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ANTITUMOR ACTIVITY AND ANTIOXIDANT STATUS OF RETICULATACIN AGAINST TESTOSTERONE INDUCED PROSTATE CANCER IN ALBINO WISTAR RAT

Saritha Surapaneni*, T. Prakash[1] and Dinesh Ghatamaneni[2]

[1]*Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bangalore - 560 107, Karnataka, India.
[2]Department of Medical Rehabilitation Science, King Khalid University, Abha, Saudi Arabia – 61481.

***Corresponding Author: Saritha Surapaneni**

Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bangalore - 560 107, Karnataka, India.

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ABSTRACT

The hydroalcoholic extract of *Annona reticulata*. *L* Bark powder was extracted by successive extraction with pet ether, chloroform and ethanol solvents and hydro alcoholic extract was collected and phytochemical evaluation are carried and isolation of reticulatacin and characteristics of active constituents by FTIR & UV Spectroscopy, HPLC, Mass Spectroscopy, 1H-NMR and 13C-NMR. Evaluation of antitumor activity against testosterone-induced prostate cancer (PC) in albino wistar rats and reticulatacin was administered for Healthy adults male Wistar albino rats were divided into five groups (n = 8). Group I was served as vehicle control (Arachis oil 1ml/kg, SC), group II was served as testosterone Depot (TD) injection (3 mg/kg, s.c in Arachis oil), group III was served as standard Finasteride (5 mg/kg, p.o, and TD 3 mg/kg, s.c in Arachis oil), group IV was served as treated with reticulatacin (50 mg/kg p.o and TD 3 mg/kg, s.c in Arachis oil), group V reticulatacin (100 mg/kg p.o and TD 3 mg/kg, s.c in Arachis oil) for 28 days to assess the preventive effect of Reticulatacin. After the treatment animal was sacrificed and the prostate was removed and homogenized. Estimation of lipid peroxidation (LOP), reduced glutathione (GSH), glutathione peroxidase activity (GPx), glutathione Reductase (GR), Catalase (CA), estimation of protein levels and the histopathological changes were observed. Reticulatacin caused a marked decrease in prostate weight of prostate cancer-induced rats whereas glandular hyperplasia occurred in the negative control. Decreased the levels of lipid peroxidation and significantly ($P < 0.05$) increased the levels of GSH, GPx, and CAT recorded a marked increase in the levels of antioxidant enzymes compared to the negative control. It also exhibited antioxidant properties and showed to be a good prophylaxis.

KEYWORDS: Reticulatacin, prostate cancer, benign hyperplasia Testosterone Depot, biochemical parameter, histopathology, antioxidant, antitumor activity.

INTRODUCTION

This prospective study was designed to examine the relationship between plasma concentrations of several major antioxidants and risk of prostate cancer.[1] An enlarged prostate means the gland has grown bigger and as the gland grows, it can press on the urethra causing difficulty in urination. The discomfort presented by this condition during urination makes its occurrence worrisome. Dihydrotestosterone (DHT), an androgen derived from testosterone through the action of 5α-reductase and its metabolite, 3α-androstanediol, seems to be the major hormonal stimuli for stromal and glandular proliferation in men with nodular hyperplasia. Experimental work has also identified age - related increases in estrogen levels that may increase the expression of DHT and the progenitor of BPH.[2] The incrimination of DTH in the pathogenesis of BPH forms the basis for the current use of 5α-reductase inhibitors in the treatment of symptomatic nodular hyperplasia. The 5α-reductase inhibitors inhibit the development of prostate cancer via a reduction in dihydrotestosterone (DHT) production.[3] Alternative therapy such as herbal medicine has been popular since the ancient time for the treatment of prostate cancer. Their popularity is based on the assumption that they are of natural source and therefore not harmful. More importantly is the fact that they are readily assessable, cheap and can be acquired without medical prescription. The phytotherapeutic agents used in the treatment of BPH could be recipes from a single plant source or could be extracts from two or more plant sources.[4] Reticulatacin is evaluated for *in vitro* study in prostate carcinoma. The structure was elucidated by spectroscopic techniques included Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC), UV and NMR. Estimation of Lipid peroxidation (LOP), Reduced glutathione (GSH), Glutathione peroxidase activity (GPx), Glutathione Reductase (GR), Catalase (CA),

estimation of protein levels and histopathology slides where analyzed.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GSH), glutathione reductase (GR), Finasteride tablets, thiobarbituric acid (TBA) were obtained from Sigma (Sigma Chemical Co., St Louis, MO), Reticulatacin, nicotinamide adenine dinucleotide phosphate reduced (NADPH), flavin adenine dinucleotide, ethylene diamine tetra-acetic acid (EDTA), nicotinamide adenine dinucleotide reduced, trichloroacetic acid (TCA), bovine serum albumin (BSA), 1,2-dithio-bis-nitrobenzoic acid (DTNB), glutathione reductase (GR) were obtained from Sigma (Sigma Chemical Co., St Louis, MO) ferric nitrate, ammonium thiocyanate, hydrogen peroxide, magnesium chloride, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate, sodium hydroxide, petroleum ether, chloroform, and ethanol were purchased from E. Merck Limited. All other chemicals and reagents were of the highest purity grade commercially available. The research was carried out in Acharya & B.M Reddy College of Pharmacy, Rajiv Gandhi University of Health Sciences, Karnataka, and Bangalore 560041.

Collection and preparation of an extract

Fresh matured bark of *Annona reticulata*. Was collected from Eturnagaram forest, Warangal district Telangana, India. The plant was identified by comparing with the authenticated herbarium of kakatiya University. The same was confirmed by comparing the authentic herbarium specimen. The bark was authenticated by Dr. Ajmeera Ragan (Professor & chair person board of studies) Department of Botany, Kakatiya University, Warangal, and Telangana, India.

Preparation of the crude extract

Bark of *Annona reticulata*. Collected, washed with water, shade dried and grounded into fine powder 200 gm of the dried bark powder was extracted using petroleum ether, chloroform, ethanol in succession using soxhlet apparatus. Each extract obtained following successive extraction was filtered using Whatman No 1 filter paper, dried to a semisolid mass using water bath and the yield of each extract thus obtained was recorded and stored in a refrigerator at 4^0C till further use. The crude extract was suspended into water and *n*-hexane successively.[5]

Phytochemical analysis

A stock concentration of 1% (W/ V) of each successive extract obtained using petroleum ether, chloroform, ethanol was prepared using the respective solvent. These extracts along with positive controls were tested for the presence of active phytochemicals of tannins, alkaloids, phytosterols, triterpenoid, flavonoids, cardiac glycosides, anthraquinones glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats are analyzed.[6]

Isolation of Reticulatacin

Preparation of Column - Soak silica gel in solvent. Pour into the column portion in wise manner (stopper must be open to escape air), during which vibration are given to the column. Pack the column tightly as possible as you can. Tap the column until no solvent appears on the top and is just about to dry the top surface of silica gel. Such as column does not stuck, even if it is longer or shorter, and also given appropriate speed of elution. You can keep the column without stopping for a while. Dissolve the sample in a small amount of easily soluble solvent and apply it on the top. Then elute with the solvent for chromatography. If elution is too slow, make some pressure on the top either by a weak pump or keeping enough amount of solvent at the top. 25 g of extract Eluted with increasing order of polarity like *n*;hexane/chloroform (8:2 v/v). 1 fraction is eluted and 0.2 mg of needles was formed.[7]

Thin - layer Chromatographic Studies (TLC)

Thin - layer chromatography was carried out on all the fractions using TLC pre-coated plates (silica gel 60 F 254) by using one way ascending technique. The plates were cut with scissors and marked with pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in methanol and capillary tubes were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent systems including (1) chloroform: methanol (9:1v/v) the plates were dried and visualized under normal day light, ultraviolet light (254 nm & 366 nm) and by iodine chamber in a mortar, grind a few crystals of iodine with some silica gel. Transfer to a jar with a plastic screw cap. Pink color spots on TLC sheet after dipping into the kedde reagent confirmation the presence of acetogenins in fraction are visualized by leaving the plate in the couple minutes until spots turn brown. Once taken out of the chamber, spots will rapidly fade away. Identification of acetogenins by using kedde reagent A: dissolve (2% W/V) 3, 5- dinitrobenzic acid (2g) in 90% ethanol and 100ml of KOH in ethanol. Kedde reagent B: dissolve sodium hydroxide (5g) in distilled water 100ml. Analytical TLC will be carried out on silica gel 60 as stationary phase and chloroform – methanol (9:1) as mobile phase. Acetogenin visualized by spraying kedde reagent on TLC plate. The retention factor (Rf) for each active compound was calculated for each fraction using the following.

Formula - Rf = Distance moved by the solute / Distanced moved by the solvent (solvent front).

Characterization of active constituents analysed by HPLC, FTIR, ^{1}H-NMR, ^{13}C-NMR and interpreted by the method.[8]

Experimental animal

Male wistar albino rats weighing between (200 - 250 g) animals were procured from registered animal breeders. The animals were housed in a polypropylene cage and

maintained at 24 ± 2ºC under 12 h light/dark conditions and relative humidity of 41-55% and provided with food and water *ad libitum*. All experiments on animals was conducted in accordance with the guidelines of CPCSEA, New Delhi, and IAEC.[9]

Acute toxicity study

Acute oral toxicity study of reticulatacin was carried out by adapting up and down method of CPCSEA, (OECD) guidelines No 425. The female Swiss albino mice weighing between 20-25 g were used for the study. The animal was continuously observed 12 hours to detect changes in autonomic or behavioral responses. Mortality was observed for 48 hours. All survived animals were observed for any sort of toxicity for 14 days after the drug administration according to main test criteria.[10]

Treatment regimen

To review the ability of reticulatacin on testosterone induced prostate cancer Wistar albino rats were randomly divided into five groups containing six animals in each group testosterone was induced in rats one week before to start the experiments. After the induction of PC, rats were divided into different groups. Group I: Arachis oil 1 ml/kg, s.c for 28 days as vehicle control. Group II: Testosterone Depot (TD) injection 3mg/kg, s.c in arachis oil for 28 days. Group III: Finasteride 5 mg/kg, p.o, and TD 3 mg/kg, s.c in arachis oil for 28 days. Group IV: Reticulatacin 50 mg/kg p.o and TD 3 mg/kg, s.c in arachis oil for 28 days. Group V: Reticulatacin 100 mg/kg p.o and TD 3 mg/kg, s.c in arachis oil for 28 days. On Day 28, the rats will be euthanized under diethyl ether anesthesia. The prostate glands were isolated and weights of right and left and total prostates was recorded and analyzed.[11]

Measurement of protein

Prostate glands were dissected and homogenates were made in phosphate buffer solution (0.01 M sodium phosphate buffer, pH 7.4, containing 0.14 M NaCl) at 1ml volume/g gland wet weight ratio of 4:1. Homogenates were centrifuged at 13,000×*g* for 20 min and supernant collected. The protein concentration in all samples will be determined by the method of Lowry *et al*. Supernant was used as source of proteins and concentration was determined by modified biuret end point assay method.[12]

Measurement of lipid peroxidation

The assay for membrane LPO was carried out by the method of Wright *et al*. (1981) with some modifications. The reaction mixture in a total volume of 3.0 ml contains 1.0 ml tissue homogenate, 1.0 ml of TCA (10%), and 1.0 ml TBA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The mixture was cooled and 5 ml of n-butanol–pyridine mixture (15:1) was added. The mixture was shaken vigorously. The tubes was shifted to ice bath and then centrifuged at 4000 for 10 min. The organic layer was taken and its absorbance was measured at 532 nm. The concentration of MDA formed is expressed as nM MDA/g wet tissue. The results was expressed as the n mol MDA formed/gram tissue by using a molar extinction co-efficient of 1.56×10^5/M/cm.[13]

Measurement of Reduced glutathione (GSH) levels

Reduced glutathione was estimated by Protein free supernatant was obtained by addition of equal volume of 10% TCA and the tissue homogenate and centrifuged at 7000 × g for 8 min. To this 1 ml of supernatant, 3 ml of 0.2 M phosphate buffer (pH 8) and 0.5 ml of 6 mM DTNB reagent were added and vortexes. The absorbance was measured at 412 nm against a blank containing TCA instead of supernatant within 15 min. The amount of glutathione is expressed as g GSH/g wet tissue.[14]

Measurement of glutathione peroxidase activity

The assay mixture consisted of 0.2 ml of brain homogenate (10%, w/v) and 0.2 ml of EDTA (0.8 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of 4 mM reduced glutathione (GSH), 0.1 ml H2O2 (30 mM) solution, 0.4 ml of 0.4 M phosphate buffer (pH 7.0). Incubated at 37◦C for 10 min, then kept the tubes at room temperature and to this add 0.5 ml of 10% trichloro acetic acid (TCA) and centrifuged at 3000 × g for 10 min, to this supernatant, added 0.1 ml of 0.04% DTNB solution. Read the optical density at 420 nm against blank.[15]

Measurement of Glutathione Reductase (GR) activity

The assay consisted of 1.65 ml of phosphate buffer (0.1 M, pH 7.6), 0.1 ml of NADPH (0.1 mM), 0.1 of ml EDTA (0.5 mM), 0.05 ml of oxidized glutathione (1 mM) and 0.1 ml of brain homogenate (10%, w/v) in a total volume of 2 ml. The enzyme activity was quantitated at room temperature by measuring the disappearance of NADPH at 340 nm and was calculated as M NADPH oxidized/min/mg protein.[16]

Measurement of Catalase activity

The assay mixture consisted of 0.1 ml of brain homogenate (10%, w/v) and added 1.9 ml of Phosphate buffer (0.05 M, pH 7). To this mixture, 1 ml of freshly prepared 30 mM H2O2 was added and changes in absorbance was measured at 240 nm for 3 min at an interval of 30 s. A control was prepared using 0.1 ml of distilled water devoid of 0.1 ml of homogenate. One unit of enzyme activity is defined as enzyme concentration required inhibiting the change in the absorbance by 50% in one min in the control sample. Activity of catalase was expressed as nM of H_2O_2 metabolized/min/mg protein.[17]

Histology

The prostate and testes was excised flushed with saline, fixed in 10% neutral buffered formalin for at least 24 h and after fixation, the specimens was dehydrated in ascending grades of ethanol, cleared in benzene, and embedded in paraffin wax. Blocks were made and 5 µm thick sections are cut from the prostate and testes. The paraffin embeds tissue sections was deparaffinised using

xylene and ethanol. The slides was washed with phosphate buffered saline and permeabilized with permeabilization solution (0.1 M citrate, 0.1% Triton X-100). These sections was stained with haematoxylin and eosin and observe under light microscope at × 40 magnifications to investigate the histo - architecture of prostate and testes of Wistar rats.[18]

RESULTS AND DISCUSSION

Our aim was to investigate the effects of reticulatacin against testosterone induced prostate cancer in rats. Testosterone is increasingly prescribed to middle aged and older men diagnosed with low circulating testosterone levels.[19] The potential of testosterone treatment to increase risk of prostate cancer, an androgen-dependent malignancy, has been raised repeatedly.[20] Probably in part because large scale testosterone therapy is a recent phenomenon and prostate cancer is a notoriously slow developing disease. Circulating testosterone levels were associated with later risk of prostate cancer in some studies.[21] Treatment of rats with sc implants containing testosterone induced a low incidence of prostate adenocarcinomas in a number of studies.[22] In the present investigation, preliminary phytochemical screening has been done in the various extracts of *Annona reticulata*. Bark showed the presence of phytochemical constituents namely the Pet ether extract gave +ve test for Sterols, Fats & Fixed oils and Chloroform extract gave +ve test for alkaloids, non-sugar moieties for glycosides, triterpenoids and tannins and Ethanolic extracts gave +ve test for flavanoids saponins, triterpenoid tannins. Isolation results of Reticulatacin: It was crystallized needles from *n*- hexane/chloroform (8:2 v/v) as white needles, Melting Point: 303 - 305ºC. It gave pale pink of colors to kedde reagent test. Hence, the compound was identified. Thin layer Chromatographic Studies TLC analysis of the fractions using solvent systems *n*; hexane / chloroform (9:1v/v), revealed the presence of promising spots as shown in TLC No. of spots -1, Rf values- 0.42. The graph represents the colorimetric study of Reticulatacin. It was observed in the range between 400-800 nm. IR cm^{-1}: 3404 (O - H Bending), 1026 (C - O - H stretching & bending), 1152 (C - O stretching), 1203 (C – C). ^{1}HNMR: (400 MHz, CDCl3), δ H 2.5 OH –Proton, δ H 3.1 – 3.9 multiplet CH2 Protons, δ H 5.5 CH Proton in (Fig. 1). Based upon the HPLC it can be finished that retention of sample and standard was initiate to be 6.06 and 6.00 respectively observed in (Fig.2, 3) by comparing the preservation factor and the reaction of the peak in the chromatogram of the standard by means of the illustration chromatogram. HPLC was an successful and quicker instrument for the inclusive or at least partial quantitative separation of individual samples. For qualitative separation and quantitation GC is exceedingly capable whereas for identification and structure elucidation GC-MS and NMR are important methods UV, IR and 1H NMR, 13C NMR, GCMS are explained in (Fig. 5, 6).[23] Circulating testosterone was elevated after 28 days of treatment. The mechanism by which testosterone acts as a tumor - promoting agent for prostate cancer in this rat model is not known, but it is possible that initiated prostate epithelial cells acquire hypersensitivity to androgens, which could provide them with a selective growth advantage resulting in a few of those cells progressing to malignancy. The ratio of large dorsolateral prostate region tumors to smaller tumors confined to the dorsolateral prostate did not change with increasing testosterone dose, suggesting that only tumor incidence was affected by increasing circulating testosterone but not tumor growth. However, dorsolateral prostate tumor multiplicity was also not affected by testosterone dose. Androgen receptor mediation is mechanistically involved because chronic treatment with the androgen receptor blocker flutamide essentially eliminated the promotional activity of testosterone in this model reflect on the size of prostate size and decreased in prostate size in treatment group (Fig.7). Free radicals and Reactive Oxygen Species generated by testosterone may play a key role in the instigation of membrane Lipid Peroxidation. Lipid Peroxidation is a marker of oxidative stress and several studies have reported that remarkable elevation in the level of MDA (Fig.9).[24] Our results also corroborated with the above mentioned findings, which showed that there is increase in the MDA level in rats treated with reticulatacin. Reduced glutathione (GSH) is a low molecular weight tripeptide cellular antioxidant, which protect against the peroxidation of lipid membrane by conjugating with the electrophile such as 4-Hydroxy-3-nonenal, produced during LPO and thus, GSH gets depleted in this conjugation reaction.[25] In this study, it was observed that the level of GSH is depleted in Reticulatacin treated group as compared to control group (Fig.11). According to present findings, Reticulatacin significantly attenuated the activities of glutathione dependent enzymes i.e. GPx and GR against testosterone induced in the prostate of Wistar rats. It also attenuated the activity of Catalase, (Fig.10) an antioxidant enzyme responsible for the break-down of hydrogen peroxide into water and oxygen.[26] Section studied shows normal prostatic tissue. The tissue bits are fragmented. Normal bilayered epithelial cells can be seen. Corpora amylacia are seen. Glands that which shown ingrowth of epithelium and epithelial intufting seen in (Fig. 14).

Table 1: Acute toxicity studies.

Cage no	No of mice	Weight of mice	1000 mg/kg dose		Route	Monitored parameters
			mg	ml		
1	1	22.22	22.22	0.22	oral	Sedation, Convulsions, Motor activity, Mortality.
2	1	22.00	22.00	0.22	oral	
3	1	22.00	22.00	0.22	oral	
4	1	22.50	22.58	0.22	oral	
5	1	22.00	22.00	0.22	oral	

Table 2: Acute toxicity studies limit test studies.

Cage no	No of mice	Weight of mice	1000 mg/kg dose		Route	Sedation After		Convulsions After		Motor activity After		Mortality After	
			mg	ml		30 min	1 hr	30 min	1 hr	30 min	1 hr	48 hr	72 hr
1	1	22.22	22.22	0.22	oral	No	No	No	No	No	No	0	0
2	1	22.00	22.00	0.22	oral	No	No	No	No	No	No	0	0
3	1	22.00	22.00	0.22	oral	No	No	No	No	No	No	0	0
4	1	22.50	22.58	0.22	oral	No	No	No	No	No	No	0	0
5	1	22.00	22.00	0.22	oral	No	No	No	No	No	No	0	0

Table 3: Effect of Reticulatacin on biochemical parameters of prostate tissue.

Treatment Groups	Dose mg/kg,p.o.	Reduced glutathione (Units/mg protein)	CAT (µmoles of H_2O_2 metabolized/mg protein/min)	MDA (nmol/gm wet tissue)	Glutathione reductase (nM/g)
1	Normal saline	0.0816 ± 0.019	0.158 ± 0.001	4.164 ± 1.667	0.559 ± 0.76
2	Testosterone 3mgkg	1.33 ± 0.200	0.0059 ± 0.001	50.39 ± 2.023	0.579 ± 0.199
3	Finasteride 5mg/kg	1.292 ± 0.054	0.0077 ± 0.004 ^{ns}	50.76 ± 1.874 ^{ns}	0.382 ± 0.02
4	Reticulatacin 50mg/kg	0.4604 ± 0.050 **	0.001 ± 0.001**	30.28 ± 1.603***	0.488 ± 0.056**
5	Reticulatacin 100mg/kg	0.3165 ± 0.101 ***	0.012 ± 0.002 ***	14.91 ± 2.090***	0.608 ± 0.20 ***

The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test.

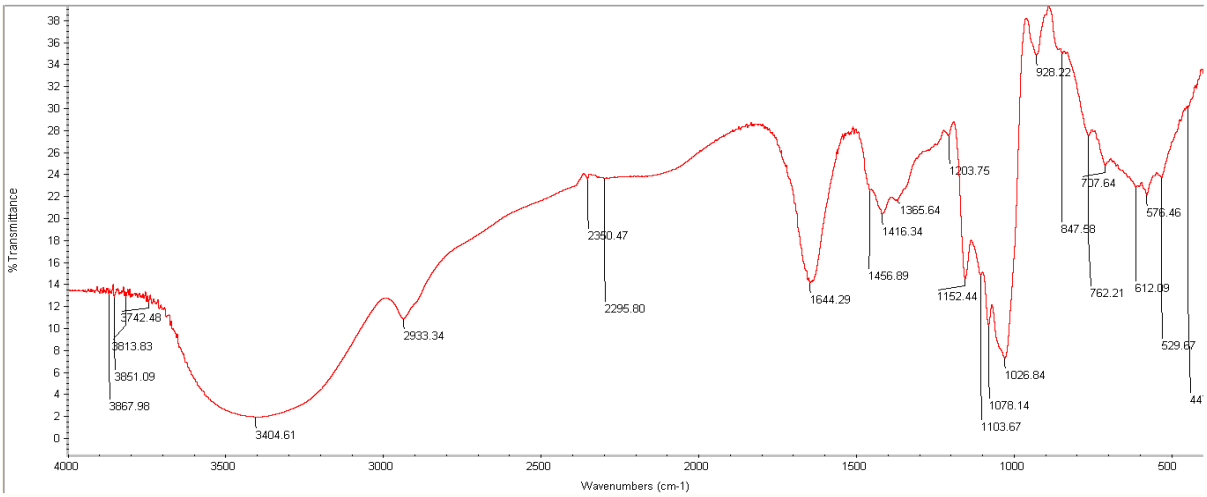


Fig. 1: Fourier transforms infrared spectroscopy (FTIR) of Sample.

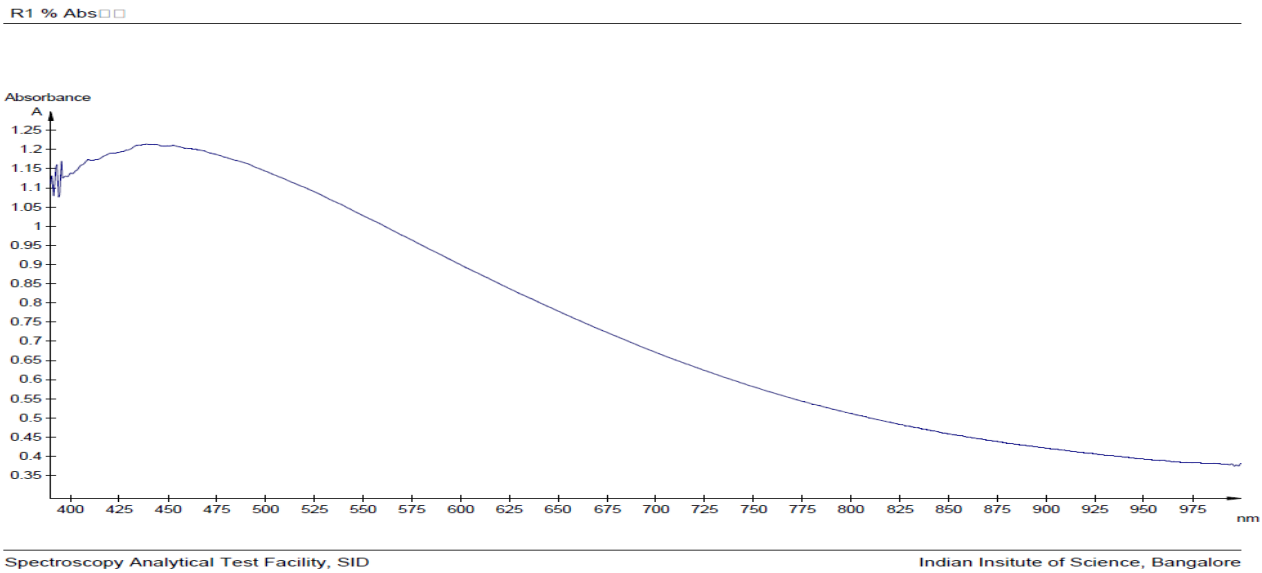


Fig. 2: UV spectrum of Reticulatacin.

This graph represents the colorimetric study of Reticulatacin. It was observed in the range between 400-800 nm.

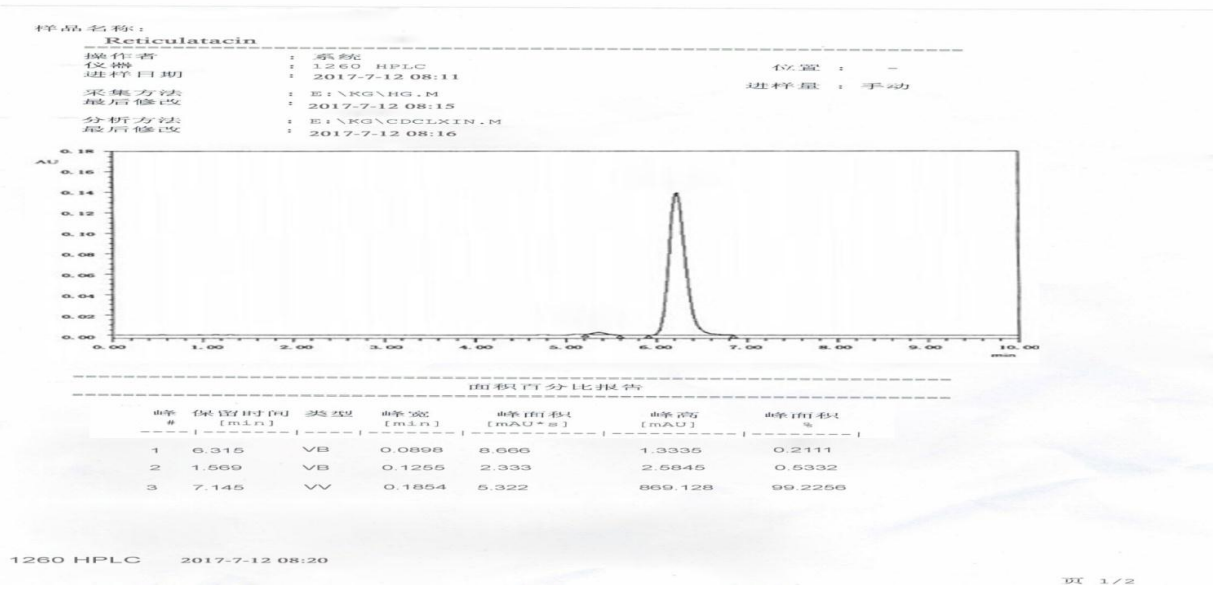
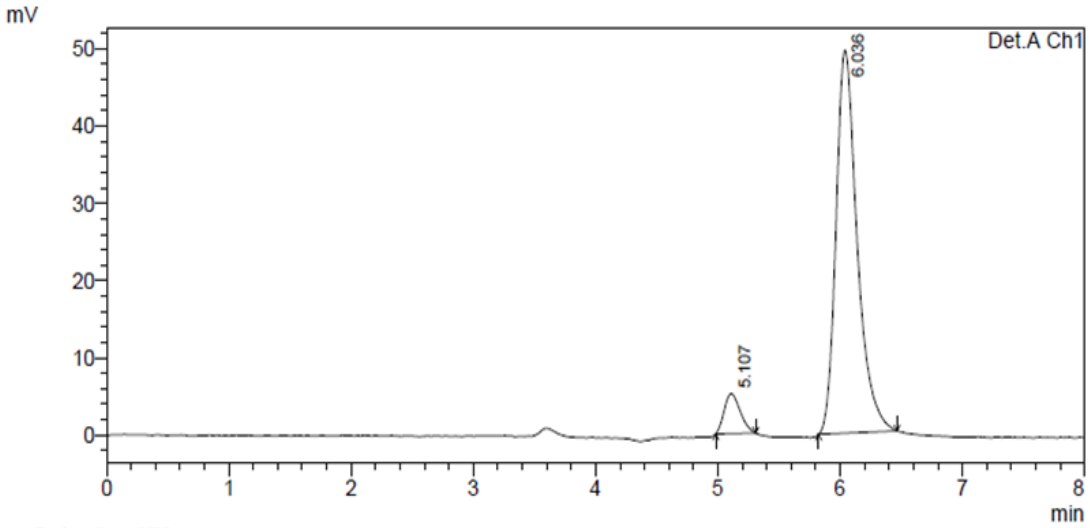


Fig. 3: HPLC chromatograms of reticulatacin Standard.



PeakTable

Detector A Ch1 275nm

Peak#	Name	Area	Ret. Time	HETP	ling Factor (1(	Resolution	k'
1		47048	5.107	23.314	1.261	0.000	0.000
2		597500	6.036	25.502	1.336	3.264	0.182
Total		644548					

Fig. 4: HPLC chromatograms of reticulatacin sample.

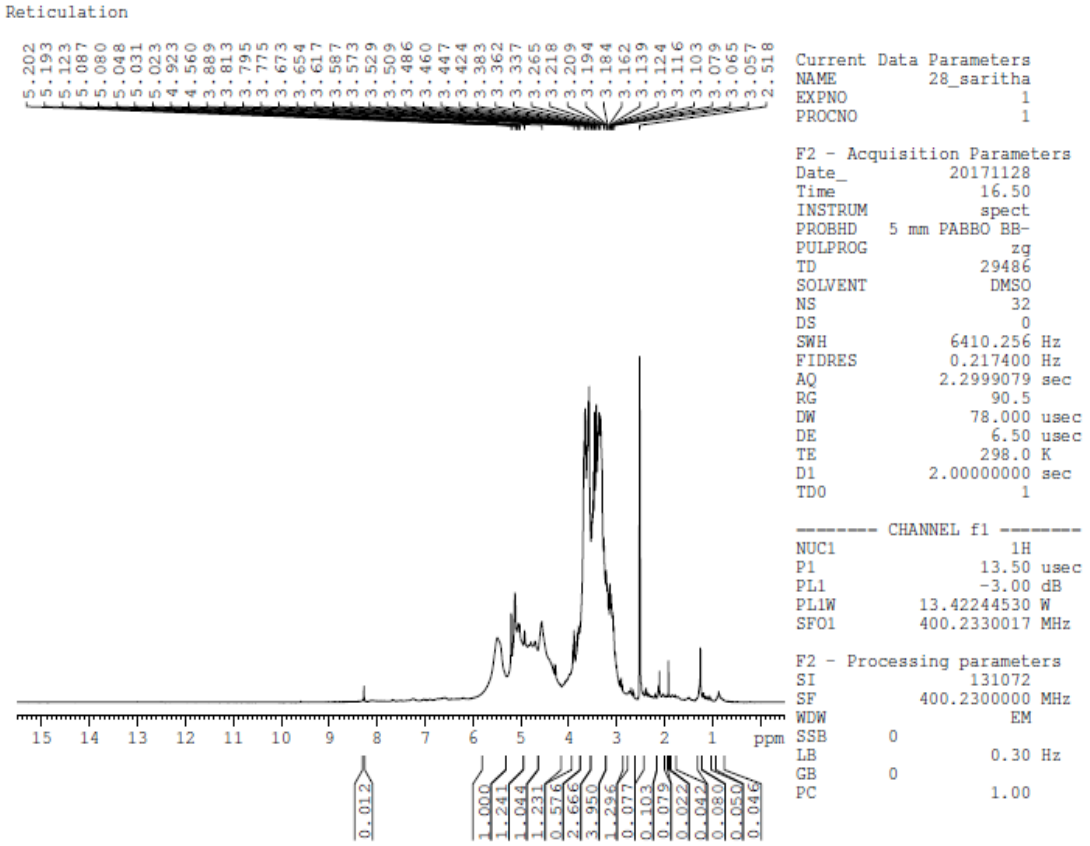


Fig. 5: 1HNMR of Reticulatacin.

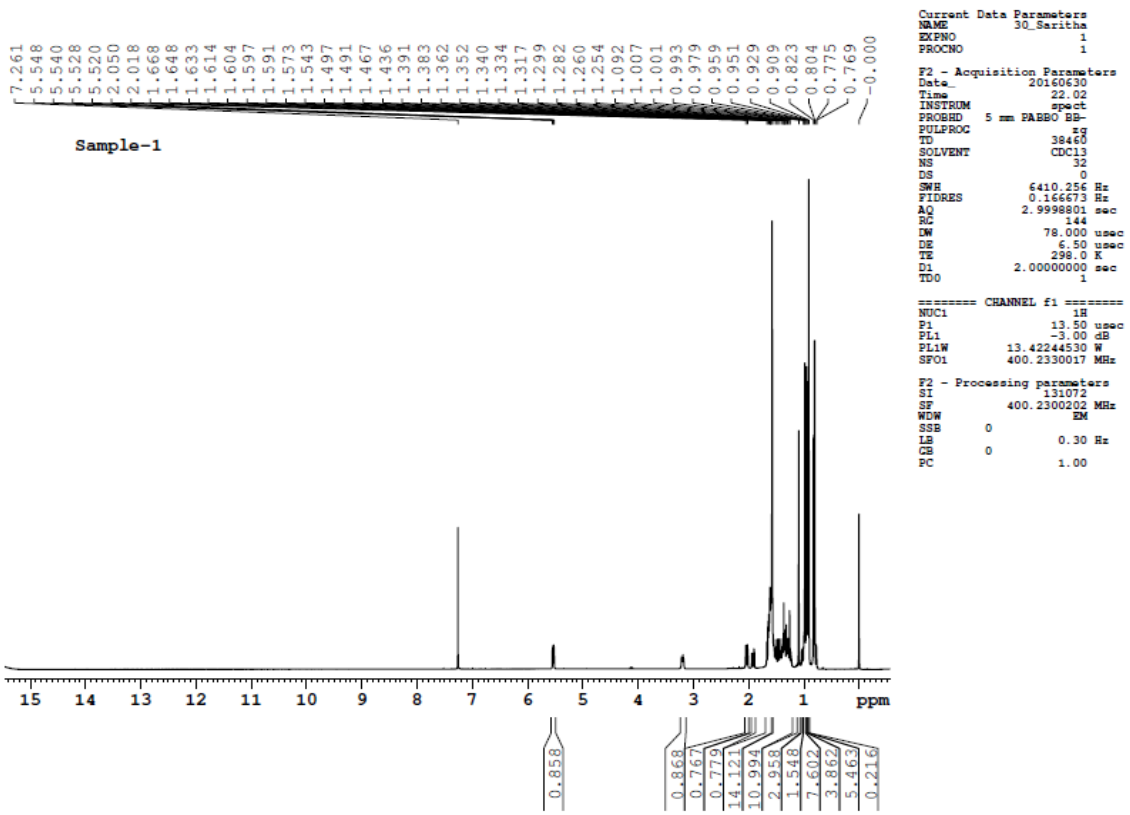


Fig. 6: ^{13}C NMR of Reticulatacin.

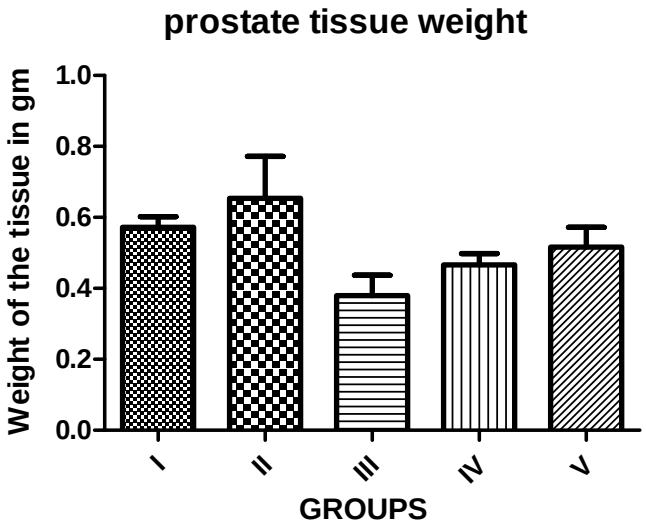


Fig. 7: Effects of Reticulatacin on prostate enlargement. Testosterone treatment increased the mean prostate weight which was decreased by supplementation of Reticulatacin (untreated control) [I], testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

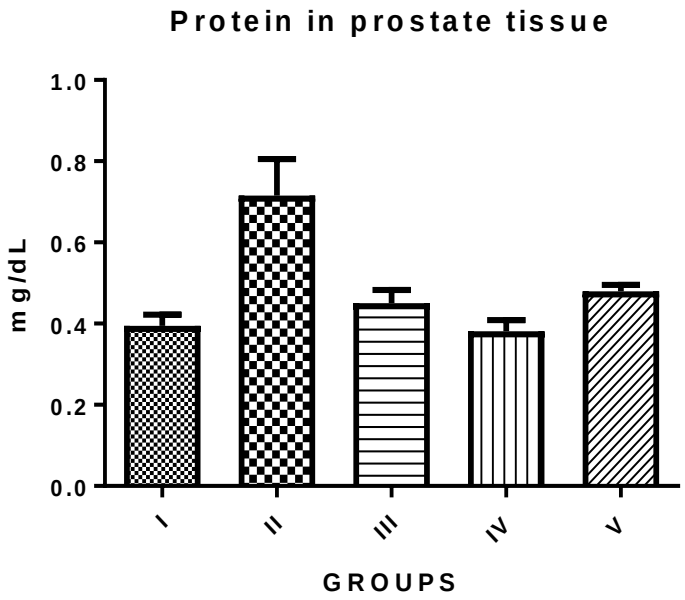


Fig. 8: Effects of Reticulatacin on Protein in prostate tissue. Testosterone treatment increased the mean prostate weight which was decreased by supplementation of Reticulatacin (untreated control) [I], testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

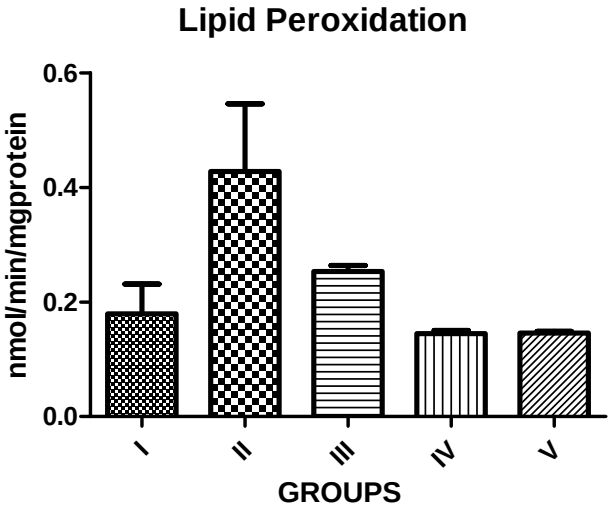


Fig. 9: Biochemical analysis of testosterone induced increase of lipid peroxidation (untreated control) [I], testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

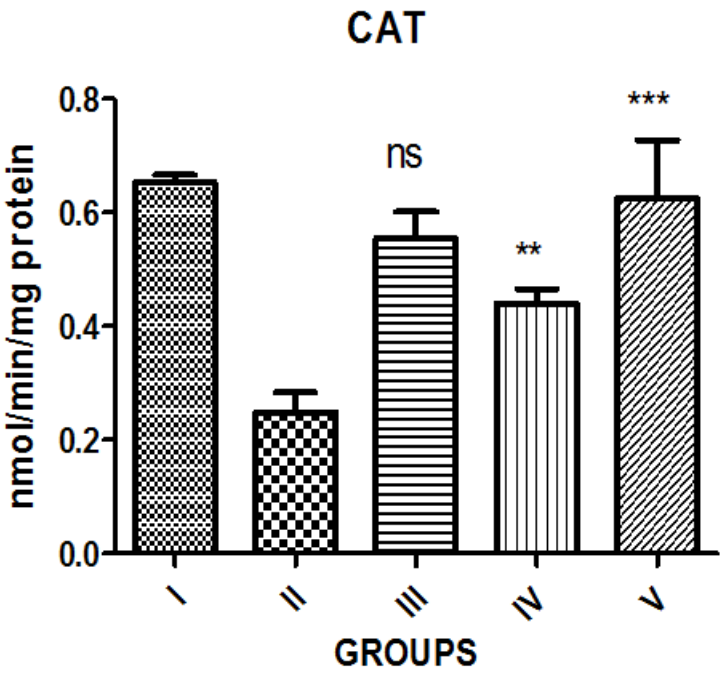


Fig. 10: Biochemical analysis of testosterone induced increase of Catalase, activity in prostate tissue (untreated control) [I], Testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

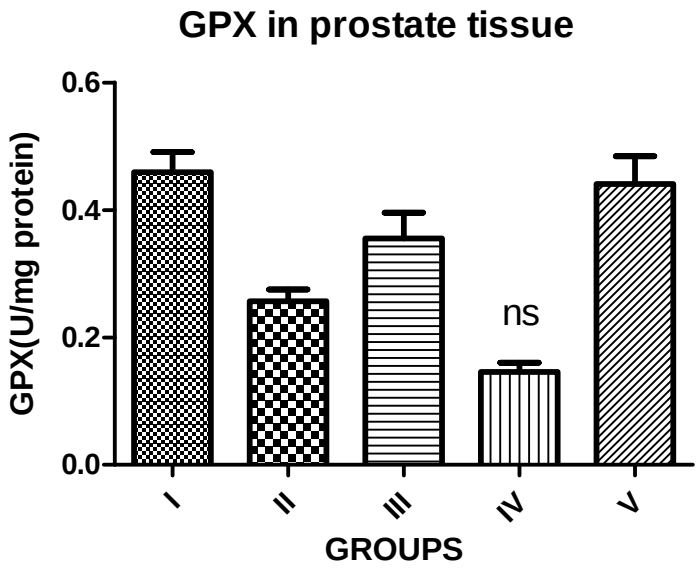


Fig. 11: Biochemical analysis of testosterone induced increase of GPX activity in prostate tissue (untreated control) [I], Testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

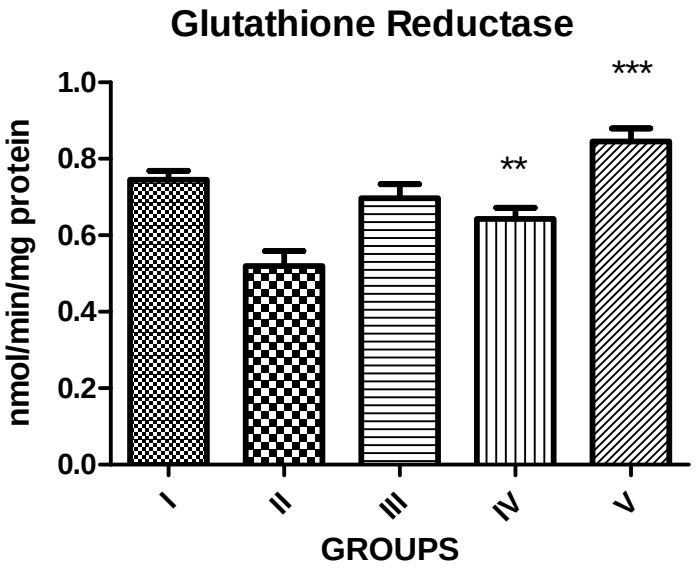


Fig. 12: Biochemical analysis of testosterone induced increase of GSH activity in prostate tissue (untreated control) [I], Testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

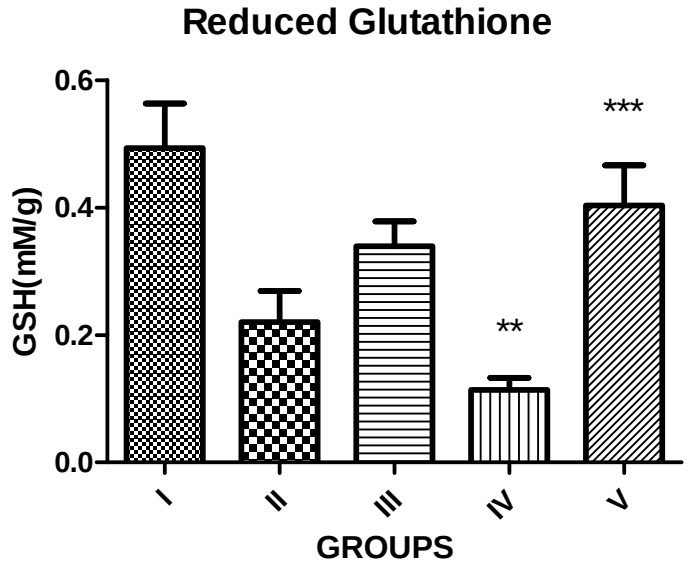


Fig. 13: Biochemical analysis of testosterone induced increase of GR activity in prostate tissue (untreated control) [I], Testosterone alone [II], Finasteride [III], Reticulatacin 50 mg/kg [IV]) Reticulatacin 100 mg/kg [V]. The values are expressed as Mean ± SD. Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. * *P<0.05*, ** *P<0.01* and * *P<0.001* as compared to control group.**

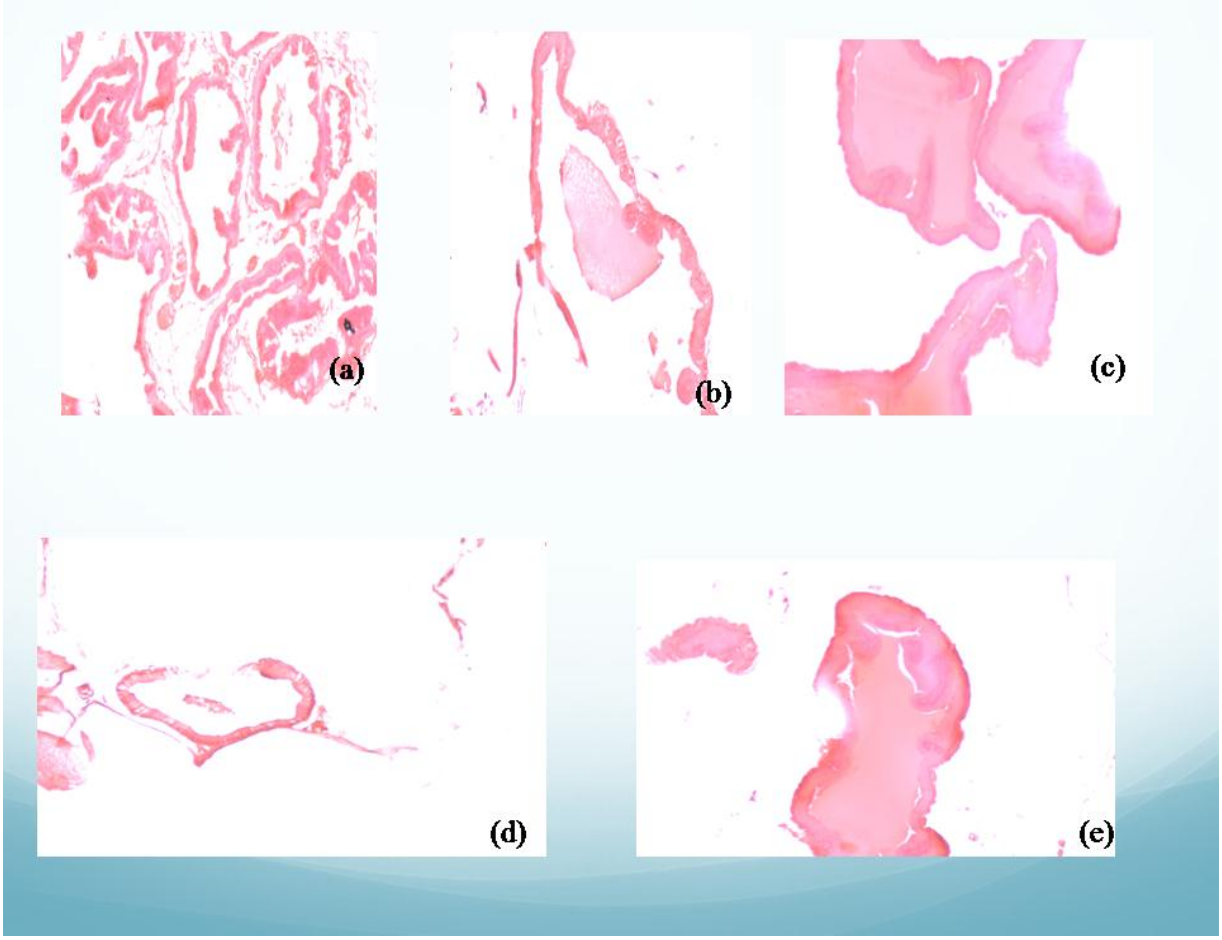


Fig. 14: Effect of Reticulatacin on histological slide (a) represents Testosterone 3mg/kg Group I showed decreased number of scalloping of the prostate secretions within the lumen. Slide (c) represents Reticulatacin 50 mg/kg treated Group II stromal shows mild inflammatory infiltration. Slide (d) represents only Reticulatacin100 mg/kg Group III showed papillary projections.

CONCLUSION

The results recommended that management with reticulatacin may progress symptoms of disease and inhibit the increased prostate size. Biochemical parameters of *In vivo* studies reported that isolated compound has produce beneficial effect on prostate cancer, which would relieve the urinary symptoms of disease. Reticulatacin could be a potential source of new treatment of prostatic hyperplasia.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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