testosterone and follicle stimulating hormone

during spermiogenesis and subsequent spermatogenesis [30,31]. However, since the extracts from the two spice plants affected these two hormones adversely, the sperm count was not spared either. According to [32,33,34], plants with high alkaloid contents were observed to be responsible for increase serum concentration of estradiol and prolactin. This has the capacity to inhibit gonadothrophin action of the testis and subsequent impairment of male fertility. It therefore means that the alkaloids inherent in the spice plants were low to cause significant increase on the concentration of these hormones.

It has also been reported that albumin level is decreased in chronic liver disease as well as in cirrhosis and nephritic syndrome [35]. Our result suggests that to maintain a high level albumin, 200mg/kg of the spice extracts should be administered. However, higher doses of the extracts reduce the albumin level in the plasma of the animal. Obviously, the efficacy of any drug is dependent on the non-toxic effects on the system of the recipient but exerting its positive effects on the target ailment [21]. Most herbs (drugs) when taken are primarily metabolized by the liver and accumulation of their products can cause cell injury and even death at high concentrations. Incidentally, this affects patients with liver diseases, where the processes of detoxification and excretion may have been dangerously altered [21]. The level of alkaline phosphatase in the plasma in the cells lining of the biliary duct of liver may rise with large bile duct obstruction, interphatic cholestasis or infiltrative substances and diseases of the liver [36]. However, the ratio of aspartate aminotransferase (AST) to alkaline phosphatase (ALP) is sometimes useful in distinguishing causes of liver damages [37]. Significantly, elevated levels of ALT might suggest the existence of viral hepatitis, congestive heart failure, liver damage, biliary duct problems [37]. Results obtained from this research showed that *P. biglobosa* and *G. latifolium* extracts caused significant reduction in the liver enzymes, especially at higher doses. It thus implies that these extracts might proffer help in reducing the incidence of liver-related diseases. Worthy of note is the fact that these effects might not be unconnected to the inherent bioactive compounds in these spice plants, which could be exploited by identification, isolation and purification.

Conclusion

Succinctly, our results are explicit indication that *P. biglobosa* and *G. latifolium* extracts might be used to enhance sperm quantity and quality, reproductive hormones and liver enzymes production, especially at lower doses of 200mg/kg. There might be disruption of biosynthetic pathways administering the extracts at higher doses. It thus implies that caution should be exercised during consumption.

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