Research Article

Testicular and prostatic function parameters in Wistar rats fed diets incorporated with 10% *Vernonia amygdalina* and *Vernonia colorata* leaves.

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**ABSTRACT:** The study is aimed at investigating the effect of dietary incorporation at 10% of *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) on testicular and prostatic functions parameters in wistar rats. Fifteen male albino rats aged 16 weeks old were placed in three groups of five animals each. Group 1 and 2 was fed 10% dietary incorporation of *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) respectively. Group 3 served as the control and was fed normal rat chow. During three weeks of feeding, daily body weight was recorded. Serum testosterone, Prostate Specific Antigen (PSA) and Prostatic acid phosphatase (PACP) concentrations were determined using standard procedures. Serum testosterone significantly decreased \((p<0.05)\) in the (VA and VC) groups compared to control. Prostate specific antigen (PSA) concentrations significantly \((p<0.05)\) decreased in the group fed diets incorporated with VA and increased significantly in the groups fed diets incorporated with VC compared to the control group. Prostatic acid phosphatase (PACP) concentrations significantly \((p<0.05)\) decreased in the VA group and significantly \((p<0.05)\) increased in the experimental animals fed diet incorporated with *Vernonia colorata* (VC) compared to the control group. The testicular weight significantly \((p<0.05)\) decreased in the test groups VA and VC relative to the control, while prostatic weight significantly \((p<0.05)\) increased in VA and VC relative to control. These results suggest that *Vernonia amygdalina* and *Vernonia colorata* may have beneficial properties in the management of prostatic conditions.

**KEYWORDS:** *Vernonia amygdalina*, *Vernonia colorata*, Albino rats, Prostate specific antigen, Testosterone, Prostatic acid phosphatase
INTRODUCTION

Plants and vegetables used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discovery (Russo et al., 2013). The use of complementary and alternative medicine for the management of benign prostatic hyperplasia (BPH) and other prostatic diseases is becoming common (Ejike and Ezeanyika, 2011a) especially among rural dwellers in Nigeria. Ejike and Ezeanyika, (2011a) reported the incorporation of 15% Teffaria occidentalis seeds into diets as a food therapy in the management of BPH. Polyherbal therapy has also been reported to decrease prostate weight, prostate index, and serum dihydrotestosterone (DHT) levels (Sun et al., 2008).

Benign prostatic hyperplasia (BPH) is an ailment that has a high incidence among elderly males (Bostwick, 2002; Roehrborn and McConnell, 2002). As a man matures, the prostate undergoes two main periods of growth, the first at early puberty when the prostate is double its original size, this is the primary growth phase which is normal and the other is around 45 years, when the gland begins to grow in a secondary growth phase it is then called benign prostatic hyperplasia (BPH) (Roehrborn et al., 2007). About 1000 species of Vernonia species have been identified including VA and VC which belongs to the family of Asteraceae and genus Vernonia (Ijeh and Ejike, 2011). Some of these species are known as iron weed in American region but in Africa, the West Coast in particular, it is known as “bitter leaf” where it both grows wild and as a domestic plant (Farombi, 2003). Research evidence has demonstrated the efficacy of Vernonia species in treating and ameliorating various diseases. These leafy vegetables VA and VC have a lot of medicinal chemoprotective and chemopreventive properties. These bioactive agents either inhibit, reverse or retard inflammatory processes (Farombi and Owoeye, 2011), Vernonia amygdalina Del have been reported to possess the various phytochemicals such as terpenes, steroids, xanthones, edotides, saponins and alkaloids, coumarins, ethanquinones, sesquiterpenes, flavonoids, phenolic acids and lignans (Farombi and Owoeye, 2011). Vernonia amygdalina has been reported to have antiplasmodial (Iwalokun, 2008; Abosi and Raseroka, 2003), antibacterial (Akinpelu, 1999; Jisaka et al., 1993b), anticancer (Yedjou et al., 2008; Gresham et al., 2008; Izembigie, 2003; Izsembi et al., 2004), antioxidant (Iwalokun et al., 2006; Nwanjo and Nwokoro, 2004; Adesanoye and Farombi, 2010) hypoglycemic (Atangwho et al., 2007; Nwanjo and Nwokoro, 2004) activities. Many studies have been conducted to determine the effects of Vernonia amygdalina on the activity of cancer cells in particular human breast cancer and prostate cancer. Vernodalineol, a sesquiterpene lactone isolated from the leaves of V.amygdalina effectively inhibited cancerous breast cells at a dose of 25-50 µg/ml (Luo et al., 2011). Opata and Izevbigie (2006) reported that water soluble extracts of Vernonia amygdalina at low concentrations inhibited human breast cancerous cells (MCF-7) cells.

Vernonia colorata commonly called “sweet bitter leaf” in Nigeria and “Country bitter leaf” in Cameroon because it is relatively less bitter compared to Vernonia amygdalina and therefore requires less processing methods. In Nigeria, the Edo call it “Oriwo”, Hausas- “Shiwarar daji” Igbos -“Onugbu anara” Yorubas -“ewuro oko”. Ethnomedicinal use of Vernonia colorata in the treatment of benign prostatic hypertrophy has been documented (Debat et al., 1966). Traditional herbal medicine across many African countries have used Vernonia colorata for the treatment of bacteria, fungal, parasitic and inflammatory disorders, syphilis, pneumonia, measles, dysentery and several skin infections in traditional medical practice (Oseni et al., 2012; Cioffi et al., 2004). This study therefore, aims at investigating the effect of dietary incorporation of Vernonia amygdalina and Vernonia colorata on prostatic and testicular functions in normal rats.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of Vernonia colorata and Vernonia amygdalina were harvested from farms around Michael Okpara University of Agriculture, Umudike and the National Root Crops Research Institute, Umudike premises both in Abia State, Nigeria. Botanical identification and authentication were done at the Herbarium unit of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were sorted, washed, rinsed with distilled water and air-dried to a constant weight and then milled to powdered form using the Thomas Whitley milling machine. The powdered samples were stored in separate air tight containers and used for feed formulation given to the rats.

Experimental procedure

Experimental animals were randomly separated into three groups of five animals each and housed in stainless steel cages with plastic base under humid tropical conditions. The animals were exposed to 12 h light/dark cycles and supplied feed and water ad libitum. The animals were allowed to acclimatize on the basal diet (Vital feed mash) and clean tap water for two weeks before the formulated diets were fed. After two weeks of acclimatization, the fifteen albino rats were randomly selected into three groups as indicated below (Table 1).
Table 1: Grouping and Feed Composition

<table>
<thead>
<tr>
<th></th>
<th>Vernonia amygdalina (g)</th>
<th>Vernonia colorata (g)</th>
<th>Vital Feed Mash (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>10</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Group 2</td>
<td>0</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Group 3</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The pellets from both diets were properly mixed and dried in an oven at 35°C to a constant weight and thereafter cooled and stored in air-tight containers. Daily body weights changes of all the rats were taken and recorded, as well as weekly fecal losses. After 21 days, the animals were humanely sacrificed and blood samples were collected by cardiac puncture. The organs such as prostate, testes, liver, heart, spleen and kidney were promptly excised and dabbed on a filter paper and weighed using a Top-loading balance for each rat that was sacrificed. The blood samples were collected and centrifuged at 2000g for 5 minutes followed by sera collection. The serum samples were refrigerated at 2-8°C until analysis.

Relative organ weight was calculated based on the following formula:

\[ \text{ROW} = \left( \frac{W_O \times 100}{W_A} \right) \]

Where ROW = Relative organ weight

\[ W_O = \text{Weight of Organ} \]
\[ W_A = \text{Weight of Animal before sacrifice} \]

Determination of Prostate Specific Antigen (PSA) Enzyme Immunoassay

The Bio-check Prostate Specific Antigen (PSA) kit was used for the quantitative determination of the PSA concentration in serum. The test is based on the principle of a Solid Phase Enzyme-Linked Immunosorbent Assay (Stowell et al., 1991; Hara and Kimura, 1989).

Determination of Testosterone Concentration

The Accu Bind ELISA microwell method was used to determine the total serum testosterone concentration in the animals by a Microplate Enzyme Immunoassay (Cummings and Wall, 1985; Dorfman and Shipley, 1956).

Determination of Prostatic Acid phosphatase

The colorimetric method was used in the determination of Acid phosphatase using methods described by (Hillman, 1971). Serum Prostatic Acid phosphatase was determined using biochemical kits purchased from RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom.

Statistical Analysis

Data were presented as Mean ± Standard Deviation (SD) of three replicates and was analyzed using Duncan’s Multiple Range Test following one-way Analysis of Variance (ANOVA) using SPSS version 20.0 software package (SPSS Inc., Chicago, U.S.A). Differences at (p<0.05) were considered significant.

RESULTS AND DISCUSSION

Figure 1 depicts serum testosterone concentrations which significantly (p<0.05) decreased in VA and VC relative to the control group. The decreases observed in testosterone concentrations could be due to the ability of both leafy vegetables to affect the cholesterol biosynthetic pathway, which is a precursor to synthesis of testosterone. Testosterone is a major androgen hormone secreted in the testes, but can be converted to an active form dihydrotestosterone (DHT) by the enzyme 5 α-testosterone reductase. It is this dihydrotestosterone that regulates prostatic function and its development. It has also been reported that phytoestrogens from medicinal plants play a role in the prevention and treatment of prostate cancers and BPH (Feng et al., 2007) through mechanisms such as inhibition of 5 alpha-reductase, 17 beta-hydroxysteroid dehydrogenase, aromatase, tyrosine specific protein kinases and DNA topoisomerase II (Morisey and Watson, 2003).
Figure 2 shows a significant (p<0.05) decrease in the prostate specific antigen (PSA) concentrations in rats fed with diets incorporated with VA relative to control and a significant (p<0.05) increase in PSA concentrations in rats fed diets incorporated with VC relative to the control group. The decreases in PSA concentration could be attributed to the fact that VA has more chemotherapeutic efficacy than groups fed with VC. It means that PSA of the VC group increased so much as weight increased and VC could not have much efficacy in reducing the PSA below the control threshold. Scientific reports by Ejike and Ezeanyika (2011b) reported that fluted pumpkin (Teliferia occidentalis) inhibited the induction of benign prostatic hyperplasia and may act by increasing the testosterone: estradiol ratio.

Figure 3 depicts that prostatic acid phosphatase (PACP) concentrations significantly (p<0.05) increased in rats fed diets incorporated with V.C. while prostatic acid phosphatase concentrations significantly (p<0.05) decreased in the group fed diets incorporated with VA relative to the control group. The possible activating factors when rats were fed diets incorporated with Vernonia colorata could be attributed to the increase observed in prostatic acid phosphatase, while the decrease observed in when rats were fed diets incorporated with V.A may lack sufficient activating factors to increase the enzymes concentration.

A significant (p<0.05) decrease in the testicular weight of animals fed at 10% dietary incorporation of VA and VC relative to the control group was shown in Figure 4. It also depicts the prostatic weight of albino rats fed diets incorporated at 10% V.A and V.C which showed a significant (p<0.05) increase compared to the control group. This ability of both leafy vegetables to affect the cholesterol biosynthetic pathway, which is a precursor to synthesis of testosterone could be attributed to the decreases observed in testicular weight. This is in line with reports that dietary incorporation at 10% levels in processed and unprocessed forms of both VA and VC had positive modulatory effect on blood lipid profile by increasing HDL (high density lipoprotein) and decreasing cholesterol concentrations (Egedigwe and Ijeh, 2010; Ijeh and Egedigwe, 2010). Also, Vernonia amygdalina contains vernomygdin and vernodalin which are sesquiterpene lactones that mediate or regulate cholesterol concentration (Izevgibie et al., 2004). VA and VC may contain certain regulatory elements that increase polypeptide formation, thus increasing prostatic protein content which increased the prostate weight.
Figure 5 depicts the significant (p<0.05) decrease in the relative kidney and spleen weight in experimental animals fed diets incorporated with VA and VC compared to the control group. The increase in the relative spleen weight suggests that feeding of the vegetable may result in increased splenic activity (Egedigwe and Ijeh, 2010). The increase in the relative kidney weight could be as a result of the induction of xenobiotic enzymes. The results of this present work agree with previous findings showing increase in kidney weights of rabbits administered extracts of *Vernonia amygdalina* (Ijeh and Adedokun, 2006; Egedigwe and Ijeh, 2010). Figure 6 shows a significant (p<0.05) decrease was observed in the relative liver weight of animals in the test groups VA and VC relative to the control group as shown in (Figure 6).

Figure 7 shows the daily body weight changes in albino rats fed diets incorporated with V.A and V.C. There was a significant (p<0.05) increase in body weight in animals fed VC than those fed diets incorporated with VA relative to the control group. This suggests that VA leaves rich in phytochemicals may be responsible for the observed weight loss in comparison to VC. Research evidence has shown that despite the characteristic bitter taste of VA, it did not affect feed intake when incorporated into diets (Atangwho et al., 2012).

**Conclusion**

At 10% of dietary incorporation these leafy vegetables, VA demonstrated a stronger efficacy than VC in reduction of serum testosterone concentrations and P.S.A. This implies that, even though V.C. could be deployed in chemotherapeutic prevention and management of prostatic dysfunctions such as benign prostatic hyperplasia and prostate cancer, *Vernonia amygdalina* could be a better chemotherapeutic agent to this regard. Therefore, a great need for affordable and reliable anticancer drugs from medicinal plants is highly recommended and much effort should be put into the scientific research for more anticancer natural products from medicinal plants. Further studies are conducted in our laboratory to ascertain the mechanism of action using both aqueous and methanol extracts of VA in the management of experimental induced BPH in rats.
REFERENCES


