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Original Research Article

Beta-D-glucan Polysaccharide Downregulates p53, and Prostate Specific Antigen Expression in Histological and Immunohistochemical study of Prostate Tumor Model

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ABSTRACT

Global increase in cancer incidence has prompted the search for natural bioactive products which are readily available, safer and more affordable. Beta-D-glucan polysaccharide (βDgP) is a phytochemical fractionate of an edible mushroom *Auricularia polytricha* known to have anti-cancer property but information about the actual mechanism underlying its therapeutic property is lacking. This research investigates the tumor regulatory effect of Beta-D-glucan polysaccharide on some tumor markers; p53 and Prostate Specific Antigen (PSA) in nitrosobis amine (NA) – induced prostate tumor model of Wister rat. Twenty-four male Wister rats were divided into four groups with six rats in each group. Group A served as normal control while treatment groups: B (5mg/kgbw nitrosobis amine only); C and D (placed on 120 mg/kgbw and 250 mg/kgbw respectively) after inducing tumor. The experiment lasted for 10 weeks. At termination, ELISA method was used to evaluate PSA levels; p53 tumor marker was assessed using immunohistochemical methods; histological alterations were examined using routine H&E technique. Results shown that p53 and PSA levels increased significantly (at $p \leq 0.05$) in group B (tumor control) when compared to normal control. Increase in papillary fronds, basal cell hyperplasia and prostatic concretions depicted cytoarchitectural alterations in tumor control group. However, expression of p53 and PSA was down-regulated significantly ($p \leq 0.05$) when compared to tumor control, and histological distortions reversed in a dose-dependent manner, following administration of graded concentration of βDgP. Findings from the present study have revealed the anti-tumor property of βDgP in NA-induced prostate tumor model in Wister rat.

Keywords: Beta-D-glucan-polysaccharide, Prostate gland, Prostate Specific Antigen (PSA), Cancer, p53.

Introduction

Cancer has become the most challenging, multifactorial and complex disease affecting man. Researchers are beaming the searchlight on both preventive and curative measures which is posting numerous challenges. Prostate tumor and accompanying conditions such as benign prostatic hyperplasia and prostate cancer has become one of the key health concerns in aging men.[1, 2]

The prostate gland function in secretion of fluid that nourishes and transport sperm cells as well as production of prostate specific antigen (PSA), a protein serine protease that function in liquefaction of semen.[3] PSA can escape into blood as a result of development of prostate tumor. Serum PSA concentration has been found by researchers to be proportional to prostate tumor volume.[4]

The p53 protein is encoded by TP53 gene, which is one of the most frequently mutated genes in human cancer. Most cancers have been reported to have inactivated p53. The functions of p53 protein includes regulation of the cell cycle, angiogenesis, cellular differentiation, immune response and apoptosis. They are also reported to regulate the genes involved in epithelial tissue development.[5,6] Since its discovery about 40 years ago, p53 have been regarded as tumor suppressors.

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Regrettably, the mutant form of p53 will lose their tumor-suppressive potentials and rather promote tumorigenesis.[7,8]

A good number of endogenous and exogenous agents activate p53 and trigger it to further regulate series of responses needed for maintaining homeostasis, critical for normal cell survival and prevention of tumorigenesis.[9,10]

Apart from chemotherapy, previous researchers have highlighted the anti-cancer activities of various plants and animal extracts in management and treatment of cancers including prostate cancer.[11]

Glucans polysaccharides are present in the cell wall of bacteria, algae, plants as well as micro and macro-fungi. Beta-D-glucan-Polysaccharide (βDgP), a bioactive phytochemical fractionates of *Auricularia polythrica*, an edible mushroom known for several medical benefits, has shown numerous therapeutic properties including antioxidant;[12] immunomodulatory and anticancer activities.[13-15]

Global increase in cancer incidence has prompted the search for natural bioactive products which are readily available, safe, and affordable. Beta-D-glucan polysaccharide (βDgP) is a phytochemical fractionate of *Auricularia polytricha*, an edible mushroom known to have anti-cancer property but information about the actual mechanism underlying its therapeutic property is lacking. This research seeks to investigate anti-tumor effect of βDgP on nitrosobis amine-induced prostate tumor model of Wister rat.

Materials and Methods

Preparation of extract

Auricularia polytricha was obtained from a central market located in Etung Local Government Area of Cross River State, Nigeria on 7th October 2022. It was authenticated in the department of biological sciences, University of Nigeria, assigned voucher number AO1/243. The fungi were dried at room temperature for one week and ground to

powder. 200g of *A. polytricha* was soaked in 1000ml of ethanol, labelled and covered for 72 hours, after which a clean filter paper (Watman No 1) was used to filter extracts. The filtrate was evaporated to dryness at 40ºC in a vacuum using a rotatory evaporator. The extract was then weighed and kept at 4^0c in refrigerator until further use. [16]

Fractionation of β-D-glucan polysaccharide

βDgP was experimentally separated from *A. polytricha* using acetyl trimethyl ammonium bromide to form a precipitated complex with the acidic polysaccharide. It was further purified through a combination of fractional precipitation with acetic acid using ion-exchange chromatography. [17]

Experimental animals

Twenty-four (24) adult male Wistar rats (six months old) with weight range of 150-220g were used for this research. The rats were divided into four groups and kept in four clean cages designated A, B, C and D with six rats in each group. The rats were allowed to acclimatize for two weeks in animal house, Department of Anatomy, Faculty of Medicine, University of Calabar, Nigeria and allowed unrestricted access to commercially available chow (livestock feed) and water.

Experimental design

Experimental animals were grouped and treated as shown in Table 1.

Ethical approval

This was obtained from Faculty of Basic Medical Sciences Animal Research Ethical Committee, University of Calabar, Calabar with the Registration Number: FBMS/EC/22/098. Accepted protocol for animal care and use in research and experimentation was followed.

Administration of agents

Group A, Normal Control was placed on 1ml of Normal saline orally, throughout the experiment. Experimental animals in Groups B received weekly intragastric treatment with 5 mg/kgbw of Nitrosibis (2-oxopropyl) amine. Groups C and D were administered 120 mg/kg.bw and 250 mg/kg.bw β-D-glucan polysaccharide respectively after treatment with Nitrosobis as shown in Table 1

Administration of extract

β-D-glucan polysaccharide administration commenced two weeks after induction of tumor by oral gastric intubation and lasted for ten weeks.

Evaluation of p53 and PSA levels in serum

Enzyme-linked immunosorbent assay (ELISA) kits for rat PSA, ELISA kit (Cloud-clone Corp., Katy, Texas, USA) was used to estimate PSA level in serum. The manufacturers protocol was followed strictly in preparing samples. Standards were added to the precoating with target-specific capture antibodies microtiter plates with optical density placed at 450 nm.

Expression of p53 was evaluated using immunohistochemical quantitative assessment based on the percentage of cells showing positive staining. P53 was localized in paraffin sections using CM-5 antibody and counterstained with methylene blue.[7]

Histopathological studies

At termination, the animals were sacrificed, and the prostate gland collected and suspended in buffered neutral formaldehyde for further processes with conventional histological techniques. Sections were cut at 5.0 µ, stained in Heamatoxylin and Eosin (H & E) and examined under a light microscope. [18]

Statistical analysis

Data obtained from this study was recorded and analyzed using one-way analysis of variance (ANOVA) with SPSS program (version 20). Post-hoc test was conducted using Fischer's Least Significant Difference (LSD) to determine statistical significance among groups. Probability level of $P<0.05$ was considered significant.

Table 1: Experimental animals were divided into four (4) groups and treated as follows:

Group	Designation	Treatment	Dose
A	Normal control (Nc)	Distilled water	3 mls
B	Tumor control (Tc)	Nitrosobis Amine (NA)	5 mg/kg.bw
C	Tc+ Low Dose βDgP	NA+β-D-glucan polysaccharide	120 mg/kg.bw
D	Tc + High Dose βDgP	NA+ β-D-glucan polysaccharide	250 mg/kg.bw

Results and Discussion

Assessment of p53 expression was done based on the degree of nuclear accumulation of p53 protein. Figure 1 shows the levels of expression of p53 (expressed in percentage) in different experimental groups. Tumor control (TC) recorded significantly higher p53 expression (59.22%) when compared to the normal control (5.32%) at $p\leq0.05$. Following the administration of βDgP, p53 tumor suppressing protein significantly decreased in a concentration-dependent manner in groups C and D.

Serum PSA level was remarkably higher in TC group (4.13 ng/L) when compared to normal control as shown in figure 2. However, PSA levels decreased significantly as groups that received 120 mg/kgbw and 250 mg/kgbw of βDgP recorded 3.11ng/L and 0.99ng/L respectively. These values were significantly lower when compared to TC group.

In the Histopathological observation (Figure 3), photomicrographs of prostate section of normal control (group A) showed normal microstructure (Figure 3A). Sections of tumor control group in fig 3B revealed decreased in lumen areas, increased papillary fronds and presence of epithelial hyperplasia in sections of prostate gland. Figure 3C which represents section of prostate of tumor models placed on 120 mg/kgbw of βDgP shows fewer papillary fronds and mild epithelial hyperplasia. Figure 3D (NA + 250 mg/kgbw βDgP) revealed prostate section with increased lumen and lesser epithelial height.

From the observations above, findings from this study reveals that expression of p53 marker was significantly increased in tumor control group (Group B) when compared to normal control (Group A). However, there was a remarkable decrease in expression of p53 cancer suppression index following administration of βDgP in a concentration dependent manner. This decline in p53 values indicates that βDgP may have the potentials to reactivate suppressed p53 functions. Researchers have reported that anti-cancer properties of polysaccharides with therapeutic value is based on their structure and molecular weights. [19] It is, therefore, reasonable to suggest that low molecular weight fragments of βDgP are capable of being bonded by complement receptors on immune system cells thus stimulating them against tumorigenic cells. Pan *et. al,*[20] Agbor *et. al*[12] and Thongsiri *et al*, [21] reported similar findings.

As observed from this study, administration of NA (5mg/kgbw) increased significantly, the serum PSA level in tumor control animals when compared to normal control group. This is a confirmation that tumorigenesis has taken place. Serum PSA concentration has been reported to be proportional to prostate tumor volume.[4] However, treatment with βDgP remarkably decreased serum concentration of PSA in a concentration-dependent manner. This may be due to the cytotoxic effect of βDgP on tumor cells directed to trigger apoptotic activities. This is in line with report by Ina *et. al.*[22]

Additionally, histopathological results revealed hyperplasia of glandular cells, decreased lumen area, and increase in papillary fronds in tumor control group which are landmarks of cytoarchitectural alteration. Treatment with 250 mg/kgbw of βDgP reversed he observed

distortions and eliminated hyperplasia in prostate glandular cells completely. This repair may have been due to a combination of both cytotoxic potentials, and antioxidant property of βDgP on tumor cells. However, further study needed to be carried out to ascertain exact mechanism for this repair. Li *et al*,[23]; Zi *et al*,[24] have recorded similar findings.

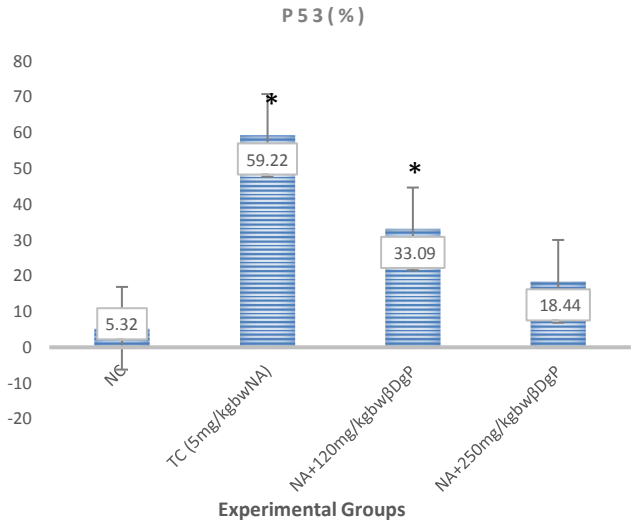
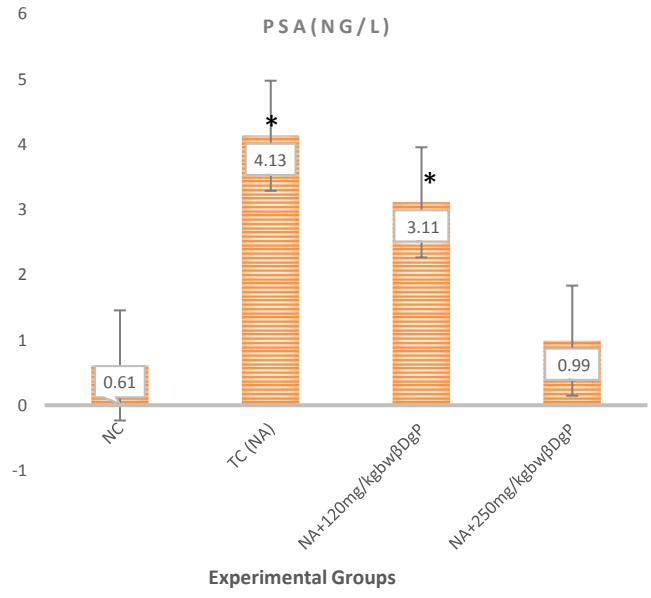


Figure 1: Comparison of expression of p53 marker (%) in different experimental groups

NC – Normal Control, TC – Tumor Control, NA – Nitrosobis Amine, βDgP – *Beta-D-glucan Polysaccharide*

Values are expressed in Mean ± SEM. N = 6. ***** = Values are remarkably increased when compared to Normal Control at p<0.05. **#** = Values are remarkably decreased when compared to Tumor Control (TC) at p<0.05.



NC – Normal Control, TC – Tumor Control, NA – Nitrosobis Amine, βDgP – *Beta-D-glucan Polysaccharide*

Figure 2: Comparison of PSA (ng/L) levels in different experimental groups

Values are expressed in Mean ± SEM. N = 6. ***** = Values are remarkably increased when compared to Normal Control at p<0.05. **#** = Values are remarkably decreased when compared to Tumor Control (TC) at p<0.05.

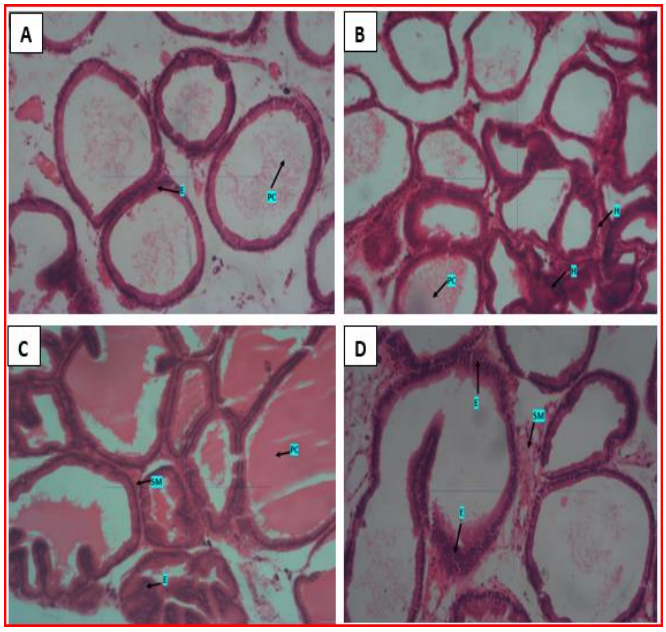


Figure 3: Histological Photomicrographs

A: Photomicrograph of section of prostate of control group showing normal epithelial cells (E). the glands appear normal with few Prostatic concretions (PC). No pathology seen: X400. B**:** Photomicrograph of tumor control (TC) group showing basal cell hyperplasia (H), few prostatic concretions (PC); reduced lumen areas and increase in papillary fronds of prostate section. MAG: X400. C: Section of prostate of tumor models placed on 120 mg/kgbw of βDgP shows fewer papillary fronds and mild epithelial hyperplasia. Few smooth muscles (SM) can be seen. X400. D: Group D (NA+250 mg/kgbw βDgP) revealed prostate section with increased lumen and lesser epithelial height Few smooth muscles (SM) are also seen. No pathology seen. X400

Conclusion

Administration of βDgP was able to decrease significantly, expression of p53 tumor-suppression index and prostate specific antigen (PSA) in NA – induced tumor model of Wister rat. This dose-dependent modulatory property was probably due to the potentials of βDgP to reactivate suppressed p53 function and their cytotoxic effect on tumor cells. However, the regulatory mechanism is still subject of investigation. All histopathological alterations associated with induced prostate tumor in this research were reversed following treatment with βDgP.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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