Original Research Article

MODULATORY EFFECT OF DOCETAXEL PLUS SAPONIN FRACTIONATE OF *VITEX DONIANA* ON PROSTATE SPECIFIC ANTIGEN AND p53 TUMOUR MARKERS EXPRESSION 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 was to investigate the modulatory effect of Docetaxel plus saponin from Vitexdoniana on the expression of p53 tumour markers and prostate specific antigen following induction of prostate toxicity in Wistar rat using Nitrosobis (2-oxopropyl) amine.

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 5mg/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 6further received subcutaneous nitrosobis (2-oxopropyl) amine 5mg/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 250mg/kg, 500mg/kg, and 750mg/kg, respectively, daily, from day 15-28

RESULT: Significant increase in prostate specific antigenand 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 antigenand p53 expression were observed in groups 4, 5, and 6, treated with docetaxel 8mg/kg plus 250mg/kg, 500mg/kg, and 750mg/kg of saponin, respectively.

CONCLUSION: Docetaxel plus Saponin fractionate of Vitexdoniana significantly reduced the serum prostate specific antigen concentrationand p53 expression in a dose dependent manner, with the group treated with 750mg/kg showing the highest decrease in the parameters tested.

Keywords: p53, Saponin, Vitexdoniana, 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]. And 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 still is still the most common cause of cancer deaths worldwide [4]. Plants have been used in traditional medicine to treat several ailments including cancers, and has

been source of several medications used currently for cancer treatment [7,8]. Vitexdonianahave potential in the treatment of several cancers, including breast cancer [9], prostate cancer [10]. Its antineoplastic effects have been attributed to several of its phytochemical contents, which saponin is one of them [10]. Saponin has been proven to be useful in treatment of several cancers such as colon cancers and pancreatic cancer [11],breast cancer [12].However, there is no study on the use of saponin from *Vitexdoniana*, nor combination of saponinwith docetaxel in treatment of prostate cancer, and modulation of p53 expression by the prostate. This study investigated the modulatory effect of docetaxel plus saponin fractionate of *Vitexdoniana* on expression and p53 tumour markers and prostate specific antigen in nitrsobis (2-oxopropyl) amine-induced prostate toxicity in Wistar rat.

MATERIALS AND METHOD

Isolation of Saponin

The stalks of the fresh leaves of Vitexdonianawere 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 [13]. Two thousand (2000) grams of the powdered form was extracted exhaustivelyand the aqueous extract filtered with Whatman No. 2 filter paper, and concentrated with a rotary evaporator at 40°C [14]. 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 [15]

Animal handling and administration of Agents

Sixty male Wistar rats with average weight of 180g were procured for the experiment. 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 5mg/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 6further received subcutaneous nitrosobis (2-oxopropyl) amine 5mg/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 250mg/kg, 500mg/kg, and 750mg/kg, respectively, daily, from day 15-28, as shown in table 1.

Table 1: showing administration of agents

Groups	1-14 days	15-28days
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 50mg/kg. Blood samples were then collected from the retro-orbital vein of the rats for haematological analysis of prostate specific antigen [16]. They were then sacrificed and the prostate gland excised and fixed immediately before immunohistochemicalanalysis for p53 expression.

Sample Analysis

The immunoenzymometric assay method was used for analysis of blood sample for prostate specific antigen. The histological slides of the prostate gland specimens were prepared using the standard histological techniques with immunohistochemical stain for p53. The slides were analyzed using a light microscope, and computer assisted stereology was used for quantitative analysis of the cells.

Statistical Analysis

This was done using Statistical Package for Social Sciences (SPSS) version 25. p-value of .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, that 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 250mg/kg, 500mg/kg, and 750mg/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 750mg/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 Saponin500mg/kg, and Docetaxel plus Saponin750mg/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.

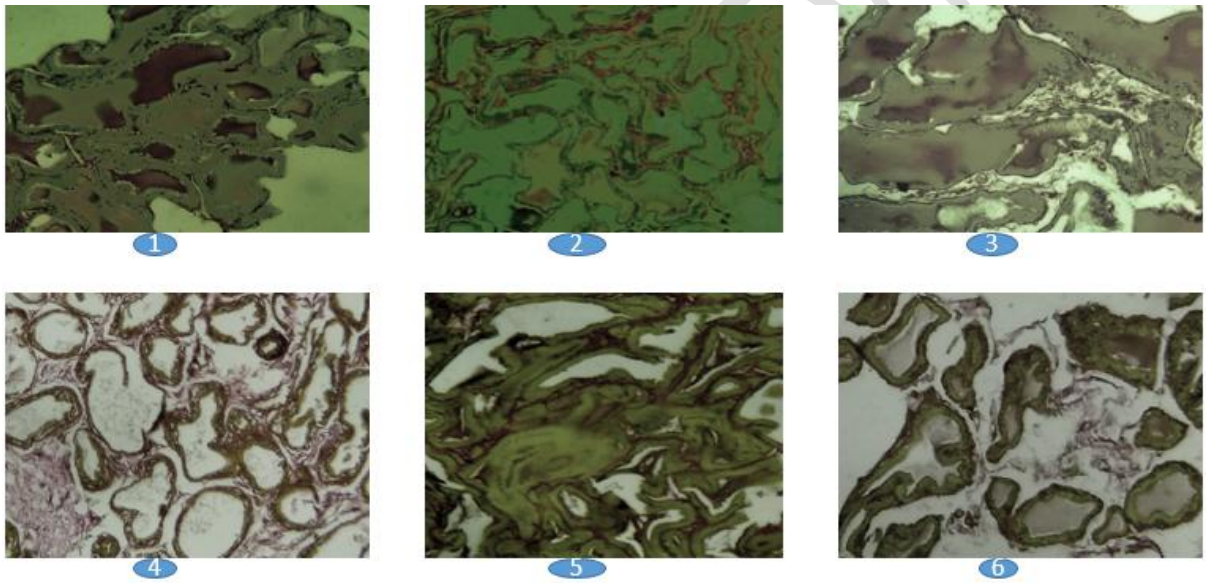

Fig. 1: Immunohistochemical assay of p53 expression in prostate gland of Wistar rat (x100) of groups 1-6.

DISCUSSION

This study observed that saponin fractionate of Vitexdoniana 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 theserum concentration of prostate specific antigen and expression of p53 bythe prostate

gland of the experimental animals.This study also observed that these effects were synergistic with docetaxel chemotherapy.These effects of saponinfractionate of Vitexdoniana may be due to the cytotoxic effect of saponin on cancer cells due to its anti-proliferation, and anti-angiogenesis effect [17]. These findings correlate with findings of Xu et al., 2016 who found that oral administration of saponinginsenoside 100mg/kg for 28-days in nude mice bearing H460 cells showed a remarkably suppressed tumor growth, and 30% reduction in tumour volume [18]. Xia et al., 2020 also reported that total saponin from Parisforestii at 2 micrograms per millilitre has a suppressive effect on the growth of prostate cancer cell lines (PC3 cell line) [19].The synergistic effect of docetaxel and saponin from Vitexdoniana 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 [20].

CONCLUSION

Saponin fractionate of Vitexdoniana 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 fractionate of Vitexdonianaas 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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