Trop J Nat Prod Res, May 2021; 5(5):952-958 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of Dietary Supplementation of Pumpkin (*Cucurbita pepo L*) Seed on Antioxidant Status, Hormonal Level and Sexual Behavior in Male Rats

Seun F. Akomolafe[1]* and Ayodeji A. Olabiyi[2]

[1]*Department of Biochemistry, Faculty of Science, Ekiti State University, Ado Ekiti, P.M.B 5363, Nigeria*
[2]*Department of Medical Biochemistry, Afe Babalola University, Ado Ekiti. P.M.B 5454, Nigeria*

ARTICLE INFO

Article history:
Received 29 January 2021
Revised 17 April 2021
Accepted 13 May 2021
Published online 03 June 2021

ABSTRACT

The seed of pumpkin (Cucurbita pepo L) is reportedly used as sex enhancer in folk medicine and to treat impotence in men. Despite all the documented importance of pumpkin seed in both nutrition and medicine, there is little or no scientific evidence of the sexual enhancement of seed in albino rats. In this study, sexual enhancing effect of pumpkin seed on healthy male rats was evaluated. Rats were fed with basal diets (NC), diet supplemented with raw pumpkin seed (5 and 10%), roasted pumpkin seed (5 and 10%) and sildenafil (5 mg/kg). Pre-treated of experimental rats with pumpkin seed for fourteen days led to a significant increment in antioxidant status, hormonal level and sexual behavior. However, processed pumpkin seed appeared to be the most promising when compared to Sildenafil, a standard aphrodisiac drug. In conclusion, these behavioral as well as biochemical parameters which revealed enhanced activities could be part of the mechanism by which the seed exerts its aphrodisiac properties.

***Keywords*:** Aphrodisiac, Hormone, Sexual Behavior, Pumpkin Seed.

Introduction

Erectile dysfunction (ED) alludes to a man's steady failure to accomplish or keep up penile erection for good sexual movement.[1] Predominance and the seriousness of ED has been archived to increment with age just as various pathogenetic factors.[2,3] Directly, around 150 million men has been assessed to be influenced by ED worldwide and it has additionally been recommended that around 322 million men will be influenced all around in the following a long time from now with about 2.5 occasions increment in Asia, Africa and South America.[4-6] Penile erection relies upon increased blood flow to the corpora cavernosa [7] and this increase in blood flow has been identified with induction by increased sacral parasympathetic-nitrergic movement and diminished sympathetic activity.[8] Trials have exhibited that nitric oxide (NO) play a middle and essential function in the vasodilator systems that cause penile erection.[9-12] NO additionally assume a function in controlling sexual conduct by advancing dopamine discharge[13-15] just as actuating luteinizing hormone-discharging hormone.[11]

Penile erection is a complex process including the transaction between penile vasculature, neural motivations, hormone levels, and cognitive behavior.[16] Sildenafil, Vardenafil, and Tadalafil have been acknowledged and utilized for adjustment of erectile dysfunction since year 1998 because of the inhibitory impact on phosphodiesterase V.[17] Be that as it may, there are a few related reactions to the utilization of these manufactured medications and this has required the requirement for elective helpful methods for overseeing ED which could be tackled from plant sources known to have different bioactive substances with strong pharmacological possibilities.[18]

*Corresponding author. E mail: purposefulseun@yahoo.co.uk
seun.akomolafe@eksu.edu.ng
Tel: +2348030460149

Citation: Akomolafe SF and Olabiyi AA. Evaluation of Dietary Supplementation of Pumpkin (*Cucurbita pepo L*) Seed on Antioxidant Status, Hormonal Level and Sexual Behavior in Male Rats. Trop J Nat Prod Res. 2021; 5(5):952-958. doi.org/10.26538/tjnpr/v5i5.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Pumpkin seed (Cucurbita pepo Linn, Family: Cucurbitaceae) is one of the most seasoned known cultivated species. It is known as pumpkin in English and locally called Elegede in Yoruba, Southwest, Nigeria.[19] This plant is local to Northern Mexico and Southwestern and Eastern USA and furthermore exists in wild structure in Europe and Asia. The young leaf that is locally called "Gboro" is regularly devoured while the mash of ready natural products has been accounted for its utilization in the administration of intestinal inflammation, stomach and liver issues and as dietary enhancement for nutrient A.[20] The seeds, also called pepitas, are little level, green consumable seeds that are frequently suggested as dietary enrichment. The seeds can be processed into fine structure and blended with grains for making bread, or, after roasting, consume as a snack. Pepitas has been archived to contain higher measures of linoleic, oleic acid, palmitic acid, stearic acids carotenoids, zinc, selenium, nutrient E, and magnesium salts[21-24] with differing wellbeing advancing properties.[25] In any case, basic biochemical guideline answerable for the administration of erectile dysfunction related with the utilization of pumpkin seed has not been accounted for. Henceforth, this examination look to research the impact of dietary supplementation of pumpkin (Cucurbita pepo L) seed on antioxidant status, hormonal level and sexual behavior in male rodents as an approach to quantify/assess its possible capacity to easing ED condition.

Materials and Methods

Sample collection and preparation
Materials

We gathered new matured pumpkin fruits from a local market in Ado Ekiti, South Western Nigeria, after authentication in the Department of plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria (Voucher number UHAE 433). The seeds were purchased August, 2019 and expelled from the fruits with table blade, washed with distilled water and left to air dry for three days at room temperature. The seeds were then broken physically, cleaned and isolated from the hulls. From that point, a segment of the seeds was roasted in an electric stove for 30 min at 100 °C (Plate 1). The raw portion was dried to constant weight in oven at 40 °C [26] (Plate 1). The two samples were independently pounded, defatted in cool n-Hexane and kept at -4^{0}C in a water/air proof holder before examination.

Besides, the proximate examinations of the samples were done to decide the nutritional content of the seed which was utilized in the eating regimen definition (Table 1). Appropriate portions of each seed powder were used for feed formulation as shown in Table 1.

Bioassays
Animals
Sixty grown-up male Wistar rodents were provided by the rearing colony of the Department of Anatomy, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria. They were first acclimatized to standard laboratory atmospheric condictions (temperature: 25 ℃, on a 12 h light/dark cycle), and took care *ad libitum* with standard laboratory diet and water. All animal experiments were acted as per the rules of the Institutional Animal Ethical Committee (Approval number AFO049SAKO10).

Experimental design
The rodents were separated into six groups of ten animals each (n = 10). Group 1: healthy control rodents put on basal eating regimen; Group 2: rodents took care of diet enriched with 5% raw pumpkin seed; Group 3: rodents took care of diet enriched with 10% raw pumpkin seed; Group 4: rodents took care of diet enriched with 5% processed pumpkin seed; Group 5: rodents took care of diet enriched with 10% processed pumpkin seed; Group 6: healthy rodents took care of basal eating regimen and administered Sildenafil citrate (5mg/kg/day). Every day feed intakes were observed. The trial kept going 14 days after which sexually active male and female animals fed standard laboratory diet were combined for a video visual account of sexual behavioral examinations. These male rodents were fasted overnight and sacrificed by decapitation, their blood collected for serum preparation followed by determination of sex hormone levels while penile tissue were processed for enzymatic and non-enzymatic activities. The sample's portions are utilized to explore the conceivable natural impact corresponding to increment in utilization of these materials.

Sexual behavior
The sexual behavioral test was done by Thawatchai *et al.*[27] as improved by Olabiyi *et al.*[28] The surveyed sexual parameters incorporate mounting number (the quantity of mounts without intromission from the time of acquaintance of the female with the male), mounting latency (the time interval between acquaintance of the female with the principal mount by the male), intromission number (the quantity of intromissions from the time of presentation of the female until the finish of the analysis), intromission latency (the stretch from the time of acquaintance of the female with the main intromission by the male), ejaculation number (the occasions the male discharged semen into the genital of the female rodent), and ejaculation latency (the span from the acquaintance of the male with female to the first run through the male discharged the semen into the female).

Determination of sexual hormone levels
Toward the finish of the 14-day investigative period, rodent blood samples were collected and serum isolated from the plasma for the test. Testosterone (T), luteinizing hormone (LH), follicle animating hormone (FSH), and prolactin (P) levels were estimated utilizing financially accessible kits from Randox (Crumlin, Dublin, Northern Ireland, UK).[28]

Antioxidant assay
The supernatant got from the penile tissues was utilized for assessment of total thiol (T-SH) content,[29] reduced glutathione (GSH) level,[29] catalase,[30] superoxide dismutase (SOD) activities.[31]

Statistical analysis
All data were tested for normality and analyzed using Graph Pad Prism 5 (Graph pad, San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to analyze the mean while appropriate post-hoc treatment was applied. Statistical significance was defined as P values less than 0.05 while the data was expressed as mean ± SD or SEM.

Results and Discussion

In this study, supplementation of diet with raw and processed pumpkin seeds (5% and 10%) caused a significant ($p < .05$) increment in both mounting number (Figure 1a), intromission number (Figure 1c) and ejaculation number (Figure 1e) when compared with the healthy control group. Nonetheless, mounting latency (Figure 1b), intromission latency (Figure 1d) and ejaculation latency (Figure 1f) were significantly ($p < .05$) brought down in rats that took raw and processed pumpkin seeds enriched diet in contrast with the healthy control group. Taken together, the mounting, intromission as well as ejaculation latencies in groups treated processed pumpkin seed enriched diet eating regimen was decreased when contrasted with the groups that took the raw seed diet, as well with that of standard drug, sildenafil citrate. Penile erection likewise relies upon the inclusion of androgens, especially testosterone which have been uncovered to have both principal and minor impacts on penile erection.[28,32]
Testosterone levels and substitution have been connected to sexual capacity, explicitly erection quality, libido, and ejaculatory function.[33] FSH directs the development of seminiferous tubules and upkeep of spermatogenesis in males. FSH is additionally fundamental for sperm formation. It bolsters the capacity of Sertoli cells, which thusly bolster numerous parts of sperm cell development. LH invigorates the emission of testosterone from gonads by binding to receptors on Leydig cells in this manner stimulating synthesis just as release of testosterone.[33] Hyperprolactinemia has been connected to erectile dysfunction and its effect announced.[34]
Reduced discharge of LH or FSH can cause a disappointment of gonadal capacity (hypogonadism), a condition that is commonly prove in males as disappointment in production of typical quantities of spermatozoa. From our finding, observed increment in FSH, LH and testosterone levels of rodent placed on raw and processed pumpkin seeds may recommend that this seed may contain bioactive constituents that could improve the precursors of sex hormones and increment circulating testosterone level.[35] It is deserving of note that hyperprolactinemia is related with low circulating levels of testosterone, which is secondary to restraint of gonadotropin-discharging hormone release by the raised prolactin levels.[36] Consequently, the significant ($p<.05$) decline saw in the level of prolactin among the rodent groups placed on processed pumpkin seed may show that dietary enrichment with processed pumpkin seed decreased prolactin level which thusly may prompt more noteworthy circling levels of testosterone, which is optional to improvement of gonadotropin-discharging hormone secretion by the low level of prolactin. Taking together, this finding may recommend that increased motivational component of sexual behavior in male rodent are connected to raised testosterone levels.[37] There was significant ($p<.05$) increment in the serum testosterone (Figure 2), LH (Figure 3) and FSH (Figure 4) levels with a significant ($p<.05$) decline in prolactin (Figure 5) level of rodents took care of raw and processed pumpkin seed diets in contrast with what is obtainable in the control group. The processed pumpkin seed caused a noteworthy lessening prolactin level while raw pumpkin seed caused a non-significant abatement in prolactin level, though there was no factual distinction for the prolactin value between 10% processed pumpkin seed and the standard drug, sildenafil citrate. All things considered, the processed pumpkin seed showed higher impact on these sexual hormonal levels than raw pumpkin seed.
Oxidative stress meddles with the NO pathway and causes vascular harm.[38,39] Antioxidant enzymes, for example, Superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase are the first line of guard against oxidative injury.[40] SOD secures tissues against oxygen free radicals by catalyzing the clearing of superoxide radicals, changing them to H_2O_2 and molecular oxygen, which harms the cell layer and other organic structures.[41] These radicals (H_2O_2 and lipid peroxide) are promptly broken by catalase and glutathione peroxidase.[42, 43] From this examination, an enhanced activity of these antioxidant enzymes was uncovered.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Raw pumpkin seed **Roasted pumpkin seed**

Plate 1: Raw and processed (roasted) pumpkin

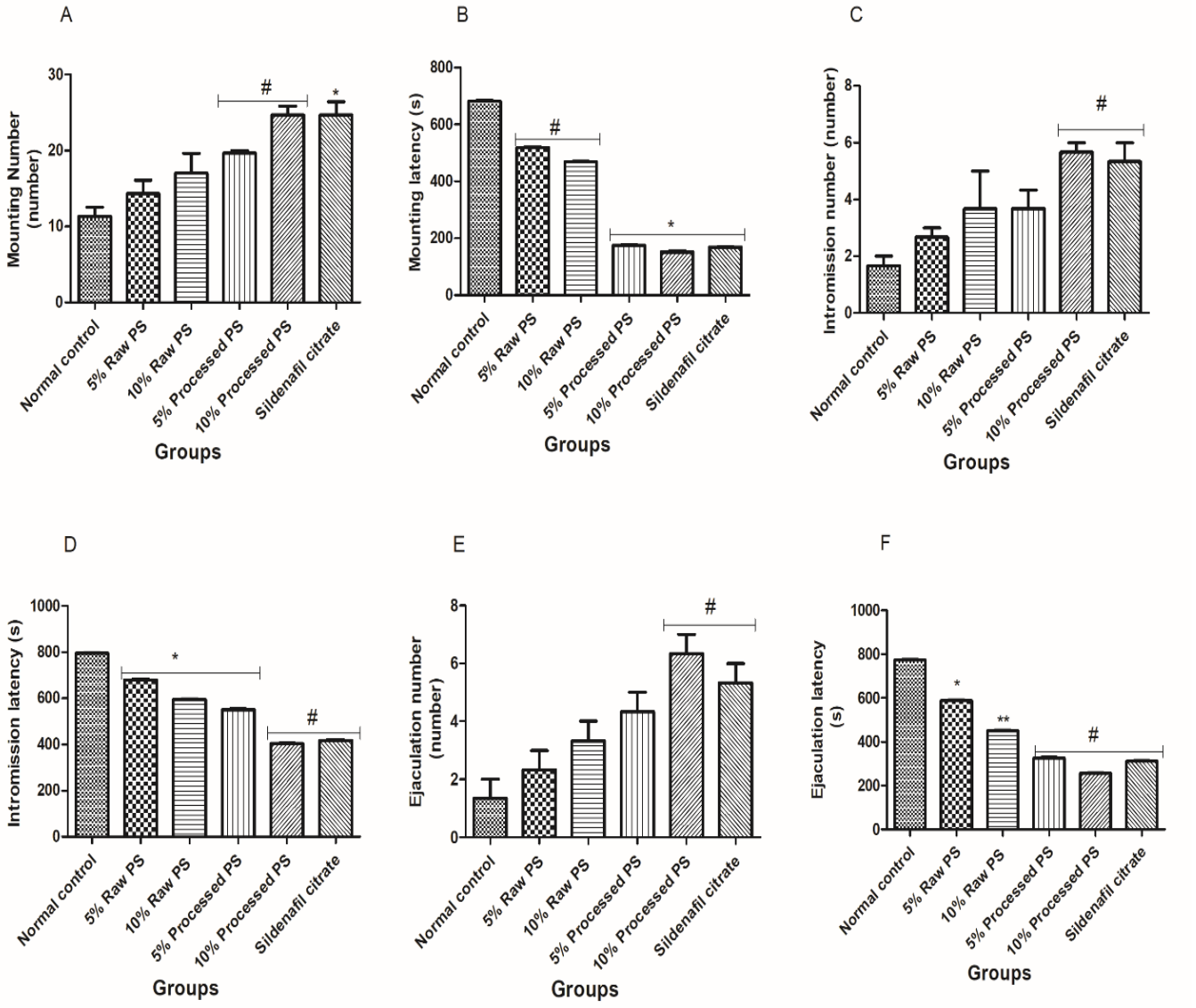

Figure 1: Effect of raw and processed pumpkin seed on mounting number (A), mounting latency (B), intromission number (C), intromission latency (D), ejaculation number (E) and ejaculation latency (F). Data are expressed as mean ± SEM. $p < 0.05$ was considered to represent a significant difference. *,# denote $p < 0.05$ and **, ##denote $p < 0.01$ from the control group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

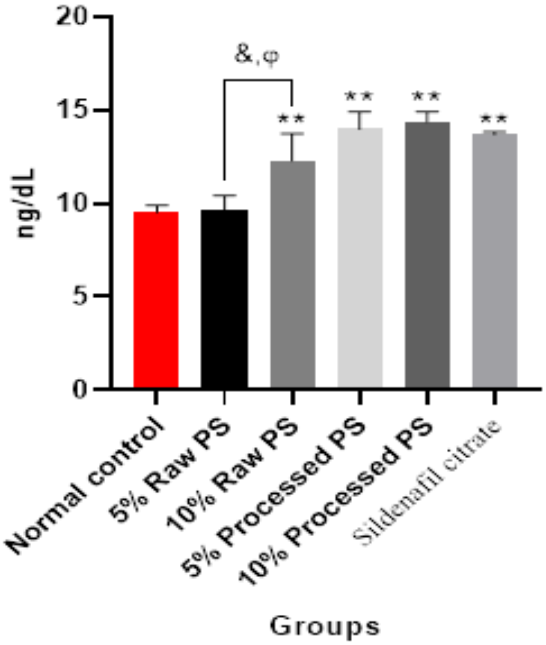

Figure 2: Effect of raw and processed pumpkin seed on testosterone level in rat's corpora cavernosa.
Data are expressed as mean ± SEM (n = 10). Values are significantly different at *p < 0.05, **p < 0.01 versus normal control group, φp < 0.05 versus 5% raw pumpkin seed, $p < 0.05 versus 5% processed pumpkin seed,&p < 0.05 versus 5% and 10% processed pumpkin seed, #p < 0.05 versus Sildenafil citrate treated group.

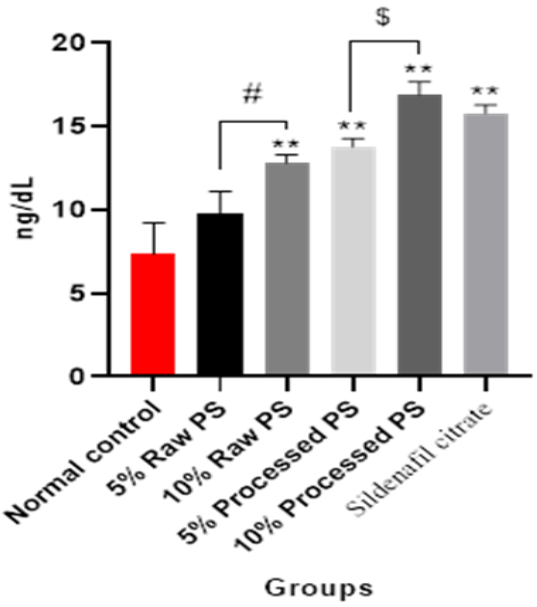

Figure 3: Effect of raw and processed pumpkin seed on luteinizing hormone level in rat's corpora cavernosa.
Data are expressed as mean ± SEM (n = 10). Values are significantly different at *p < 0.05, **p < 0.01 versus normal control group, φp < 0.05 versus 5% raw pumpkin seed, $p < 0.05 versus 5% processed pumpkin seed,&p < 0.05 versus 5% and 10% processed pumpkin seed, #p < 0.05 versus Sildenafil citrate treated group.

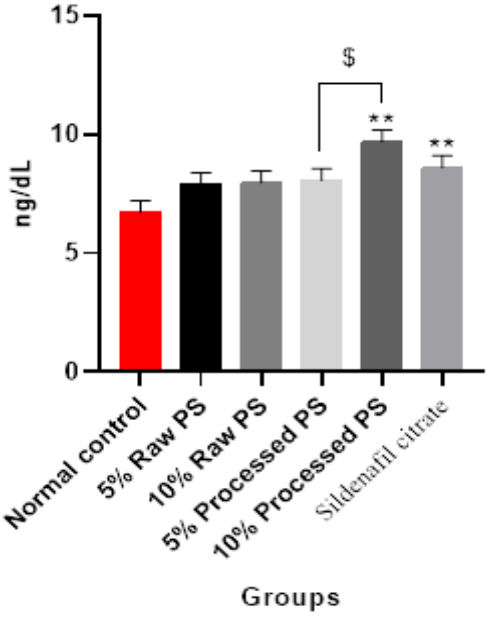

Figure 4: Effect of raw and processed pumpkin seed on follicle stimulating hormone level in rat's corpora cavernosa.
Data are expressed as mean ± SEM (n = 10). Values are significantly different at *p < 0.05, **p < 0.01 versus normal control group, φp < 0.05 versus 5% raw pumpkin seed, $p < 0.05 versus 5% processed pumpkin seed,&p < 0.05 versus 5% and 10% processed pumpkin seed, #p < 0.05 versus Sildenafil citrate treated group.

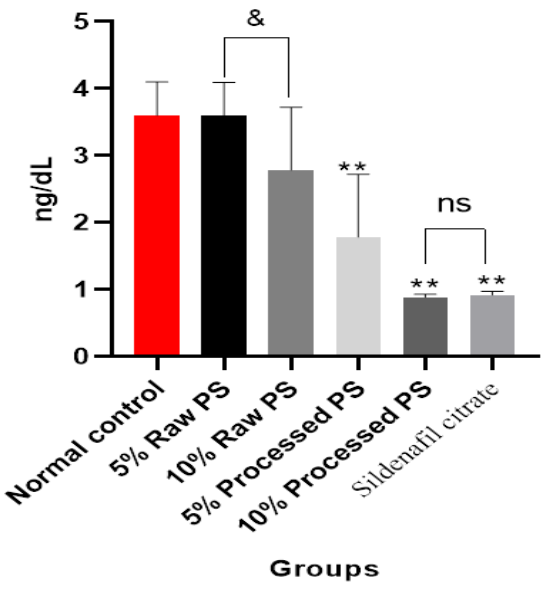

Figure 5: Effect of raw and processed pumpkin seed on prolactin level in rat's corpora cavernosa.
Data are expressed as mean ± SEM (n = 10). Values are significantly different at *p < 0.05, **p < 0.01 versus normal control group, φp < 0.05 versus 5% raw pumpkin seed, $p < 0.05 versus 5% processed pumpkin seed,&p < 0.05 versus 5% and 10% processed pumpkin seed, #p < 0.05 versus Sildenafil citrate treated group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

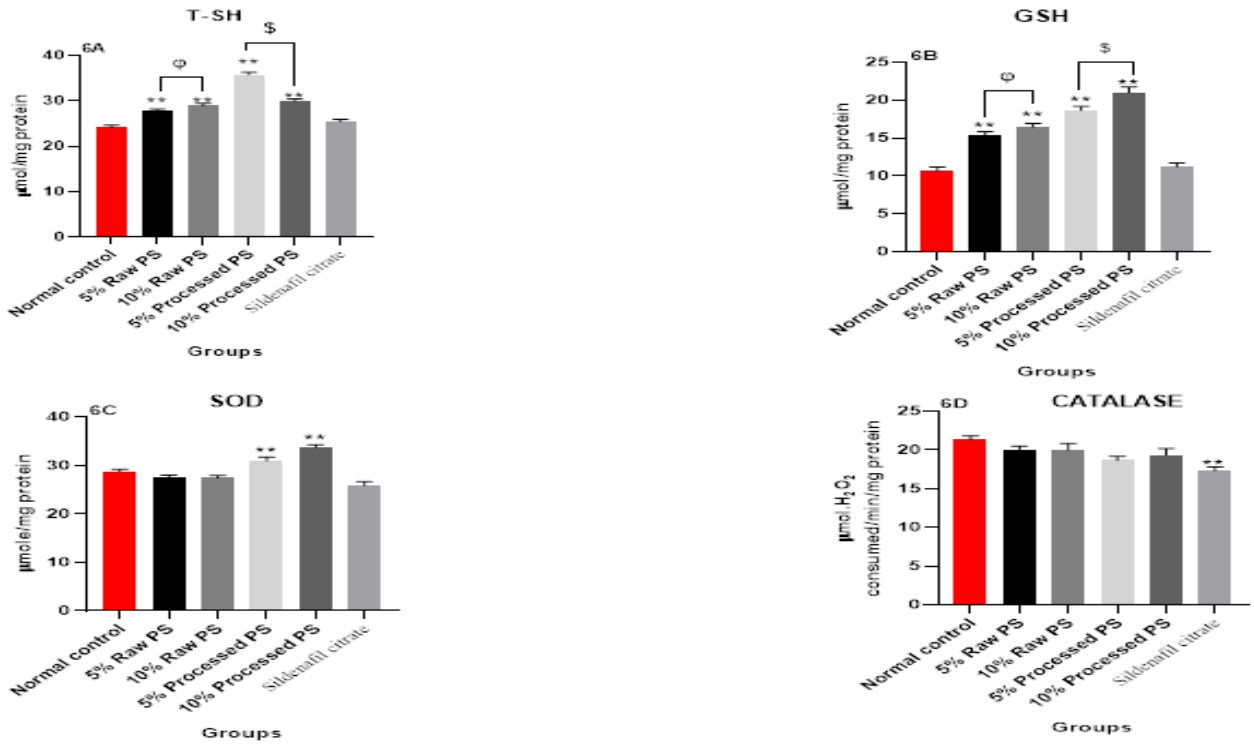

Figure 6: Effect of raw and processed pumpkin seed on total thiol (T-SH) level (A), reduced glutathione (GSH) level (B), superoxide dismutase (SOD) activity (C) and catalase activity (D) in rat's corpora cavernosa. Data are expressed as mean ± SEM (n = 10). Values are significantly different at *p < 0.05, **p < 0.01 versus normal control group, φp < 0.05 versus 5% raw pumpkin seed, $p < 0.05 versus 5% processed pumpkin seed, and p < 0.05 versus 5% and 10% processed pumpkin seed, #p < 0.05 versus Sildenafil citrate treated group.

Table 1: Diet formulation for basal and supplemented diets for control and test groups (g/100g)

Ingredients	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Skimmed Milk (g)	40	38.47	36.92	38.47	36.92	40
Oil (g)	10	9.84	9.68	9.84	9.68	10
Premix (g)	4	4	4	4	4	4
Corn Starch (g)	46	42.69	39.40	42.69	39.40	46
Pumpkin seed1 (g)	-	5	10	-	-	-
Pumpkin seed2 (g)	-	-	-	5	10	-
Sildenafil citrate	-	-	-	-	-	5mg/kg/day
Total (g)	100	100	100	100	100	100

Note: Skimmed milk = 32% protein; The vitamin premix (mg or IU/g) h was the following composition; 3200 IU vitamin A, 600 IU vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 1 mg vitamin B2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin H2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. Group 1: Control rats placed on basal diet; Group 2: rats placed on a basal diet supplemented with 5% raw pumpkin seed for 14 days; Group 3: rats placed on a basal diet supplemented with 10% raw pumpkin seed for 14 days; Group 4: rats placed on a basal diet supplemented with 5% roasted pumpkin seed for 14 days; Group 5: rats placed on a basal diet supplemented with 10% roasted pumpkin seed for 14 days; Group 6: Rats placed on basal diet and then administered sildenafil citrate (5mg/kg/day). Pumpkin seed 1 = Raw pumpkin seed, Pumpkin seed 2 = Roasted pumpkin seed.

Along these lines, the sexual improving impact of the comprehensive eating regimens may in part be related to the antioxidant possibilities of pumpkin seed which may have set off the endogenous antioxidant enzyme activities[44,45] and hormones.[46] The impact of raw and processed pumpkin seeds on antioxidant status of the corpus cavenosum in healthy rodents is presented in Figure 6a–d. In this examination, higher SOD action, TSH and GSH levels were seen in rodents taken 5% and 10% processed pumpkin seed with no significant ($p>0.05$) change in CAT activity among the groups.

Conclusion

The improved sexual behavior, hormonal changes, and antioxidant capacities showed by this seed in rodent model, could be a piece of the conceivable mechanism by which they apply their aphrodisiac properties, by and by, the processed pumpkin seed displayed higher impact on these biochemical indices than raw pumpkin seed. Hence, further studies are still ongoing on the characterization and toxicity of the seed to ascertain its safety.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to acknowledge the Department of Biochemistry, Ekiti State University, Ado Ekiti, Ekiti State for providing laboratory and infrastructural support.

References

1. Hatzimouratidis K, Amar E, Eardley I. Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. Eur Urol. 2010; 57(5):804-814.
2. Cheng JYW, Ng EML, Chen RYL, Ko JSN. Prevalence of erectile dysfunction in Asian populations: A meta-analysis. Int J Imp Res. 2007; 19(3):229-244.
3. He LJ, Zhang C, Li WY, Song GH, Jiang T, Geng F. Erectile function of male rats in different age groups: an experimental study. *Zhonghua nan ke xue* = Nat J Androl. 2013; 19(4):296-299.
4. Aytac IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectiledysfunction between 1995 and 2025 and some possible policy consequences. BJU Int. 1999; 84:50-56.
5. Ismail EA and El-Sakka AI. Innovative trends and perspectives for erectile dysfunctiontreatment: a systematic review. Arab J Urol. 2016; 14:84-93.
6. Ademiluyi AO, Oyeniran OH, Jimoh TO, Oboh G, Boligon AA. Fluted Pumpkin (*Telfairia occidentalis*) seed modulate markers of erectile function in isolated rat's corpus cavernosum: Influence of polyphenol and amino acid constituents. J Food Biochem. 2019; 43(11):e13037.
7. Moore K and Anne A. Essential clinical anatomy (3rd ed., p.265). Lippincott Williams & Wilkins, University of Oxford MedicalSchool. 2007.
8. Pocock G and Richards C. Human physiology. The Basis of Medicine(3rd ed., p. 63). London, UK: Oxford University Press. 2006.
9. Azadzoi KM, Kim N, Brown ML, Goldstein I, Cohen RA, Saenz DE Tejada I. Endothelium-derived nitric oxide and cyclooxygenaseproducts modulate corpuscavernosum smooth muscle tone. J Urol. 1992; 147:220-225.
10. Kim N, Vardi Y, Padma-Nathan H, Daley J, Goldstein J, Saenz DE Tejada I. Oxygen tensi_on regulates the nitric oxide pathway.Physiological role in penile erection. J Clin Invest. 1993; 91:437-442.
11. McCann SM, Mastronatfi C, Walczewska A, Karanth S, Rettori V. The role of nitricoxide in reproduction. Braz J Med Biol Res. 1999; 32:1367-1379.
12. Saenz De Tejada I. Molecular mechanisms for the regulation ofpenile smooth muscle contractility. Int J Imp Res. 2002; 1:6-10.
13. Cirino G, Fusco F, Imbimbo C, Mirone V. Pharmacology oferectile dysfunction in man. Pharmacol Ther. 2006; 111:400-423.
14. Lorrain DS, Matuszewich L, Howard RV, Du J, Hull EM. Nitric oxide promotesmedial preoptic dopamine release duringmale rat copulation. Neuro Rep. 1996; 8:31-34.
15. Williams SK and Melman A. Novel therapeutic targets for erectiledysfunction. Maturitas. 2012; 71:20-27.
16. Steidle C, Schwartz S, Jacoby K, Sebree T, Smith T, Bachand R. AA2500 testosterone gel normalizes androgen levels in agingmales with improvements in body composition and sexual function. J Clin Endocrinol Metab. 2003; 88:2673-2681.
17. Seftel AD. Phosphodiesterase type 5 inhibitors: Molecular pharmacologyand interactions with other phosphodiesterases. Curr Pharmacol Design. 2005; 11:4047-4058.
18. Omojokun OS, Oboh G, Ademiluyi AO. Effects of drying on cholinesterasesand angiotensin-I converting enzyme inhibitory potential and phenolic constituents of African mistletoe (*Loranthus bengwensis* L) leaves from kolanut host tree. J Food Biochem. 2018; 2018:e12510.
19. Oloyede FM. Growth, yield and antioxidant profile of pumpkin (*Cucurbita pepo* L.) leafy vegetable as affected by NPK compound fertilizer. J Soil Sci Plant Nutr. 2012; 12(3):379-387.
20. Sarkar S and Guha D. Effect of ripe fruit pulp extract of *Cucurbita pepo* Linn. in aspirin induced gastric and duodenal ulcer in rats. Indian J Exp Biol. 2008; 46(9):639-645.
21. Pier GP, Giorgia M, Francesco G, Erica L, Ryszard A. Antioxidative activities and phenolic compounds of pumpkin (*Cucurbita pepo*) seeds and amaranth (*Amaranthus caudatus*) grain extracts. Nat Prod Res. 2017; 31(18):2178-2182.
22. Mirjana A, John VC, Agniezska T, Roland V. Phenolic compounds and some quality parameters of pumpkin seed oil. Eur J Lipid Sci Technol. 2010; 112(2):208-217.
23. Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003; 51:2866-2887.
24. Brawley OW and Barnes ST. Potential agents for prostate cancer chemoprevention. *Epidemiol Rev*. 2001; 23:168-172.
25. Caili F, Huan S, Quanhong L. A review on pharmacological activities and utilization technologies ofpumpkin. Plant Foods Hum Nutr. 2006; 61:73-80.
26. Akomolafe SF, Oyeleye IS, Molehin OR, Ogunsuyi OB. Phenolic composition and inhibitory ability of methanolic extract from Pumpkin (*Cucurbita pepo* L.) seeds on Fe^{2+}-induced thiobarbituric acid reactive species in albino rat's testicular tissue - *In Vitro*. J Appl Pharm Sci. 2016a; 6(9):115-120.
27. Thawatchai P, Jintanaporn W, Sitthachai I, Supaporn M, Wipawee T. Moringa Oleifera leaves extract attenuates male sexual dysfunction. Am J Neurosci. 2012; 3:17-24.
28. Olabiyi AA, Oboh G, Adefegha SA. Effect of dietary supplementation of tiger nut (*Cyperus esculentus* L.) and walnut (*Tetracarpidium conophorum* Mull. Arg.) on sexualbehavior, hormonal level, and antioxidant status in male rats. J Food Biochem. 2016; 00:e12351.

29. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophy. 1957; 82:70-77.
30. Claiborne A. Catalase activity. In R. A. Greenwald (Ed.), Handbook of methods for oxygen radical research. 1985. 283-284 p.
31. Misra HP and Fridovich I. The role of superoxide anion in theauto oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170-3175.
32. Buvat J, Maggi M, Gooren L, Guay AT, Kaufman J, Morgentaler A, Zitzmann M. Endocrine aspects of male sexual dysfunctions.The J Sex Med. 2010; 7:1627-1656.
33. Jacob Rajfer MD. Relationship between testosterone and erectiledysfunction. Rev Urol. 2000; 2:122-128.
34. Scott IZ, Jacob R. Hyperprolactinemia and erectile dysfunction. Rev Urol. 2000; 2:39-42.
35. Gannon JR and Walsh TJ. Testosterone and sexual function. Urol Clin North Am. 2015; 43:217-222.
36. Leonard MP, Nickel CJ, Morales A. Hyperprolactinemiaand impotence: Why, when and how to investigate. J Urol. 1989; 142:992.
37. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ. Long-term testosterone gel (AndroGel)treatment maintains beneficial effects on sexual function andmood, lean and fat mass, and bone mineral density inhypogonadalmen. J Clin Endocrinol Metab. 2004; 89:2085-2098.
38. Jeremy JY, Angelini GD, Khan M, Mikhailidis DP, Morgan RJ, Thompson CS, Bruckdorfer KR, Naseem KM. Platelets, oxidant stress and erectile dysfunction: An hypothesis. Cardiovasc Res. 2000; 46:50-54.
39. Jones RW, Rees RW, Minhas S, Ralph D, Persad RA, Jeremy JY. Oxygen free radicals and the penis. Exp Opin Pharmacother. 2002; 3:889-897.
40. Rodrigo R. Prevention of postoperative atrial fibrillation: Noveland safe strategy based on the modulation of the antioxidant system. Front Physiol. 2012; 3:93.
41. Arivazhagan P, Thilagavathy T, Pannerselvam C. Antioxidantlipoate and tissue antioxidants in aged rats. J Nutr Biochem. 2000; 11:122-127.
42. Meister A and Anderson ME. Glutathione. Annu Rev Biochem. 1983; 52:711-760.
43. Sehirli AO, Sener G, Satiroglu H, Ayanoglu-Dulger G. Protectiveeffect of N-acetylcysteine on renal ischemia/reperfusioninjury in the rat. J Nephrol. 2003; 16:75-80.
44. Harris ED. Regulation of antioxidant enzymes. J Nutr. 1992; 122:625-626.
45. Miyasaka CK, DE-Souza JAA, Torres RP, Mancini-Filho J, Lajolo FM, Curi R. Effect of the administration of fish oil bygavage on activities of antioxidant enzymes of rat lymphoid organs. Gen Pharmacol. 1998; 30:759-762.
46. Pereira B, Costa-Rosa LFBP, Bechara EJH, Newsholme P, Curi R. Changes in TBARS content and superoxide dismutase,catalase and glutathione peroxidase activities in the lymphoidorgans and skeletal muscles of adrenodemedullated rats. Braz J Med Biol Res. 1998; 31:827-833.