Evaluation of Efficacy of Ten Herbal Sperm Boosters Using Male Swiss Albino Mice as Experimental Model

*1 Iroanya O.O, 1 Adewole H.A, 1 Nwoko C.L, 1Folarin O.A and 1Adelabu MA

1Department of Cell Biology and Genetics, University of Lagos, Akoka Yaba, Lagos Nigeria

*Author for Correspondence: oiroanya@unilag.edu.ng; onyiog@yahoo.com

ABSTRACT

Infertility in humans has rapidly increased prompting researchers to screen herbal extracts and formulations to improve fertility. The aim of this project is to ascertain the efficacy of Addyzoa and some local herbal fertility enhancers on sperm and their effects on some biochemical parameters. Adult Swiss albino male mice were placed in 11 cages of 5 animals each. Animals in cage 1 were administered only water i.e. control group, group 2 was administered Addyzoa, an Ayurvedic fertility enhancer while animals in groups 3 to 11 received local herbal fertility enhancers at different concentrations for 45 days. On the 46th day, the animals were sacrificed. Some sperm and biochemical parameters were assayed. In groups 3, 6 and 11, the animals showed significant (P ≤ 0.05) increase in sperm count while groups 6 and 11 exhibited significant (P ≤ 0.05) increase in sperm motility compared to groups 1 and 2. Photomicrographs showed different sperm morphological aberrations. ALP levels of groups 3, 4 and 6 was significantly (P ≤ 0.05) low while ALT concentration of groups 7 and 11 was significantly (P ≤ 0.05) low compared to group 1. Group 1 showed significant (P ≤ 0.05) increase in AST concentration compared to groups 7, 8 and 10. These results suggests that the herbal boosters administered to groups 3, 6 and 11 improved the quality and quantity of sperm possibly by enhancing asthenozoospermia, increased sperm formation thereby increasing oligozoospermia, supporting and improving teratozoospermia and size thereby preventing DNA damage to sperms.

Keywords: Oligozoospermia, Teratozoospermia, Asthenozoospermia, Fertility Enhancers, Sperm.

INTRODUCTION

Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples. Males are found to be solely responsible for 20-30% of infertility cases and contribute to 50% of cases overall (Agarwal et al. 2015). The causes of male infertility are multifactorial, this includes anatomical and genetic defects, testicular injury and disease, sperm disorders, hormonal dysfunction, aging, and environmental- and lifestyle-related factors. Poor lifestyle and unhealthy habits such as increase in the intake of drugs, chronic alcoholism, wearing of tight underpants, frequent hot baths, sedentary lifestyles can affects the quality of sperm. Sperm count (number of sperm contained in a certain volume), morphology (size and shape) and motility (ability of the sperm to propel in a forward direction for a sustained period of time) helps to determine the quality of the sperm (Barratt, 2007; WHO, 2010).

The use of medicinal plants in the prevention and management of ailments has increased in recent times. This could be attributed to the fact that people believe that these plants usually have low side effects, are cost effective, easily accessible to the local populace and have a holistic approach towards the treatment of diseases compared to conventional drugs (Iroanya and Okpuzor, 2016). Studies conducted on the use of herbal plants in the treatment of infertility in men have proven to be effective in the production of healthier sperm thereby increasing or improving fertility. Plants like the Horny goat weed (Chen et al. 2014), American ginseng (Akram et al. 2012), Withania somnifera Panax ginseng (Park et al. 2002) have been used to improve sperm quality. Foods rich in essential fatty acids especially decosahexaenoic acid (DHA), L-carnitine, folic acid, Vitamins C, E, A, D, zinc, selenium and coenzyme Q10 has been found to improve sperm quality (Akmal et al. 2006; Agarwal and Sekhon, 2010; Wang et al. 2010; Blomberg et al. 2011). Some phytochemicals e.g. escin (Fang et al. 2010) and anthocyanin...
(Jang et al. 2012) have been reported to improve sperm quality. The therapeutic potential of these plants can be ascribed to their phytochemical constituents.

Addyzoa is a proprietary herbo-mineral, spermatogenic, anti-oxidant Ayurvedic medicine manufactured by Charak Pharmaceuticals. Addyzoa is indicated for the treatment of male functional impotency and infertility, Oligosperma, Azoosperma, senile and sexual debility, Impotency after recanalization and Post-vasectomy syndrome as listed by the manufacturer. Addyzoa has multifaceted free radical scavenging action and therefore, helps to minimize the damage to the sperm cells due to free radicals. It supports the process of sperm formation by maintaining the functions of male reproductive organs and improving the hormonal levels of testosterone. Treatment with Addyzoa resulted in a significant improvement in total and progressive motility in the semen of men with idiopathic oligoasthenoteratospermia (iOATs) after 3 months of therapy (Kumar et al. 2011). Some plants used in preparation of Addyzoa e.g. Withania somnifera (Paul et al. 2016), Tinospora cordifolia (Upadhyay et al. 2014) and Phyllanthus Emblica (Li et al. 2015) are potent antioxidants. Withania somnifera and Emblica officinalis have been shown to have potential role in managing oxidative stress-associated with male infertility (Dutta and Sahu, 2013; Kumar et al. 2015).

The effect of Addyzoa capsules on some sperm characteristics and biochemical parameters using Swiss albino mice was compared to some local herbal sperm boosters commonly marketed in Oshodi main market in Lagos State Nigeria.

**MATERIALS AND METHODS**

**Experimental Animals**

Adult male Swiss albino mice (55) weighing an average of 25.5 ± 1 g were purchased from the Laboratory animal centre of the College of Medicine, University of Lagos Nigeria. They were maintained under standard laboratory conditions at the Experimental Animal House of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos with dark and light cycle (12/12 hrs.). The animals had access to standard rat chow bought from Ladokun feeds, Ibadan, Nigeria and clean water *ad libitum*. The Guidelines in the *Guide for the Care and Use of Laboratory Animals* by the National Research Council was followed. The experiment was carried out between May and July, 2015.

**Experimental Design**

After an adaptive period of 1 week, the animals were randomly divided into 11 groups (5 mice/group). Animals in group 1 received 10 ml kg⁻¹ distilled water (p.o.) and served as the control group. Animals in groups 2 – 11 were administered Addyzoa, New Life perfection power-store, A-plus herbal remedy, Chukatrin herbal powder, Dr. Samas Kemly 5/6 manpower, Ultimate booster, Kick and start, Kolorogun Herbal, Evaking herbs Energizer and Easyboost extra power herbal mixture respectively by gavage for 45 consecutive days.

**Chemicals and reagents:**

Some herbal preparations used in boosting male fertility were purchased from Oshodi market in Lagos state Nigeria. They are Chukatrin herbal powder (Chucks Herbal Ventures, Awkunanaw, Enugu State), A-plus herbal remedy (Kayfahd Herbalceuticals, Abeokuta Ogun state), New Life perfection power store (New Life Herbals World, Kumasi Ghana), Easy boost extra power herbal mixture (Easy Cure Herbal, Ibadan), Evaking herbs Energizer (Eva Kings Natural, Awka), Kolorogun Herbal (Sam Herbal and Spiritual Home. Ipaja, Lagos State), Dr. Samas Kemly 5/6 manpower (Bobola Nig. Ent. Ibadan, Oyo State), Ultimate Booster (K. K. Ultimate herbs, Umuocha Delta State), Kick and Start (Doctor “B” Herbs roots and Leaf Research Co, Ibadan Oyo, State) and Addyzoa (Charak Pharma Pvt Ltd., India). The biochemical parameters were assayed using Randox® kits. All other reagents used were purchased from Sigma-Aldrich Corp. (St. Louis, MO USA) otherwise stated.

**Analytical Methods**

**Biochemical assays**

On the 46th day, whole blood was drawn...
from the animals by puncturing the retro-orbital venous sinus after mild ether anesthesia using uncoated microhaematocrit tube. Serum was used for the determination of albumin, serum alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, creatinine, total bilirubin, total protein, triglyceride and urea according to the method specified on the Randox® kit.

**Semen analysis**

All the mice were then euthanized by cervical dislocation and the cauda epididymis were quickly excised for sperm collection since this is the primary sperm storage site prior to ejaculation. Sperm count, motility and morphology were assayed according to WHO guidelines (WHO, 2010). Semen analysis was performed by suspending the caudal epididymis in 1ml of phosphate buffer saline and making several incisions in it. The caudal epididymis was emptied using fine sterile forceps to gently squeeze the tubule before removing it. The solution was pipetted several times so as to homogenize the sperm suspension. The sperm suspension was incubated at 37 °C for 5 minutes so that the sperm can swim up. Sperm motility was estimated by placing a drop of the suspension on a clean warm glass slide and viewing under a light microscope (Nikon, H600L, Tokyo, Japan) at 400X magnification. The percentage of motile sperm was estimated using the method described by Uzunhisarcikli et al. (2007).

Slides were prepared from a drop of the sperm suspension and stained with 1 % eosin for 5 minutes (Narayana et al. 2005). It was viewed under a light microscope at 1000X magnification for morphological defects in the head, neck, mid-piece or tail regions. For each animal, 250 sperm cells with aberrations were screened and the result was expressed as percentage of sperm cells with aberrations per 250 cells.

The improved Neubauer haemocytometer was used to count the sperm cells in the cauda epididymis. Sperm count was recommended protocol of the WHO manual under a light microscope (WHO, 2010). To express the number of sperm cells per epididymis (million/epididymis) the total sperm count in 8 big squares was determined and multiplied by $3 \times 5 \times 10^5$ (Narayana et al. 2005).

**Statistical analysis**

The results were expressed as Mean ± SEM for five rats. The statistical analysis of the data was performed using SPSS package version 16.0, while comparison between means was determined using ANOVA. The significance of differences among all groups was determined by the Tukey HSD test and difference at $P \leq 0.05$ were considered statistically significant. All the assays were compared to either Addyzoa as positive control or distilled water as negative control.

**RESULTS**

**Semen analysis**

Table 1 shows the effect of some herbal preparations on Semen analysis. Animals in group 11 demonstrated significant ($P \leq 0.05$) increase in sperm count compared to groups 1 and 2. Compared to group 2, animals in groups 1, 4, 7, 8 and 9 showed a significant ($P \leq 0.05$) decrease in sperm count. Higher values were shown in groups 3, 6 and 11 (100 ± 2.24, 102.5 ± 1.4 and 128.5 ± 4.5 respectively) compared to groups 1 (63.8 ± 0.8) and 2 (90 ± 3.5). Groups 2, 3, 6, and 11 demonstrated significant ($P \leq 0.05$) increase in motility while group 8 was significantly ($P \leq 0.05$) decreased compared to control group 1.

The % Sperm Motility rate for the mice in groups 3, 6 and 11 (71.5 ±1.5, 70.5 ±1.5 and 70.5 ± 1.4 respectively) were significantly ($P \leq 0.05$) high compared to groups 1 and 2 (45 ± 2 and 59 ± 1.14 respectively). The control and addyzoa groups showed significant ($P \leq 0.05$) increase in % sperm motility compared to groups 4, 5, 7, 8, 9 and 10.

The % of sperm cells with aberrations per 250 cells were attenuated in group 6 (15 ± 1.7) compared to groups 1 and 2 (18.25 ± 1.4 and 18.5 ± 0.8 respectively). There was a significant ($P \leq 0.05$) increase in aberration in groups 3, 4, 5, 7, 9, 10 and 11 compared to groups 1 and 2.
Table 1: Effect of some herbal preparations on Semen analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group</th>
<th>Sperm Count (mean x 10^6)</th>
<th>%Sperm Motility (mean)</th>
<th>% Sperm Morphology (mean % of sperm cells with aberrations per 250 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>63.8 ± 0.8 (b)</td>
<td>45 ± 2 (b)</td>
<td>18.25 ± 1.4</td>
</tr>
<tr>
<td>Addyzoa</td>
<td>2</td>
<td>90 ± 3.5 (a)</td>
<td>59 ± 1.14 (a)</td>
<td>18.5 ± 0.8</td>
</tr>
<tr>
<td>Newlife perfection power-store</td>
<td>3</td>
<td>100 ± 2.24 (a)</td>
<td>71.5 ± 1.5 (a, b)</td>
<td>31 ± 1.7 (a, b)</td>
</tr>
<tr>
<td>A-plus herbal remedy</td>
<td>4</td>
<td>61.25 ± 0.58 (b)</td>
<td>1.75 ± 0.1 (a, b)</td>
<td>26.25 ± 0.8 (a, b)</td>
</tr>
<tr>
<td>Chukatrin herbal powder</td>
<td>5</td>
<td>85 ± 3.7 (a)</td>
<td>1.9 ± 0.2 (a, b)</td>
<td>30.25 ± 1.28 (a, b)</td>
</tr>
<tr>
<td>Dr. Samas Kemly 5/6 manpower</td>
<td>6</td>
<td>102.5 ± 1.4 (a)</td>
<td>70.5 ± 1.5 (a, b)</td>
<td>15 ± 1.7</td>
</tr>
<tr>
<td>Ultimate booster</td>
<td>7</td>
<td>62.5 ± 1.3 (b)</td>
<td>2.75 ± 0.1 (a, b)</td>
<td>26.75 ± 1.75 (a, b)</td>
</tr>
<tr>
<td>Kick and start</td>
<td>8</td>
<td>23 ± 2.03 (a, b)</td>
<td>3.2 ± 0.22 (a, b)</td>
<td>18.75 ± 0.95</td>
</tr>
<tr>
<td>Kolorogun Herbal</td>
<td>9</td>
<td>57.25 ± 1.89 (b)</td>
<td>26 ± 2.1 (a, b)</td>
<td>48.75 ± 1.7 (a, b)</td>
</tr>
<tr>
<td>Evaking herbs Energizer</td>
<td>10</td>
<td>86 ± 4.6 (a)</td>
<td>33 ± 2.4 (a, b)</td>
<td>35.75 ± 1.8 (a, b)</td>
</tr>
<tr>
<td>Easyboost extra power herbal mixture</td>
<td>11</td>
<td>128.5 ± 4.5 (a, b)</td>
<td>70.5 ± 1.4 (a, b)</td>
<td>37 ± 1.4 (a, b)</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for 5 rats. The Mean difference is significant at the 0.05 level. (a) = p ≤ 0.05 as compared to the control group. (b) = p  0.05 as compared to Addyzoa group. The significance of differences among all groups was determined by the Tukey HSD test.

The types of abnormalities seen in the cauda epididymidal sperm of some of the experimental animals are clustered sperm cell, sperm cell with no tail and or head, and twisted tail sperm cell (figure 1 - 4). Sperm cells with no head was seen in groups 4 and 7 as shown in figure 4. In groups 5 and 7, abnormalities observed are tailless sperm cell (figure 1) and sperm cell with twisted tail (figure 2) while some sperm cell in group 4 were clustered as shown in figure 3.

Figure 1: sperm cell without tail- as seen in groups 5 and 7
Figure 2: sperm cell with twisted tail- as seen in groups 5 and 7

Figure 3: Clustered sperm cells- as seen in group 4

Figure 4: sperm cell without head- as seen in groups 4 and 7

Figures 1- 4: Photomicrographs showing sperm abnormalities seen in some of the experimental groups

Table 2 shows the effect of some herbal preparations on liver function of rats after 45 days of administration. The albumin levels of animals in group 9 were significantly ($P \leq 0.05$) low compared to those in groups 1 and 2. There was significant ($P \leq 0.05$) increase in the albumin levels in groups 2, 3, 4, 5, 6, 7 and 10 compared to group 1. Groups 3, 4 and 6 showed significantly ($P \leq 0.05$) lower levels of ALP compared to group 1. ALT level in group 1 was significantly ($P \leq 0.05$) low compared to groups 7 and 11. There was significantly ($P \leq 0.05$) low level of AST in groups 7, 8 and 10 compared to group 1. Group 1 demonstrated a significantly ($P \leq 0.05$) low level of total bilirubin compared to groups 5, 7 and 9 while group 11 was significantly ($P \leq 0.05$) low compared to group 1.
The concentration of cholesterol was significantly ($P \leq 0.05$) attenuated in group 1 compared to groups 2, 3, 5, 6, 7, 8 and 10 as shown in Table 3. Groups 1, 4, 5, 8 and 11 showed significantly ($P \leq 0.05$) lower cholesterol concentration compared to group 2. The HDL cholesterol concentration in groups 2, 3, 6, 7, 8 and 10 were significantly ($P \leq 0.05$) high compared to group 1 while group 2 showed significantly ($P \leq 0.05$) high concentration of HDL cholesterol compared to groups 1, 4, 5, 9 and 11. The concentration of LDL cholesterol in groups 2, 7, 8 and 10 were significantly ($P \leq 0.05$) increased compared to group 1.

<table>
<thead>
<tr>
<th>SAMPLE (group)</th>
<th>ALBUMIN (g/L)</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>T. BIL. (L)</th>
<th>T. PROT. (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>26.88 ± 0.6$^b$</td>
<td>124.94 ± 1.6</td>
<td>118.4 ± 5.6</td>
<td>153.4 ± 5.3</td>
<td>1.5 ± 0.2</td>
<td>67.76 ± 1.2</td>
</tr>
<tr>
<td>Addyzoa (2)</td>
<td>31.66 ± 0.9$^a$</td>
<td>106.44 ± 1.3</td>
<td>137.38 ± 1.2</td>
<td>142.42 ± 3</td>
<td>1.92 ± 0.2</td>
<td>64.84 ± 1.4</td>
</tr>
<tr>
<td>Newlife perfection power-store (3)</td>
<td>30.68 ± 0.7$^a$</td>
<td>100.18 ± 12.5$^a$</td>
<td>138 ± 16.6</td>
<td>132.84 ± 1.9</td>
<td>2 ± 0.07</td>
<td>71.44 ± 0.81</td>
</tr>
<tr>
<td>A-plus herbal remedy (4)</td>
<td>31.44 ± 0.54$^a$</td>
<td>92.84 ± 1.47$^a$</td>
<td>110.22 ± 2.35</td>
<td>151.19 ± 7.6</td>
<td>1.7 ± 11</td>
<td>64.06 ± 1.09</td>
</tr>
<tr>
<td>Chukatrin herbal powder (5)</td>
<td>30.92 ± 0.71$^a$</td>
<td>107.04 ± 2.43</td>
<td>130.31 ± 2.65</td>
<td>134.21 ± 3.2</td>
<td>2.3 ± 0.1$^a$</td>
<td>65.6 ± 3.42</td>
</tr>
<tr>
<td>Dr. Samas Kemly 5/6 manpower (6)</td>
<td>33.3 ± 0.63$^a$</td>
<td>83.3 ± 0.77$^{(a,b)}$</td>
<td>124.5 ± 3.2</td>
<td>147.7 ± 1.3</td>
<td>2.06 ± 0.08</td>
<td>66.44 ± 2.1</td>
</tr>
<tr>
<td>Ultimate booster (7)</td>
<td>32.02 ± 0.3$^a$</td>
<td>139.66 ± 3.3$^b$</td>
<td>151.28 ± 10$^a$</td>
<td>123.57 ± 11$^a$</td>
<td>2.18 ± 0.23$^a$</td>
<td>67.86 ± 0.77</td>
</tr>
<tr>
<td>Kick and start (8)</td>
<td>30.02 ± 1.1</td>
<td>125.42 ± 1/44</td>
<td>115.5 ± 4.7</td>
<td>123.2 ± 7.1$^b$</td>
<td>1.8 ± 0.1</td>
<td>68.84 ± 2.2</td>
</tr>
<tr>
<td>Kolorogun Herbal (9)</td>
<td>23.48 ± 0.5$^{(a,b)}$</td>
<td>104.82 ± 2</td>
<td>97.06 ± 1.5$^b$</td>
<td>158.1 ± 4.2</td>
<td>3.22 ± 0.16$^{(a,b)}$</td>
<td>69.18 ± 0.9</td>
</tr>
<tr>
<td>Evaking herbs Energizer (10)</td>
<td>31.72 ± 0.68$^a$</td>
<td>114.96 ± 1.27</td>
<td>138.42 ± 6.8</td>
<td>123.8 ± 4.6$^a$</td>
<td>1.82 ± 0.1</td>
<td>75.28 ± 1.2$^b$</td>
</tr>
<tr>
<td>Easyboost extra power herbal mixture (11)</td>
<td>27.92 ± 0.3$^b$</td>
<td>111.6 ± 3.1</td>
<td>167.94 ± 3.6$^a$</td>
<td>171.18 ± 9.1</td>
<td>1.24 ± 0.07$^b$</td>
<td>64.98 ± 1</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for 5 rats. The Mean difference is significant at the 0.05 level. ($a$) = $P \leq 0.05$ as compared to the control group. ($b$) = $P \geq 0.05$ as compared to Addyzoa group. The significance of differences among all groups was determined by the Tukey HSD test.

Key: ALP= Alkaline phosphatase; ALT= Alanine transaminase; AST= Aspartate transaminase. T. PROT.= Total protein; T. BIL. = Total Bilirubin.
concentration was significantly ($P \leq 0.05$) attenuated in groups 1, 5, 6, 9 and 11 compared to group 2. The creatinine concentration in groups 2, 4, 7, 9, 10 and 11 were significantly ($P \leq 0.05$) high compared to group 1 while it was significantly ($P \leq 0.05$) attenuated in groups 1, 3, 4, 5, 6, 7, 8, 9 and 11 compared to group 2. Groups 8 and 9 significantly ($P \leq 0.05$) showed increased triglyceride concentration compared to groups 1 and 2. There was significant ($P \leq 0.05$) increase in concentration of Urea in group 10 compared to groups 1 and 2.

Table 3: Effect of some herbal preparations on some biochemical parameters

<table>
<thead>
<tr>
<th>SAMPLE (group)</th>
<th>CHO (mmol/L)</th>
<th>HDL CHO (mmol/L)</th>
<th>LDL CHO (mmol/L)</th>
<th>CREA (mmol/L)</th>
<th>TRIG (mmol/L)</th>
<th>BUN (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>2.57 ± 0.1(b)</td>
<td>1.61 ± 0.03(b)</td>
<td>0.5 ± 0.03(b)</td>
<td>27.49 ± 1.3(b)</td>
<td>2.5 ± 0.1(b)</td>
<td>6.56 ± 0.4</td>
</tr>
<tr>
<td>Addyzoa (2)</td>
<td>3.69 ± 0.2(a)</td>
<td>2.57 ± 0.1(a)</td>
<td>0.62 ± 0.01(a)</td>
<td>56.73 ± 0.9(a)</td>
<td>3.02 ± 0.03(a)</td>
<td>6.22 ± 0.2</td>
</tr>
<tr>
<td>Newlife perfection power-store (3)</td>
<td>3.35 ± 0.11(a)</td>
<td>2.18 ± 0.11(a)</td>
<td>0.53 ± 0.04</td>
<td>26.51 ± 0.39(b)</td>
<td>3.12 ± 0.06(a)</td>
<td>7.28 ± 0.15</td>
</tr>
<tr>
<td>A-plus herbal remedy (4)</td>
<td>2.84 ± 0.14(b)</td>
<td>1.67 ± 0.1(b)</td>
<td>0.53 ± 0.02</td>
<td>32.46 ± 0.85(a,b)</td>
<td>2.56 ± 016</td>
<td>7.54±0.17</td>
</tr>
<tr>
<td>Chukatrin herbal powder (5)</td>
<td>3.07±0.03(a,b)</td>
<td>2.08±0.04(b)</td>
<td>0.44±0.01(b)</td>
<td>23.67±0.53(b)</td>
<td>2.83±0.04</td>
<td>5.58±0.04</td>
</tr>
<tr>
<td>Dr. Samas Kemly 5/6 manpower (6)</td>
<td>3.55±0.1(a)</td>
<td>2.5±0.03(a)</td>
<td>0.5±0.01(b)</td>
<td>30.1±0.3(b)</td>
<td>3.1±0.05(a)</td>
<td>5.38±0.17</td>
</tr>
<tr>
<td>Ultimate booster (7)</td>
<td>3.57±0.15(a)</td>
<td>2.14±0.24(a)</td>
<td>0.62±0.02(a)</td>
<td>37±1.05(a,b)</td>
<td>2.98±0.02(a)</td>
<td>6.34±0.38</td>
</tr>
<tr>
<td>Kick and start (8)</td>
<td>3.15±0.13(ab)</td>
<td>2.13±0.1(a)</td>
<td>0.7±0.03(a)</td>
<td>25.46±1(b)</td>
<td>3.6±0.18(a,b)</td>
<td>7.46±0.18</td>
</tr>
<tr>
<td>Kolorogun Herbal (9)</td>
<td>4.11±0.07(a)</td>
<td>1.74±0.12(b)</td>
<td>0.5±0.01(b)</td>
<td>31.38±0.2(a,b)</td>
<td>4.3±0.1(a,b)</td>
<td>7.24±0.16</td>
</tr>
<tr>
<td>Evaking herbs Energizer (10)</td>
<td>3.41±0.11(a)</td>
<td>2.23±0.08(a)</td>
<td>0.64±0.02(a)</td>
<td>63.26±1.1(a,b)</td>
<td>2.16±0.14(b)</td>
<td>11.28±0.93(a,b)</td>
</tr>
<tr>
<td>Easyboost extra power herbal mixture (11)</td>
<td>2.99±0.02(b)</td>
<td>2.03±0.08(b)</td>
<td>0.5±0.003(b)</td>
<td>45±0.03(ab)</td>
<td>2.54±0.05(b)</td>
<td>7.44±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for 5 rats. The Mean difference is significant at the 0.05 level. (a) = P ≤ 0.05 as compared to the control group. (b) = P ≤ 0.05 as compared to Addyzoa group. The significance of differences among all groups was determined by the Tukey HSD test.

**Key:**
CHO= Cholesterol; HDL CHO= High-density lipoprotein cholesterol  
LDL CHO= Low-density lipoprotein cholesterol  
CREA= Creatinine ; BUN= Blood urea nitrogen

**DISCUSSION**
Addyzoa is indicated to increase Sperm Count, Enhance Sperm motility, improve Sperm Morphology (prevents DNA damage to sperms), Increase sexual desire, enhance the chances of pregnancy. It is used to improve Oligospermia...
(Low sperm count), Asthenospermia and Teratospermia (Defective sperm morphology). The primary mechanism of action in Addyzoa is antioxidant protection which hunt down free radicals and protect sperm cells from damage. Some of the key ingredients in Addyzoa supports the healthy formation of sperm, improve sperm shape, increase count and motility, protect sperm from oxidative damage, helps generate healthy new sperm cells, add to semen density and helps to prevent the DNA damage to the sperm (Fang et al. 2010; Blomberg et al. 2011; Akram et al. 2012; Agarwal et al. 2015). Addyzoa supports spermatogenesis by maintaining the functions of male reproductive organs and improving the hormonal levels of testosterone. In this study, Addyzoa supported the healthy formation of sperm, improved sperm shape, increase count and motility, by significantly (P < 0.05) increasing sperm motility and count compared to the normal control.

Sperm count is one of the most sensitive tests for spermatogenesis and gives the cumulative result of all stages in sperm production, which highly correlates with fertility (Meistrich et al. 1982). Easyboost extra power herbal mixture demonstrated a significant (P < 0.05) increase in mean sperm count, percentage motility and sperm cell abnormalities compared to Addyzoa and the control groups. This suggests that it was able to increase sperm count and motility by supporting the formation of healthy sperm cells though it did not improve the shape of the sperm cells. Dr. Samas Kemly 5/6 manpower significantly (P ≤ 0.05) increased the mean sperm count, percentage motility and decreased the mean % of sperm cells with aberrations per 250 cells compared to Addyzoa and the control groups. This herbal formulation presented the best result compared to all the formulations used in this study. It is suggested that Dr. Samas Kemly 5/6 manpower recovers the loss of semen quality and sperm function. This is surprising since some of the plants used in the formulation are reported to possess antifertility properties e.g. Azadirachta indica (Meliaceae) seeds and Cassia alata L. (Caesalpinaceae) flower (Dehghan et al. 2005; Jain and Ali, 2007). These results indicated that some of the herbal formulations increased the sperm motility by increasing the percentage of the sperm with higher motility grade therefore does not cause any defect to the morphology of the sperm produced. A reduction in sperm count suggests changes in sperm maturation and sperm production (Mishra and Singh, 2009).

Albumin binds to toxins and heavy metals, thus preventing them from damaging the body. On administration of Newlife perfection power-store, A-plus herbal remedy, Chukatrin herbal powder, Dr. Samas Kemly 5/6 manpower, Ultimate booster and Kick and start, there was significant (P ≤ 0.05) increase in albumin concentration compared to the control group indicating the increased regulation of the colloidal osmotic pressure of blood. A-plus herbal remedy reduced the levels of ALP, ALT and AST and also increased the concentration of albumin thereby improving the liver function on its administration.

Some of the herbal formulations and Addyzoa induced some alterations in the kidney functions characterized by some signs of injury, e.g. significant (P ≤ 0.05) increase in concentration of plasma creatinine, triglyceride and or urea. However, few of the herbal formulations improved the kidney function e.g. Chukatrin herbal powder which improved the function of the kidney compared to Addyzoa. Increase in the chance of heart and circulation problems can be associated with high triglyceride, total and LDL cholesterol level. Addyzoa, Ultimate booster and Kick and start significantly (P < 0.05) increased the levels of triglyceride, cholesterol and LDL cholesterol indicating the chances of an increase in problems associated with the heart and circulation.

Cholesterol, a recognized modulator of sperm functions, is a key molecule in the mammalian physiology of particular importance for the reproductive system as it is the common precursor for steroid hormone synthesis. Imbalanced cholesterol levels may particularly affect post-testicular events since cholesterol homeostasis regulation is crucial for post-testicular sperm maturation (Whitfield et al. 2015). On administration of some of the herbal formulations especially Ultimate booster, there was significant increase in the mean % of sperm cells with aberrations per 250 cells, total and
LDL cholesterol, with decrease in sperm count and motility. This collaborates the findings of Gupta and Dixit which concluded that hypercholesterolemia affects the testicular function and aortic endothelium (Gupta and Dixit, 1988). The study suggests that hypercholesterolemia in the epididymis caused by genetic or abiotic factors may be the cause unexplained male infertility.

CONCLUSION
This study suggests that some of the herbal extracts may actually inhibit spermatogenesis while others may enhance the testicular functions and thereby increase the quality and quantity of spermatocytes without causing any defect to the sperm morphology. The use of herbal formulations should be thoroughly investigated as this study without any doubt brings new insights on the effect of some commonly used herbal sperm boosters on male fertility. The pharmacologic activities of the herbal formulations are not clear so further investigation should be performed to understand their unknown pharmacologic effects.

REFERENCES


