Original Research Article

SYNERGISTIC EFFECT OF DOCETAXEL PLUS SAPONIN FRACTION OF *VITEX DONIANA* ON PROSTATE SPECIFIC ANTIGEN AND p53 IN NITRSOBIS (2-OXOPROPYL) AMINE-INDUCED PROSTATE TOXICITY

RUNNING TITLE
Effect of Docetaxel Plus Saponin on Prostate Specific Antigen and p53

ABSTRACT

AIM: This study investigated the synergistic effect of docetaxel plus saponin fraction of *Vitex doniana* on prostate specific antigen and p53 in nitrsobis (2-oxopropyl) amine-induced prostate toxicity in Wistar rat.

METHODOLOGY: Twenty-four (24) male Wistar rats with elevated serum prostate specific antigen level were selected from a group of sixty (60) rats pretreated with subcutaneous Nitrosobis (2-oxopropyl) amine 5 mg/kg daily for 4 weeks. The selected 24 male Wistar rats were then grouped into 6 groups of four (4) rats each. Group 1 was given 1ml normal saline daily from day 1-28. Groups 2, 3, 4, 5, and 6 further received subcutaneous nitrosobis (2-oxopropyl) amine 5 mg/kg daily from day 1-28. In addition, groups 3, 4, 5, and 6 were given weekly intravenous docetaxel 8 mg/kg on day 15 and 22. In addition to docetaxel, groups 4,

5, and 6 were further treated with oral saponin at 250 mg/kg, 500 mg/kg, and 750 mg/kg, respectively, daily, from day 15-28. Immunoenzymometric assay method was used for analysis of blood sample for prostate specific antigen. The prostate tissues were subjected to immuno study using the ImmunoCruz Staining System (Lab Vision Corporation, Fremont, CA, USA). The quantitative evaluation of p53 was done by calculating the percentages of p53-immunostained nuclei (labeling index).

RESULT: Significant increase in prostate specific antigen and p53 expression were observed in group 2 (treated with Nitrsobis (2-oxopropyl) amine alone) when compared with group 1 (control). Dose dependent decrease in prostate specific antigen and p53 expression were observed in groups 4, 5, and 6, treated with docetaxel 8 mg/kg plus 250 mg/kg, 500 mg/kg, and 750 mg/kg of saponin respectively.

CONCLUSION: Docetaxel plus Saponin fraction of *Vitex doniana* significantly reduced the serum prostate specific antigen concentration and p53 expression in a dose dependent manner, with the group treated with 750 mg/kg showing the highest decrease in the parameters tested.

Keywords: p53, Saponin, *Vitex doniana*, Docetaxel, prostate specific antigen

INTRODCUTION

The tumour suppressor gene, p53, is usually activated following pathological injury to DNA of cells [1]. The level of expression of p53 is minimal in normal cells, but the level gets elevated in conditions that lead to tumour development such as activation of oncogenes, genotoxic stress, and acute stress [2]. Nitrosobis (2-oxopropyl) amine is a known genotoxic agent which has been used to induce prostate cancer [3]. Prostate cancer ranks as the most common diagnosed cancer in men worldwide, and accounts for 13% of cancer deaths in men [4]. It is associated with increased p53 expression [5]. Docetaxel is among the various options

available for the treatment of prostate cancer [6]. But despite these available options, prostate cancer is still the most common cause of cancer deaths in men worldwide [4]. Plants have been used in traditional medicine to treat several ailments including cancers, and has been a source of several medications used currently for cancer treatment [7,8]. *Vitex doniana* (Black plum) is a deciduous tree which belongs to the family *Lamiaceae* and has potential in the treatment of several cancers, including breast cancer [9] and prostate cancer [10]. Its antineoplastic effects have been attributed to several of its phytochemical contents, such as saponin, hydoxycinnamic acid, allicins, flavonoids and terpenoids [10,11]. Saponin has been proven to be useful in the treatment of several cancers such as colon cancers, pancreatic cancer [12] and breast cancer [13]. However, there is no study on the use of saponin from *Vitex doniana*, nor combination of saponin with docetaxel in treatment of prostate cancer, and modulation of p53 expression by the prostate. This study investigated the synergistic effect of docetaxel plus saponin fraction of *Vitex doniana* on prostate specific antigen and p53 in nitrsobis (2-oxopropyl) amine-induced prostate toxicity in Wistar rat.

MATERIALS AND METHOD

Isolation of Saponin

Vitex doniana leaves were gotten from *Vitex doniana* tree in Enugu Metropolis, Enugu, Nigeria. It was authenticated in the Department of Botany, University of Nigeria. The stalks of the fresh leaves of *Vitex doniana* were removed, and the leaves washed with distilled water and then air-dried at room temperature. Subsequently, the leaves were pulverized to fine powdered form using mortar and pestle. Ungrounded fibres were removed by passing the powdered form through a sieve with little pores [14]. Two thousand (2000) grams of the powdered form was extracted exhaustively and the aqueous extract filtered with Whatman No. 2 filter paper, and concentrated with a rotary evaporator at 40°C [15]. Then the extract

was concentrated under reduced pressure and partitioned successively using n-hexane, ethyl acetate, and n-Butanol. The n-Butanol soluble fraction and the aqueous part afford the major saponin triterpene fraction. The crude extracts were applied separately to columns of Diaion HP-20 which were then washed with Water-Methanol in various ratios (0, 50, 85, and 100) and finally with acetone. The fractions found to have the same pattern were mixed together and separated further by silica gel column chromatography with ethyl acetate-Methanol-water (40:10:1 v / v / v). Then the saponin compounds was separated by HPLC on Octadecylsilyl column using Methanol-water as eluent [16]

Animal handling and administration of Agents

Sixty male Wistar rats with average weight of 180g were procured for the experiment from the Animal house of Department of Anatomy, University of Nigeria. The rats were handled carefully according to the protocol of the Committee for the purpose of control and supervision of experiments on Animals. They were housed in netted iron cages and kept at temperature of 25°C, humidity 60-70%, and they had 12-hour light and dark cycles throughout the experiment. They were allowed 2 weeks for acclimatization before the commencement of the experiment, and during this time, they had free access to rat chow and water. They were given subcutaneous Nitrosobis (2-oxopropyl) amine 5mg/kg daily for 4 weeks. At the end of the 4 weeks, blood samples were collected by aid of capillary tube via the medial canthus for the determination of the serum prostate specific antigen level.

Then twenty-four (24) male Wistar rats with elevated serum prostate specific antigen level were selected from the sixty (60) rats pretreated with subcutaneous Nitrosobis (2-oxopropyl) amine 5 mg/kg daily. The selected 24 male Wistar rats were then grouped into 6 groups of four (4) rats each for the next phase of the experiment which lasted for 28 days.

Group 1 was given 1ml normal saline daily from day 1-28. Groups 2, 3, 4, 5, and 6 further received subcutaneous nitrosobis (2-oxopropyl) amine 5 mg/kg daily from day 1-28. In addition, groups 3, 4, 5, and 6 were given weekly intravenous docetaxel 8mg/kg on day 15 and 22. In addition to docetaxel, groups 4, 5, and 6 were further treated with oral saponin at 250 mg/kg, 500 mg/kg, and 750 mg/kg, respectively, daily, from day 15-28, as shown in table 1.

Table 1: showing administration of agents

Groups	**1-14 days**	**15-28 days**
1	Normal saline 1ml	Normal saline 1ml
2	Nitrosobis amine 5 mg/kg	Nitrosobis amine 5 mg/kg
3	Nitrosobis amine 5 mg/kg	Nitrosobis amine 5 mg/kg+Docetaxel 8 mg/kg/week
4	Nitrosobis amine 5 mg/kg	Nitrosobis amine 5 mg/kg +Docetaxel 8 mg/kg + Saponin 250 mg/kg
5	Nitrosobis amine 5 mg/kg	Nitrosobis amine 5 mg/kg +Docetaxel 8 mg/kg + Saponin 500 mg/kg
6.	Nitrosobis amine 5 mg/kg	Nitrosobis amine 5 mg/kg +Docetaxel 8 mg/kg + Saponin750 mg/kg

Sample Collection

The rats were anaesthetized at the end of the experiment using intraperitoneal thiopentone at 50 mg/kg. Blood samples were then collected from the retro-orbital vein of the rats for haematological analysis of prostate specific antigen [17]. They were then sacrificed and the

prostate gland excised and fixed immediately before immunohistochemical analysis for p53 expression.

Sample Analysis

The immunoenzymometric assay method was used for analysis of blood sample for prostate specific antigen. The prostate tissues were cut to sections of 5 µm and subjected to immuno study using the ImmunoCruz Staining System (Lab Vision Corporation, Fremont, CA, USA).

The quantitative evaluation of p53 was done by calculating the percentages of p53-immunostained nuclei (labeling index) in each selected section for all the groups using this formula: Number of labeled nuclei x100/total number (labeled + unlabeled) of nuclei. The measurements were carried out using an Olympus (Leica Microsystems GmbH, Wetzlar, Germany) microscope that has a 3100 oil-immersion lens (numerical aperture 1.4) at a final magnification of X1200 and using the stereologic software GRID (Interactivision; Silkeborg, Denmark).

Statistical Analysis

This was done using Statistical Package for Social Sciences (SPSS) version 25. p-value of 0.05 or less was considered significant.

RESULTS

Result of prostate specific antigen

The prostate specific antigen showed significant increase in the group treated with nitrosobis (2-oxopropyl) amine (group 2) when compared to the normal control, group 1, which received normal saline throughout the period of the experiment. Decrease in the prostate specific antigen level were observed in group 3, treated with Nitrosobis (2-oxopropyl) amine plus docetaxel alone (group 3), but this decrease was not statistically significant when

compared with group 2. The prostate specific antigen in groups 4, 5, and 6, that received additional treatment of oral saponin 250 mg/kg, 500 mg/kg, and 750 mg/kg, respectively, showed a dose dependent statistically significant decrease, when compared to group 2 treated with Nitrosobis (2-Oxopropyl) amine alone, as shown in table 2. Group 6, treated with 750 mg/kg of saponin, were observed to have the highest effect.

Table 2: showing the values of prostate specific antigen and quantitative representation of p53 expression

Assay	**1**	**2**	**3**	**4**	**5**	**6**
PSA	3.71±0.24	9.67±0.23	8.55±0.23	7.40±0.18	4.91±0.25	4.38±0.65
P53	5.75±0.48	21.00±0.41	18.50±0.65	15.25±0.25	14.25±0.48	10.00±0.41

Result of immunohistochemical analysis of p53 expression

The immunohistochemical assay for p53 expression, as shown in figure 1, was subjected to computer assisted stereology, and quantitative value of p53 was obtained, as shown in table 2. This study observed that there was a significant increase in the expression of p53 in group 2, treated with nitrosobis (2-oxopropyl) amine when compared with group 1, which received normal saline throughout the period of the experiment. There was significant decrease in p53 expression in groups 5, and 6, treated with Docetaxel plus Saponin 500 mg/kg, and Docetaxel plus Saponin 750 mg/kg, respectively, when compared to group 2. Also there was significant decrease in p53 expression in groups 5, and 6, when compared to group 3, treated with docetaxel only. These significant decreases observed are in dose dependent manner.

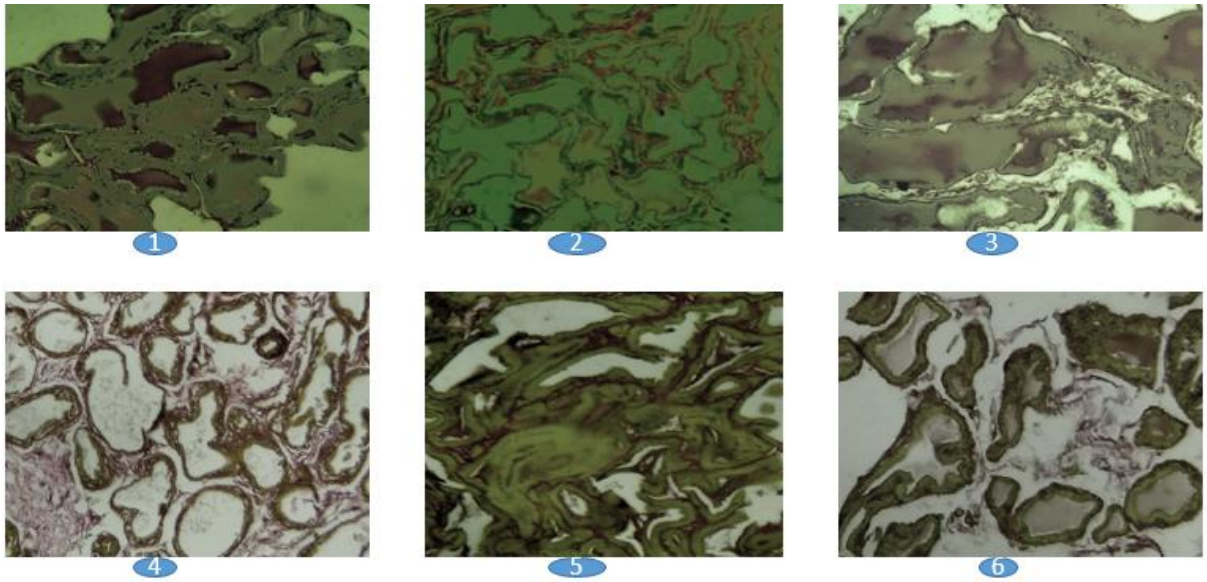

Fig. 1: Immunohistochemical assay of p53 expression in prostate gland of Wistar rat (x100) of groups 1-6.

DISCUSSION

This study observed that saponin fractionate of *Vitex doniana* leaf is inhibitory to the expression of p53 tumour marker and prostate specific antigen induced by Nitrosobis (2-oxopropyl) amine in prostate gland of Wistar rat, as shown by its modulatory effect on the serum concentration of prostate specific antigen and expression of p53 by the prostate gland of the experimental animals. This study also observed that these effects were synergistic with docetaxel chemotherapy. These effects of saponin fraction of *Vitex doniana* may be due to the cytotoxic effect of saponin on cancer cells due to its anti-proliferation, and anti-angiogenesis effect [18]. These findings correlate with findings of Xu et al., 2016 who found that oral administration of saponin ginsenoside 100 mg/kg for 28-days in nude mice bearing H460 cells showed a remarkably suppressed tumor growth, and 30% reduction in tumour volume [19]. Xia et al., 2020 also reported that total saponin from *Paris forestii* at 2 micrograms per millilitre has a suppressive effect on the growth of prostate cancer cell lines (PC3 cell line) [20]. The synergistic effect of docetaxel and saponin from *Vitex doniana*

observed in this study is in consonance with the study by Wenner et al., 2011 who observed that Polysaccharide-K fractionate of *Trametes versicolor* when combined with docetaxel has enhanced prostate cancer cells apoptosis, and also improved the immune responses against the prostate cancer cells from transgenic adenocarcinoma of mouse prostate- bearing mice [21].

CONCLUSION

Saponin fraction of *Vitex doniana* has a modulatory effect on the expression of p53 tumour marker, and prostate specific antigen, with a possible resultant effect on the reduction of prostate cancer burden in the experimental animals. Also it has a synergistic effect on the anticancer action of docetaxel chemotherapy. Hence, following purification, the use of saponin fraction of *Vitex doniana* as a single agent, or as a combination with docetaxel chemotherapeutic agent in the treatment of prostate cancer may be of great importance in the reduction of tumour burden on prostate cancer patients.

CONSENT

It is not applicable.

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