Effects of *Viscum album* (mistletoe) from three host plants (cocoa, kola and coffee) on semen quality of wistar albino rats

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**ABSTRACT**

Toxicity evaluation of medicinal plants is useful to know their safety profile. Male infertility arising from administration of drugs of plant origin is a major health concern. In the management of chronic health issues, the adverse effects of these agents are often down-played in favour of the beneficial medicinal values. This encourages repeated administration or consumption of part or whole plant products. *Viscum album* (mistletoe) is a popular medicinal plants used in the treatment of various chronic diseases. However, there is paucity of research on the effect of mistletoe from different host plants on male fertility. The study investigated the effects of aqueous extracts of *Viscum album* obtained from three host plants (Cocoa, Kola and Coffee) on semen parameters of Wistar rats. Animals were divided into four groups Control, Cocoa, Kola and Coffee respectively. Group 1 served as control (received distilled water, 10 ml/kg), groups 2, 3, 4 and 5 had four sub-groups each. Each sub-group received 400, 800, 1600 and 3200 mg/kg doses of extract respectively daily for 24 days. Five animals were allotted to each group (control) and sub-group (extracts). Administration of 400, 800, 1600 and 3200 mg/kg doses of extract of *Viscum album* obtained from three host plants (Cocoa, Kola and Coffee) caused dose-dependent significant ($P<0.05$, 0.01) decrease in semen quality (count, motility, morphology, concentration and viability) respectively. However, semen volume was not significantly altered ($P>0.05$). The results suggest that *Viscum album* obtained from the three host plants adversely affected sperm parameters in male wistar rats. Thus, people who consume the plant extracts should be careful and medical practitioners who prescribe *Viscum album* in the management of chronic diseases should be cautious.

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**Capsule Summary:** Administration of 400, 800, 1600 and 3200 mg/kg doses of extract of *Viscum album* obtained from cocoa, kola and coffee plants caused dose-dependent significant ($P<0.05$, 0.01) decrease in semen quality in rats.

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**INTRODUCTION**

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Human existence cum propagation and reproduction/fertility are mutual inclusive. It is understandable therefore, that infertility is a prominent medical issue that takes its toll on social life in many parts of the world (Akinola et al., 2010). In most instances, the causal factors are multi-factorial or vague. Prominent of the factors implicated are exposure to heavy metals, extreme scrotal temperature, consumptions of conventional drugs, administration of drugs from plants and a host of other factors (Olayemi, 2010).

Male infertility arising from administration of drugs of plant origin is a major health concern (Olayemi, 2010). In the management of chronic health issues, the adverse effects of these agents are often down-played in favour of the beneficial medicinal values (Erhirhie et al., 2015). This encourages repeated administration or consumption of part or whole plant products.

On record are the temporary and permanent anti-fertility effects of some plant extracts in laboratory animals. Such effects include step-wise decrease in weight of parts of the reproductive organs or in the volume of the products from the organs. Other observations were changes in morphology of the cells of the reproductive organs, alteration of the spermatogenesis process (Kulsheshtha and Mathur, 1990; Raji et al., 2003), inhibition of reproductive hormone production (Muragavel and Akbarsha, 1991) leading to different changes in testicular histology (Pakrashi et al., 1985).

Viscum album which belongs to the family Santalaceae is commonly known as European mistletoe, common mistletoe or mistletoe. This plant is originally native to Nigeria, Europe, North Africa, Western and Southern Asia (Jurin et al., 1993). As semi-parasitic evergreen growing on host tree, it depends on the host for minerals and water only but synthesizes its carbohydrate using green leathery, oblong leaves (Osadebe and Uzochukwu, 2006). It is a unique parasitic tree that have broad leaves such as poplar, apple, lime, hawthorn, poplar and similar plants. The chemical make-up of mistletoe may differ according to host tree species, the time of harvest and the process employed in processing it. Such chemical constituents include caffeic acid, alkaloids, amines, phenols, flavonoids, terpenoids and viscosuloxins, flavonoids, flavonol aglycones, lectins, triterpenes, saponins, caffeic acid, acetylsalicyline derivatives, vitamin C, histamine, resins, thionins, cardenolides (Jolanta and Przemyslaw, 2015).

Mistletoe has been in use medicinally for ages to cure ailments such as symptoms of menopause, infertility, cancer, nervous tension, asthma, hypertension, headache, diabetes and dermatitis (Obatomi et al., 1994; Grossarth-Maticek and Ziegler, 2007). Immune system modulating ability of extracts of mistletoe is also well documented (Jurin et al., 1993; Ladokun et al., 2015).

To the best of our knowledge, there is paucity of information on the effect of Viscum album on male fertility. This prompted this present study to investigate the effect of extracts of mistletoe from three different hosts plants (Cocoa, kola and Coffee) on semen parameters in Wistar rats.

MATERIAL AND METHODS

Plant Collection

Procurement and authentication of fresh leaves of mistletoe plant (Viscum album) from cocoa, coffee and kola was done at Cocoa Research Institute of Nigerian (CRIN), Ibadan, Oyo state, Nigeria.

Extract Preparation

Fresh leaves of mistletoe plant (Viscum album) were air dried and powdered. A portion (50 g) of the powdered leaves was weighed into a beaker and 500 ml warm distilled water was added, stirred for 20 minutes and allowed to stand for 24 hours. It was then filtered using Whatman’s filter paper and the filtrate obtained was concentrated.

Experimental Animals

Wistar albino rats weighing between 260-280g were used for the study. The animals were allowed free access to clean drinking water and standard feed (rat chow, Vital feed®, Nigeria) diet ad libitum. The rats were weighed before commencement of the experiment and thereafter every four days.

Extract administration

Animals were divided into four groups Control, Cocoa, Kola and Coffee respectively. Group 1 served as control (received distilled water, 10 ml/kg), groups 2, 3, 4 and 5 had four sub-groups each. Each sub-group received 400, 800, 1600 and 3200 mg/kg doses of extract orally respectively daily for 24 days. Five animals were allotted to each group (control) and sub-group (extracts).

Semen Collection

On the 25th day, all the rats were sacrificed through cervical dislocation. Semen examination was carried out using a modified method of Zemjanis (1970) as described by Olufisayo and Oluremi (2008). Briefly, a drop of semen obtained from the epididymis by gentle pressure was placed on pre-warmed slide. A drop of sodium citrate buffer (2.9%) was added to the semen and cover slip applied to evaluate motility under x40 of microscope. The semen sample was also stained with Eosin-Nigrosin to evaluate the ratio live to dead cells. This sample was used to estimate sperm abnormalities. The epididymis was then submerged in a graduated test-tube containing 5 ml of Formol saline. The volume of semen was evaluated as the measure of displacement of formol saline. The entire epididymis was then crushed in formol saline and this mixture was used to evaluate spermatozoa concentration using the improved Neubauer haemocytometer.

Data analysis

Results are presented as mean ± standard error of mean (SEM). Data were subjected to one way analysis of variance.
RESULTS AND DISCUSSION

Effect of Viscum album (Va) extract from cocoa (Theobromae cacao) on epididymal semen parameters of Wistar rats.

Administration of extract caused dose dependent significant increase (P<0.05, 0.01) in spermatozoa abnormalities in rats administered the extract compared with control (Table 1). There was no statistically significant difference (P>0.05) in the semen volume of Wistar rats in the test groups compared with those in the control group, following the administration of Viscum album extract from cocoa (Table 1). Administration of extract caused significant decrease (P<0.01) in sperm concentration of Wistar rats (Table 1). The extracts caused dose dependent significant decrease (P<0.05, 0.01) in active sperm motility in Wistar rats. The percentage livability of the spermatozoa was not significantly (P>0.05) affected by the extracts.

Effect of Viscum album (Va) extract from kola (Kola nitida) on epididymal semen parameters of Wistar rats

There was dose dependent significant increase (P<0.01) in spermatozoa abnormalities in rats administered the extract compared with control (Table 2). No significant difference (P>0.05) was observed in the semen volume of rats administered the extract (400, 800, 1600 and 3200 mg/kg) compared with rats in the control group (Table 2). Administration of extract caused significant decrease (P<0.01) in sperm concentration in rats when compared with control group (Table 2). Administration of extracts caused dose dependent significant decrease (P< 0.01) in active sperm motility in Wistar rats (Table 2). The percentage livability of the spermatozoa was significantly (P<0.05) reduced when compared with control group (Table 2).

Effect of Viscum album (Va) extract from coffee (Coffee Arabica) on epididymal semen parameters

There was dose dependent significant increase (P< 0.05, 0.01) in spermatozoa abnormalities in rats administered the extract compared with control (Table 3). No significant difference (P>0.05) was observed in the semen volume of rats administered the extract (400, 800, 1600 and 3200 mg/kg) compared with rats in the control group (Table 3). Administration of extract caused significant decrease (P<0.05, 0.01) in sperm concentration of Wistar rats when compared with control group (Table 3). Administration of

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Abnormal spermatozoa (%)</th>
<th>Semen volume (mL)</th>
<th>Sperm conc. x10^7cell/mL</th>
<th>Sperm motility (%)</th>
<th>Livability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 10 ml/kg</td>
<td>9.8±0.1</td>
<td>0.52±0.006</td>
<td>141.3±4.2</td>
<td>93.3±2.9</td>
<td>98.0±0.00</td>
</tr>
<tr>
<td>Va, 400 mg/kg</td>
<td>11.4±0.4*</td>
<td>0.52±0.06NS</td>
<td>91.3±4.6**</td>
<td>66.7±5.8*</td>
<td>92.7±4.6NS</td>
</tr>
<tr>
<td>Va, 800 mg/kg</td>
<td>11.4±0.3**</td>
<td>0.51±0.006NS</td>
<td>79.0±6.6**</td>
<td>56.7±5.8**</td>
<td>92.7±4.6NS</td>
</tr>
<tr>
<td>Va, 1600 mg/kg</td>
<td>12.6±0.5**</td>
<td>0.51±0.006NS</td>
<td>79.0±6.6**</td>
<td>53.3±5.8**</td>
<td>94.3±4.0NS</td>
</tr>
<tr>
<td>Va, 3200 mg/kg</td>
<td>13.5±0.6**</td>
<td>0.52±0.006NS</td>
<td>73.0±6.2**</td>
<td>33.3±11.5**</td>
<td>96.0±1.7NS</td>
</tr>
</tbody>
</table>

Values are presented as mean ± Standard error of mean (SEM), n=5. *P<0.05, **P<0.001: Significantly different when compared with control group. NS: Not significantly different from control group.

Table 2: Effect of Viscum album extract from kola (Kola nitida) on epididymal semen parameters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Abnormal spermatozoa (%)</th>
<th>Semen volume (mL)</th>
<th>Sperm conc. x10^7cell/mL</th>
<th>Sperm motility (%)</th>
<th>Livability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 10 ml/kg</td>
<td>9.8±0.1</td>
<td>0.52±0.01</td>
<td>141.3±4.2</td>
<td>93.3±2.9</td>
<td>98.0±0.00</td>
</tr>
<tr>
<td>Va, 400 mg/kg</td>
<td>12.1±0.40**</td>
<td>0.52±0.000NS</td>
<td>102.0±5.30**</td>
<td>70.0±0.00**</td>
<td>95.0±0.00**</td>
</tr>
<tr>
<td>Va, 800 mg/kg</td>
<td>12.6±0.20**</td>
<td>0.51±0.01NS</td>
<td>91.0±3.60**</td>
<td>67.7±2.50**</td>
<td>88.3±0.00**</td>
</tr>
<tr>
<td>Va, 1600 mg/kg</td>
<td>13.7±0.20**</td>
<td>0.51±0.01NS</td>
<td>93.3±5.80**</td>
<td>56.7±5.30**</td>
<td>86.7±2.80**</td>
</tr>
<tr>
<td>Va, 3200 mg/kg</td>
<td>14.3±0.60**</td>
<td>0.51±0.01NS</td>
<td>79.3±3.10**</td>
<td>46.7±5.80**</td>
<td>81.7±2.90**</td>
</tr>
</tbody>
</table>

Values are presented as mean ± Standard error of mean (SEM), n=5. *P<0.05, **P<0.001: Significantly different when compared with control group. NS: Not significantly different from control group.
extracts (800, 1600 and 3200 mg/kg), except 400 mg/kg caused dose dependent significant decrease (P< 0.01) in active sperm motility in Wistar rats (Table 3). The percentage livability of the spermatozoa was significantly (P<0.05) reduced following the administration of 1600, and 3200 mg/kg when compared to control group (Table 3).

DISCUSSION

The present study investigated the effects of Viscum album obtained from three host plants (Cocoa, Kola and Coffee) on semen parameters of Wistar rats. The data revealed that Viscum album extract from the three host plants did not have any significant effect on sperm quantity. However the extracts from the three host plants dose dependently reduced semen quality (Tables 1, 2 and 3). The observation in this study suggest that long term exposure to Viscum album, especially at high doses may cause spermatozoa abnormality which may cause infertility. It may be inferred that administration of the extracts would not affect the fluidity of semen. The increase in abnormality of sperm morphology observed suggests that the extract could disrupt spermatogenesis (Olufisayo and Oluremi, 2008). This may be due to the presence of spermatoxic phytoconstituents in Viscum album from the three sources. In a nutshell, it may be inferred that the Viscum album from the three host plants may have inherent spermatoxic agents which may be from their source (host plants).

Although, a study carried out by Ofem et al. (2014) revealed that modest and restricted doses of Viscum album (mistletoe) extract at 150mg/kg, 300mg/kg, and 450 mg/kg respectively increased serum levels of testosterone, LH and FSH but decreased prolactin concentrations in rats. Results of Ofem et al (2014) may be due to low dose levels (150 mg/kg – 450 mg/kg) of the extract administered to animals as against high dose levels (400, 800, 1600 and 3200 mg/kg) administered to animals in this present study. Also different sources of the mistletoe could have accounted for the different observations.

CONCLUSIONS AND RECOMMENDATIONS

This study revealed that long term administration of Viscum album extract, especially those from Cocoa, Kola and Coffee may affect sperm quality which may cause infertility. Medical practitioners who prescribe Viscum album in the management of chronic diseases should be cautious. A withdrawal study is needed to investigate if the spermatoxic effects of the extracts in Wistar rats could be reversible. Effect of high doses of Viscum album extracts on hormonal parameters is needed to authenticate previous findings. Similar studies in female animals is also necessary.

REFERENCES


Jolanta, N., Przemyslaw, O., 2015. Phytochemical profile and therapeutic potential of *Viscum album* L. Natural Product Research DOI: 10.1080/14786419.2015.1022776


