International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 3, March 2018

ISSN: 2394-4404

Kidney And Liver Function Indices Of Prosopis Africana Seed Extract On Testosterone And Estradiol Induced Benign Prostatic Hyperplasia In Adult Male Rats

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Abstract: ***Benign prostate hyperplasia (BPH) is present in 50% of men more than 50 years, 95% in men of more than 70 years and in 5-10% of cases, it leads to obstruction of the urethra which makes life unbearable for the patient. We investigated the effect of Nigerian indigenous plant, Prosopis africana (PA) on BPH. BPH was induced in male rats weighing 250-350g through exogenous administration of testosterone and estradiol by subcutaneous injection. A total of 30 rats were divided into five groups. One group was used as a control and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH. Groups 1 and 2 were treated with different doses of PA extracts and group 3 received finasteride, all by gavages for forty-five days, while group 4 was left untreated, group 5 served as normal control. After forty-five days of treatment with PA extract, the rats were anaesthetised by short contact with trichloromethane vapour. Blood was collected by cardiac puncture and the sera cautiously centrifuged and used for the determination of different biochemical indices. The level of urea and creatinine were significantly (P<0.05) reduced when compared to the BPH control. No significant differences in serum concentration of AST, ALT, ALP, and GGT were recorded in all treatment groups relative to BPH control. Conclusively, the extract of Prosopis africana seed has nephroprotective effect on kidney and no toxic effect on the liver. It seems that benign prostate hyperplasia do not have any damaging effect on the liver.***

Keywords: Urea, creatinine, AST, ALT, ALP, and GGT

I. INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common condition in elderly men, with an estimated prevalence of up to 85% (Lee, 2008). According to autopsy studies, approximately 80% of elderly men develop microscopic evidence of the disease (Parsons and Kashefi, 2008). About half of the patient with microscopic changes develop an enlarged prostate gland and as a result have a difficulty emptying the content of the urinary bladder (Platz *et al.*, 2002).

When benign prostatic hyperplasia is found in its earlier stages, there is a lower risk of developing such complications. Local conversion of testosterone to its metabolite dihydrotestosterone (DHT), catalyzed by the enzyme steroid 5-alpha-reductase (5-AR), is implicated as a causative factor in BPH. DHT exerts its effects by binding to androgen receptors in the nucleus of prostate cells, stimulating cellular growth and division (McConnell *et al.*, 1994). It is interesting to note that DHT binds many times stronger than testosterone to these androgen receptors (Metcalf *et al.*, 1989) and thus it is more likely that DHT is the preferred substrate for these receptors.

Many people suffering from some diseases prefer herbal medicines instead of orthodox drugs and a significant number of such individuals attest to the effectiveness of such herbal remedies. The use of complementary and alternative medicine (CAM) for the treatment of BPH is becoming popular. It is estimated that 30% of men diagnosed with prostate disease in North America use some CAM products (Nickel *et al.*, 2008), while such products constitute approximately 50% of all medicines prescribed for BPH in Italy (Di-Silverio *et al.*, 1993) and almost 60% of such prescriptions in Germany and Austria (Buck, 1996). This research was designed to study the efficacy of *Prosopis africana* (PA), a Nigerian indigenous plant in the management of BPH/prostate diseases.

II. MATERIALS AND METHODS

PLANT MATERIAL

Prosopis seeds were purchased from Ishibori market in Ogoja Local Government of Cross river State, Nigeria. The seeds (500g) was sorted, cleaned and boiled for 5h using a gas cooker and allowed to cool to room temperature. The boiling helps to soften the hulls for easy removal and separation of the cotyledons. After it was dehulled and decorticated, the dehulled and boiled seeds were washed again with clean water. The processing of decorticated seeds was done by hand squeezing the seeds and washing with clean water. The wet decorticated seeds were kept in a large polythene sacks to exclude air and was fermented for three days according to the method described by (Achi, 1992). The fermentation was done at room temperature for 72h. The fermented seeds were then sun-dried to a constant weight and milled using hammer mill to produce *Prosopis* seed flour (Yusuf *et al.*, 2008). The flour was kept in a refrigerator at 4^{o} C prior to use.

HORMONES

Testosterone propionate Brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China. Estradiol valerate (by Medipharm Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E 2 (puregynon depot) were used for the induction of prostate enlargement at a dose of 400µg T and 80µg E2 (Bernoulli, 2008). This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for gross examination of prostate enlargement.

ANIMALS

A total of thirty (30) Wistar rats weighing between 250-350g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The rats were used for the experiment. The rats were acclimatized for two weeks before the experiment commenced. The rats were exposed to approximately 12-hour light/dark cycles under humid tropical conditions, given tap water and feed *ad libitum*, and were housed in standard plastic cages (six per cage) throughout the 45-day duration of the study. The animal room was well be ventilated with a temperature range of 27 ± 2^{0}C.

INDUCTION OF BPH

BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks according to (Bernoulli, 2008) with modification by (Mbaka *et al.*, 2013).

EXPERIMENTAL DESIGN

The animals were divided into five (5) groups each comprised of six (6) male rats. Four groups were induced with BPH which were grouped as group 1 to group 4). Groups 1 and 2 received 50 and 100mg kg^{-1} body weight (bw) of *Prosopis africana* extract; group 3 received finasteride (orthodox drug) at 0.1mg kg^{-1}; all by gavages for forty five days, group 4 was left untreated for forty five days before sacrifice to assess possible reversal of the exogenous induction and group 5 served as normal control. The animals were weighed prior to the commencement of the experiment and subsequently every week till the end of the experiment. The fluid and water intake was taken daily till the end of the experiment.

- ✓ *DETERMINATIONS OF BIOCHEMICAL PARAMETERS*

After 45 days, the rats were anaesthetized by a brief exposure to trichloromethane vapour and bled by cardiac puncture. The sera were carefully separated and used for the determination of various biochemical analyses.

ASSAY OF SERUM ASPARTATE AMINOTRANSFERASE (AST) ACTIVITY

The assay of the blood serum aspartate aminotransferase (AST) activity was assayed by examining the level of oxaloacetate hydrazone produced with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957).

ASSAY OF SERUM ALANINE AMINOTRANSFERASE (ALT) ACTIVITY

Serum Alanine aminotransferase (ALT) activity was analyzed by checking the level of pyruvate hydrazone produced with 2, 4-dinitrophenylhydraine (Reitman and Frankel, 1957).

ASSAY OF SERUM ALKALINE PHOSPHATASE (ALP) ACTIVITY

The activity of ALP was assayed by using the kinetic colorimetric technique of optimized Deutsche Gesellschaft for Klinische Chemie (DGKC) by German Society of Clinical Chemists/Dewtsche Gessellschaft fu klinische chemie (1972).

ISSN: 2394-4404

ASSAY OF GAMMA GLUTAMYL TRANSFERASE (Γ-GT) ACTIVITY

The serum activity of this enzyme was assayed by using the kinetic colorimetric method as described (Persijin and Van der-Silk, 1976).

DETERMINATION OF UREA CONCENTRATION

Serum urea concentration was estimated using the Agape assay kit procedure as explained by Tobacco, (1979).

DETERMINATION OF SERUM CREATININE CONCENTRATION

Serum creatinine concentration was estimated using the Agape diagnostic kit procedure as described by Allen, (1982).

STATISTICAL ANALYSIS

The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and the probability tested at 95 percent level of confidence (ie $P < 0.05$).

III. RESULTS

EFFECT OF EXTRACT ON KIDNEY FUNCTION TEST IN BPH-INDUCED RATS

Results of the serum urea and creatinine assessment for the treated and controlled groups are shown in Table 1.

EFFECT OF EXTRACT ON SERUM UREA CONCENTRATION OF BPH-INDUCED RATS

Serum urea concentrations in (mg/dl) were 26.41±2.81 for BPH control, 17.69±1.07 for normal control, 21.28 ± 1.72 for 50mg PA, 21.79 ± 2.03 for 100mg PA OG and 18.97±1.07 for finasteride. There was a significant (P<0.05) increase in level of serum urea level in BPH control group when compared with normal control. The value of the dose of finasteride was similar to the normal control. The results revealed that all the treated groups exhibited reduction in the level of urea concentration.

EFFECT OF EXTRACT ON SERUM CREATININE CONCENTRATION OF BPH-INDUCED RATS

Serum creatinine concentrations (in mg/dl) were 1.96±0.33 for BPH control, 0.67±0.35 for normal control, 1.03±0.19 for 50mg PA, 1.03±0.08 for 100mg PA and 0.83±0.15 for finasteride. There was a significant (P<0.05) increase in level of serum creatinine level in BPH control group when compared with the normal control. All the values of treated groups were similar to the normal control. The results revealed that all the treated groups exhibited reduction in the level of creatinine concentration.

EFFECT OF BPH ON SERUM ENZYME ACTIVITIES

Table 2 showed no significant different in the activities of serum hepatic toxic indicator enzymes, ALT, AST, ALP and γ-GT, indicating normal liver function.

EFFECT OF EXTRACT ON ALANINE AMINOTRANSFERASE (ALT) ACTIVITY OF BPH-INDUCED RATS

Serum ALT concentrations in U/L were26.56±1.50 for BPH control, 24.20±5.18 for mormal control, 26.09±0.89 for 50mg PA, 26.15±1.13 for 100mg PA and 26.07±1.14 for finasteride. All the values were similar to the normal control showing no damage to the liver.

EFFECT OF EXTRACT ON ASPARTATE AMINOTRANSFERASE (AST) ACTIVITY OF BPH-INDUCED RATS

Serum AST concentrations in U/L were 36.82±1.27 for BPH control, 36.01±0.99 for normal control, 7.02±0.94 for 50mg PA, 36.70±0.90 for 100mg PA and 35.55±3.18 finasteride. All the values were similar to the normal control showing no damage to the liver.

EFFECT OF EXTRACT ON ALKALINE PHOSPHATASE (ALP) ACTIVITY OF BPH-INDUCED RATS

Serum ALP concentrations in U/L were244.58±2.40 for BPH control, 244.12±2.97 for normal control, 244.26±0.76 for 50mg PA, 244.41±2.05 for 100mg PA and 244.14±2.62 for finasteride. All the values were similar to the normal control showing no damage to the liver.

EFFECT OF EXTRACT ON GAMMA GLUTAMYL TRANSFERASE (Γ-GT) ACTIVITY OF BPH-INDUCED RATS

Serum γ-GT activity in U/L were21.15±0.60 for BPH control, 21.15±0.97 for normal control, 20.81±0. 76 for 50mg PA, 20.77±1.42 for 100mg PA and 21.17±1.71 for finasteride. All the values were similar to the normal control showing no damage to the liver.

Group	Urea (mg/dl)	Creatinine (mg/dl)
BPH + 50mg PA	21.28 ± 1.72^{cd}	1.03 ± 0.19^{b}
BPH + 100mg PA	21.79 ± 2.03^{d}	1.03 ± 0.08^{b}
BPH + Finasteride	18.97 ± 1.07^{ab}	0.83 ± 0.15^{ab}
BPH Control	26.41 ± 2.81^{e}	1.96 ± 0.33^{c}
Normal Control	17.69 ± 1.07^{a}	0.67 ± 0.35^{a}

Values are expressed as mean ± SD (n = 6) BPH (Benign prostate hyperplasia), PA (*Prosopis africana*). [a, b, c, d, e] Values with different superscripts are statistically different at P<0.05.

Table 1: Effect of PA extract and finasteride on kidney function parameters

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
BPH + 50mg PA	26.09 ± 0.89^{a}	37.02 ± 0.94^{a}	244.26 ± 0.76^{a}	20.81 ± 0.76^{a}
BPH + 100mg PA	26.15 ± 1.13^{a}	36.70 ± 0.90^{a}	244.41 ± 2.05^{a}	20.77 ± 1.42^{a}
BPH + Finasteride	26.07 ± 1.14^{a}	35.55 ± 3.18^{a}	244.14 ± 2.62^{a}	21.17 ± 1.71^{a}

BPH Control	26.56±1.50[a]	36.82±1.27[a]	244.58±2.40[a]	21.15±0.60[a]
Normal Control	24.20±5.18[a]	36.01±0.99[a]	244.12±2.97[a]	21.15±0.97[a]

Values are expressed as mean ± SD (n = 6). BPH (Benign prostate hyperplasia), PA (Prosopis africana), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT), [a] Values with same superscript are not statistically different at P>0.05.

Table 2: Effect of PA extract and finasteride on serum liver enzyme activities

IV. DISCUSSION

Certain tissue cells contain some characteristic enzymes, which enter the blood, only when the cells in which they are contained are damaged. The clinical significance of these enzymes lies in their diagnosis of diseases such as myocardial infection, hepatitis and jaundice (Saunder, 2007). Serum alanine amino transferase (ALT) is known to increase when there is liver disease and it has been used as a tool for measuring hepatic necrosis (Yakubu *et al.*, 2003). Aspartate amino transaminase (AST) is predominantly localized within the cells of kidney, muscle, heart and liver parenchamal cells. An increase in serum AST might connote acute liver damage or liver cytolysis (Nelson and Cox, 2008). Alkaline phosphate a marker enzyme for the plasma enzyme and endoplasmic reticulum is frequently used to access the integrity of the plasma membranes such that any alteration in the activity of the enzymes in the tissue and serum would indicate likely damage to the external boundary of the cells (plasma membrane) (Yakubu *et al.*, 2008).

The Gamma–glutamyl transferase is a membrane bound enzyme that transfer the γ-glutamyl moiety to a number of acceptor substrate (L–amino acid, peptides, glutathione). The enzyme is most abundant in the liver of many animal species, however it is also found to be present in other tissues such as kidney and human seminal fluid (Tate and Meister, 1975). The enzymes get leaked out to the serum during tissue damage and hence a kinetic property of the enzyme showed a high activity during liver disease (Tanaka, 1974). The γ-Glutanyl transferase had been successful used to study the disease state of the liver because the activity occur earlier and persist longer than other enzymes, and hence a simple, sensitive and direct means of detecting the final state of the liver.

The extract did not affect the activities of the serum toxicity marker enzymes, ALT, AST, ALP and GGT indicating normal liver function. This implies that benign prostatic hyperplasia might not have adverse effect on the liver function and that the extract had no toxicity effect on this organ (Iwalokun *et al.*, 2006). Earlier studies showed that oral administration of the aqueous extract of some plant could accelerate the reversion of liver damage through reduction of liver marker enzymes, including aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, lactate dehydrogenase and bilirubin indices in liver biochemical tests (Arhoghro *et al.*, 2009). Therefore it might be logical to suggest the extract has hepatoprotective effect.

The functional capacity or integrity of the kidney can be accessed using biomarkers like urea, uric acid and creatinine (Yakubu *et al.*, 2003).The concentrations of urea and creatinine which are biomarkers of kidney function were estimated in this study. It was noted that there was increase in the levels of urea and creatinine in the BPH induced animals when compared to the normal animals which is a sign of kidney damage. The administration of the extract was able to reduce the levels of urea and creatinine in the treated groups. Previous report by (Hardik *et al.*, 2014) showed a similar progression. Urea is the major nitrogen-containing material product of protein catabolism. The significant reduction ($P< 0.05$) in the serum urea concentration following the administration of the extract at various doses may be due to the repair mechanism in the kidney (Yakubu *et al.*, 2003).

Creatinine concentration is considered a significant maker of renal dysfunction (Ugwu *et al.*, 2013). The constancy of endogenous creatinine production and its release into the body fluids at a constant rate, and constancy of plasma levels of creatinine over 24 hours of a day, makes creatinine a useful endogenous substance where clearance may be measured as an indication of glomerular filtration rate (Dasofunjo *et al.*, 2013). Increase in creatinine content of the serum BPH control suggests glomerular and tubular dysfunction, nephropathy or injuries which were corrected in the extract and drug treated groups. Therefore it might be logical to suggest the extract has nephroprotective effect.

V. CONCLUSION

The extract of *Prosopis africana* seed has nephroprotective effect on kidney and no toxic effect on the liver. It seems that benign prostate hyperplasia do not have any damaging effect on the liver.

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