Spermato-protective role of honey in Rabbit

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Abstract
This study was designed to evaluate the effect of Cola nitida and Cola acuminata on testicular tissues and semen characteristics, such as sperm count, sperm motility, sperm morphology and sperm viability. Twenty (20) rabbits with average weight 1.4kg were used for the study. The rabbits were divided into five (5) groups; group 1 served as control, 0.064g/ml of aqueous extract of cola nitida and 0.066 g/ml of cola acuminata were given to rabbits in groups 2 and 3 respectively orally for 9 consecutive weeks. 0.066g/ml of cola acuminata mixed with natural honey was given orally to rabbits in group 4 for 9 consecutive days. 0.1ml of pure honey was given orally to rabbits in group 5 for 9 weeks. Parameters of sperm qualities such as number of total sperm cells, sperm viability, sperm morphology and sperm motility were assessed. Also testicular histology was assessed. The total sperm counts, number of viable sperm cells and number of viable sperm cells were insignificantly decreased in groups given cola nitida and acuminata respectively when compared with control. Also, number of sperm cells with defective morphology was increased in groups given cola nitida and cola acuminata when compared with control. However, in groups given cola acuminata mixed with honey and group given pure honey alone, the number of total sperm cells, viable sperm cells and motile sperm cells were increased while the number of defective sperm cells was reduced when compared with the control. The testicular features of groups given cola nitida and cola acuminata alone showed histopathological defects when compared with the control. However, groups given honey alone and honey plus cola showed improved histology. The implication of these observations may be that honey possesses spermato protective properties.

Key words: Semen quality, colanuts, honey, caffeine.

Introduction:

Kola nuts are the seed-pods of various evergreen trees that are native to Africa (1). They are important in various social and religious customs and may also be used to counteract hunger and thirst. In Nigeria for instance, the rate of consumption of kola nut especially by students is very high as a principal stimulant to keep awake and withstand fatigue (2). (3) reported that caffeine, theobromine and theophylline found in kola nuts are xanthine stimulants. Caffeine was first isolated from green coffee beans in 1820. Kolanuts have been shown to contain high concentration of caffeine and theobromine as active constituents. However, caffeine an important bioactive constituent of kolanut has been implicated as a risk factor for delayed conception (4,5,6). It was reported that there is no association between sperm quality, smoking habits, coffee drinking, moderate alcohol intake, exposure to heat (sauna, hot baths, type of underwear, sedentary activities) or physical activities in men. However, this assertion contradicts the report that caffeine impaired semen quality (7) and increased motility (8). This disagreement therefore, led to this study to assess possible effects of cola species namely cola nitida and cola acuminata on the semen quality using experimental rabbits. Also modulatory effect of honey on this effect was also determined.

Materials and methods:

Experimental animals

Twenty male (20) rabbits of average weight 1.4kg were used for experiment. These rabbits were housed in a well ventilated cage inside the animal house section of Ladoke Akintola University of Technology, Ogbomoso. The animals were grouped into five with each group having four rabbits each and marked with different colour for identification i.e Blue, Red, Black and Green and also the group which is unmarked. They were all being fed with pellet and portable water (borne hole).
Preparation of Kolanuts Extract (Cola Nitida and Cola Acuminata)
Three hundred and twenty grammes (320g) of cola nitida was grinded and dissolved in 500ml of distilled water to make 0.64g/ml aqueous extract of cola nitida. Six hundred and sixty gramme (660g) of cola acuminata was dissolved in 1000ml of distilled water to make 0.66g/ml aqueous extract of cola acuminata.

Administration of Aqueous Extract of Cola Species and Honey
Rabbits in group 1 were neither given extract nor given honey and they serve as controls. To the rabbits in group 2, 0.1ml of aqueous extract of cola nitida was administered orally to each rabbit for the study period. To the rabbits in group 2, 0.1ml of aqueous extract of cola acuminata was given for the period of the study. Rabbits in group 4 were given 0.1ml of cola acuminata containing small quantity of honey. The fifth group contained rabbits that were given 0.1ml of honey. This was designed to mimick how human consume kola nuts i.e. the consumption of the whole seed without removing any part. The administration was twice per day for a period of 9 weeks. The prepared extracts of cola nitida and cola acuminata and the honey were always kept in the refrigerator after each day’s administration to prevent it from getting contaminated.

Histological process
The organs were cut in slabs of about 0.5mm thick transversely and fixed in bourn’s fluid for a day after which it was transferred to 70% alcohol for dehydration. After six hours it was transferred to 90% alcohol and left over night. From 90% to 3 changes of absolute alcohol for 1 hour each, then into xylene for about 10 hours and later transferred into fresh xylene for about 30min the tissues were placed in two changes of molten paraffin for 20min each in an oven at 57˚C. They were placed vertically in molten paraffin which was inside a metal mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks serial sections were cut using rotary microtone at 5micron section were floated on a water bath to spread out and later picked into albumenized slide and dried on a hot plate at 52˚C. Slides were put in staining rack and placed in staining well containing xylene to dewax, absolute alcohol (2 changes). 10% alcohol and then to water for 5minutes after which they were stained with haemotoxyline for 3 minutes. Excess haemotoxyline was washed off with water and differentiated with 1% acid alcohol. Sections were stained with eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and cleared in xylene to remove all traces of water. Then a mountant was placed on the surface of slide and covered with 22 by 22mm cover slip.

Sperm Analysis
At the end of the study period, the animal were sacrificed by cervical dislocation. Immediately after the testicles were removed from each rabbit, the sperm specimens were collected by aspiration from the epididymis. This involves making an incision in the caudal of right ductus deference of the testicle. Two drops of semen were placed on the microscope slide and two drops of warm 2.9% sodium citrate were added. This was then covered with the cover slip and examine under the microscope using x40 objective with reduced light. Sperm count, motility, viability and morphology were carried out using the new improved neubeur’s haemocytometer counting chamber.

Statistical Analysis
Quantitative data are presented as a Mean± SD. Sperm counts, morphology, viability and motility, of control and experimental groups are compared using one-way analysis of variance (ANOVA) to find the statistical difference among their means. A value of P<0.05 was considered to be statistically significant.

Results:
Table 1: Effect of administration of aqueous extracts of Cola species, Cola acuminata mixed honey and honey alone on sperm characteristics

<table>
<thead>
<tr>
<th>Sperm Characteristics</th>
<th>Control</th>
<th>Cola -nitida</th>
<th>Cola acuminata</th>
<th>Cola acuminata + honey</th>
<th>Honey</th>
<th>P. value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.count</td>
<td>61.67±13.11</td>
<td>55.42±1.81</td>
<td>57.27±4.50</td>
<td>64.70±2.45</td>
<td>67.13±0.42</td>
<td>2.63</td>
<td>NS</td>
</tr>
<tr>
<td>S.viability</td>
<td>87.66±5.35</td>
<td>83.92±2.79</td>
<td>56.58±28.77</td>
<td>94.27±0.69</td>
<td>94.48±3.86</td>
<td>5.152</td>
<td>NS</td>
</tr>
<tr>
<td>S.morphology</td>
<td>15.63±4.74</td>
<td>18.55±2.95</td>
<td>51.30±29.19</td>
<td>8.70±1.87</td>
<td>6.17±0.91</td>
<td>7.4193</td>
<td>NS</td>
</tr>
<tr>
<td>S.motility</td>
<td>84.34±4.76</td>
<td>81.16±2.94</td>
<td>48.67±29.17</td>
<td>91.27±1.89</td>
<td>93.74±0.99</td>
<td>7.4178</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2: Pairwise comparisons of sperm characteristics between different experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. count</th>
<th>S. viability</th>
<th>S. morphology</th>
<th>S. motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control VS Cola nitida</td>
<td>0.541 (NS)</td>
<td>0.601 (NS)</td>
<td>0.644 (NS)</td>
<td>0.648 (NS)</td>
</tr>
<tr>
<td>Control VS Cola acuminate</td>
<td>0.701 (NS)</td>
<td>0.168 (NS)</td>
<td>0.138 (NS)</td>
<td>0.137 (NS)</td>
</tr>
<tr>
<td>Control VS Cola acuminata + Honey</td>
<td>0.837 (NS)</td>
<td>0.140 (NS)</td>
<td>0.099 (NS)</td>
<td>0.099 (NS)</td>
</tr>
</tbody>
</table>
The mean sperm counts in the groups 2 and 3 were reduced compared with mean sperm counts in control. The mean sperm counts in group 4 i.e rabbit given kolanut mixed with honey and group 5 (rabbits given honey alone.

**Histopathology**

*The connective tissues and spermatogenic cells were normal*
Discussion:
The observation of reduced sperm counts, sperm motility, sperm viability and increased number of sperm cells with defective morphology in the rabbits given Cola nitida and Cola acuminata may be an indication that kolanut extract may negatively affect sperm characteristics in rabbits. Although, each of the bioactive substances in the kolanut was not extracted and tested against sperm characteristics to ascertain what could be directly responsible for the effect, however reports of previous studies such as those of (7) showed that caffeine (an important bioactive substance in kolanut) impaired semen quality. This is in accordance with the finding of (9) and (10) that catechin (a component of kolanut extract) could improve boar sperm viability and sperm motility. The improved sperm counts, sperm motility, sperm viability and reduced sperm cells with defective morphology in group given honey alone and group given honey along with kolanut (table 1) may be an indication that honey may have spermatoprotective properties. Several studies have shown that honey possesses properties that improve semen quality. However reports of previous studies such as those of (11) showed that honey increases spermatogenesis which may be due to the effect of increase in sorbitol dehydrogenase that convert sorbitol to fructose which metabolize via the glycolytic pathway in sperm to make ATP.

From the results, administration of honey showed protective properties and boost sperm qualities in the experimental rabbits. More works should be done using large number of experimental animals to be able to ascertain the effectiveness of honey in boosting sperm qualities.

Reference


