Comparative study on the effects of aqueous extracts of Viscum album (mistletoe) from three host plants on hematological parameters in albino rats.

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Abstract
Background: The extract of the leaves of Viscum album (mistletoe) has been used for centuries in traditional medicine in many parts of the world. However, like many medicinal plants, the belief that things of natural origin are safe may not be entirely true. The blood is a good indicator of health and pathological mirror of the entire body.

Objective: Therefore, in this study, the acute effects of extracts of mistletoe, harvested from three hosts, on haematology indices of wistar albino rats was investigated.

Methods: Graded doses (400, 800, 1600 and 3200mg/kg body weight) of aqueous extracts of mistletoe from three different host plants, coffee (Coffee arabica), kola (Kola nitida), cocoa (Theobromae cacao), were administered orally to wistar albino rats for 14 days. Full haematological parameters were evaluated on whole blood collected from rats twenty four hours after the administration of the last dose.

Results: Mistletoe from kola caused a concentration dependent and statistically significant (p<0.05) reduction in platelets count in rats. Administration of mistletoe extract from cocoa and coffee led to reduction in hemoglobin concentration. Reductions in packed cell volume (PCV) and red blood cell (RBC) and increase in white blood cells (WBC) were also observed in rats administered all the extracts.

Conclusion: Increase in the WBC count observed in rats administered mistletoe suggests that mistletoe extract contains cellular agents that could stimulate the production of leucocytes and could serve as immune booster. However, there is need to be cautious in administration at high doses to prevent the risk of anaemia.

Key words: Viscum album, coffee arabica, kola nitida, theobromae cacao, haematology.

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Introduction
By the World Health Organization estimates, increasing population world-wide is depending on herbal medicine as a source of primary health care. Herbal medicine is used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used. The reasons for this, especially in developing nations, include ease and cost of assessing orthodox medicine as well as cost of procuring prescribed medications.

Viscum album (mistletoe) is a hemi parasitic shrub, frequently globular in shape. It grows on the branches of other trees, to which it is attached by a swelling called a haustorium. As a hemi-parasite it depends on its host for water and mineral nutrients but is able to photosynthesize (create its own carbohydrates using sunlight) because it has green leaves and stem. Two prominent types of V. album, European and American, contain very similar protein constituents but have different medicinal uses. Both species contain lectins (viscum/agglutinin), protein toxins, alkaloids and polysaccharides. The lectins are structurally similar to to ricin and abrin. According to Franz the lectins are cytotoxic glyco-proteins, they cause cells to agglutinate and inhibit protein synthesis on the ribosomal level. The lectins are dual chain molecules. Chain A inhibits protein synthesis and chain B activates macrophages and releases lymphokines from lymphocytes. Both chains have been reported to inhibit allergen-induced histamine release from leukocytes and collagen-induced serotonin release from platelets. According to Bussing and Schietzel, the amounts and biological activity of V. album lectins are dependent on the host tree, manufacturing process, and time of harvest.

The extract of the leaves of Viscum album has been used for centuries in traditional medicine in many parts of the world. In some parts of Africa, the plant is used both in the traditional and complementary management of many diseases. In Nigeria, the extract of the leaves of V. album is used in traditional medicine for the treatment of several ailments including hypertension, improve cardio-vascular functions; prevention and management of stroke. A number of biological effects, such as anticancer, antimecobacterial, antiviral, apoptosis inducing and immunomodulatory activities have been reported for mistletoes. It has also been reported to be effective in the management of chronic metabolic disorders such as diabetes and free radical scavenging. Clinical studies reported changes in immune parameters in humans during and after Viscum album application.

Blood is a good indicator to determine the health of an organism. It is also a good pathological mirror of the entire body. Cellular component of blood is valuable in immunotoxicology to evaluate immunotoxic potential of a compound. To this end, haematological parameters are important in establishing the body's functional status as a result of exposure to toxicants. Therefore, in this study, the acute effects of extracts of mistletoe, harvested from three hosts, on haematology of wistar albino rats was investigated.

Methods
Experimental plant
The fresh leaves of mistletoe plant (Viscum album) from host plants coffee, cocoa and kola were obtained from Cocoa Research Institute of Nigerian (CRIN) Ildi Ayunre Ibadan, Oyo state, Nigeria. Authentication of the plants was done in the same institute.

Preparation of aqueous extract
The fresh leaves of mistletoe plant (Viscum album) were sorted out to remove extraneous material and rinsed with water to remove debris and dust particles. They were air dried and pulverized. A portion (50g) of the powdered leaves was weighed into a beaker and 800ml of warm distilled water was added and stirred for 20 minutes. This was left to stand for 24 hours. It was then filtered using whatman filter paper to obtain the filtrate. Fresh extracts were prepared every 72 hours.

Experimental animals
Sixty-five (65) healthy male Wistar albino rats weighing between 150g and 200g were used for the study. Animals were allowed access to portable drinking water and rat Chow (Vital feed®, Nigeria) ad libitum unless where otherwise stated. Handling of rats was as approved by the University's ethics committee.

Pilot toxicity / acute toxicity test
The animals were divided into 12 test groups of 5 rats each. The 13th group (A) served as control and was administered 0.2ml distilled water throughout the period of the experiment. The 12 groups were subdivided to four (B-E). Each sub-divisions were administered increasing doses of extracts of mistletoe from different host plants. The rats in the test groups B, C, D and E were administrated 400, 800, 1600 and 3200mg/kg body weight of the extracts respectively. Feed and water were withdrawn prior to administration of the extract and restored after. The rats were observed for death or change in behavior throughout the experiment. Administration of the extracts and distilled water were done once daily for 14 days between 7.00 - 9.00 hr.
Collection of blood samples for analysis
Twenty four hours after the last administration of extracts (15th day of study), blood samples were collected through the orbital plexus of the rats, under anaesthesia into anti-coagulant bottles for haematological analysis. Haematological parameters were evaluated on whole blood using an automated analyser (Mindray Auto hematolgy Analyser BC-5500).

Results

TABLE 1-Effect of Viscum album (mistletoe) extracts from coffee (Coffee arabica) on hematological parameters in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Doses mg/kg body weight</th>
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<tbody>
<tr>
<td></td>
<td>Control (A)</td>
</tr>
<tr>
<td>PCV %</td>
<td>44.75±1.97</td>
</tr>
<tr>
<td>RBC ×10^12/μL</td>
<td>7.98±0.30</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>15.43±0.63</td>
</tr>
<tr>
<td>MCV fL</td>
<td>55.90±0.87</td>
</tr>
<tr>
<td>MCH pg</td>
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</tr>
<tr>
<td>MCHC g/dL</td>
<td>34.68±0.23</td>
</tr>
<tr>
<td>WBC ×10^3/μL</td>
<td>11.08±2.34</td>
</tr>
<tr>
<td>Neutrophils ×10^3/μL</td>
<td>0.78±0.41</td>
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<tr>
<td>Lymphocytes ×10^3/μL</td>
<td>8.84±1.25</td>
</tr>
<tr>
<td>Monocytes ×10^3/μL</td>
<td>0.64±0.63</td>
</tr>
<tr>
<td>Eosinophils ×10^3/μL</td>
<td>0.91±0.48</td>
</tr>
<tr>
<td>Platelets ×10^12/μL</td>
<td>1042.25±61.65</td>
</tr>
</tbody>
</table>

*Significantly different from control at p<0.05

The effects of the extract of mistletoe from Kola on platelets, PCV, RBC and WBC on rats were the same as those administered extracts of mistletoe from coffee.

TABLE 2- Effect of Viscum album (mistletoe) extracts from kola (Kola nitida) on hematological parameters in rats.

<table>
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Administration of mistletoe from coffee (800mg/kg - 3200mg/kg body weight) led to a decrease in platelet counts in rats (Table1). Also observed was a dose-dependent and statistically significant reduction in haemoglobin concentration of rats administered 1600mg/kg and 3200mg/kg of the extract. The extract caused reductions in packed cell volume (PCV) and red blood cell count (RBC). An increase in white blood cell (WBC) was observed in rats following the administration of the extract.

The data was subjected to statistical analysis with the level of significance set at p < 0.05 using statistical package for social sciences (SPSS) to determine any significant relationship between the groups. Results are expressed as mean ± standard deviation (SD).
Administration of extracts from cocoa caused a decrease in platelets, reduction in haemoglobin concentration, PCV, RBC and increase in WBC in rats.

Discussion

Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems. They can also be used to explain blood relating functions of chemical compound/plant extract. Such laboratory investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational research disease diagnosis, prevention and treatment.

From the results of this study, administration of mistletoe from three host plants i.e coffee, kola and cocoa led to a decrease in platelet counts in rats (Tables1, 2 and 3). Administration of mistletoe from kola caused a concentration dependent and statistically significant (p<0.05) reduction in platelet count in rats at 400mg/kg, 800mg/kg, 1600 and 3200mg/kg respectively. Also, reduction in platelet counts in rats given mistletoe from cocoa was also statistically significant (p<0.05) at 400mg/kg, 800mg/kg and 1600mg/kg. Reduction in platelet counts in experimental animals has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin. Reduction in PCV was statistically significant in rats administered 3200mg/kg of mistletoe from cocoa and coffee and the reduction in RBC was significant in rats administered 1600mg/kg and 3200 mg/kg of mistletoe from coffee. The observed decrease in PCV is believed to be as a result of the decreased RBC. Decrease in haematological indices in exposed animals indicates destruction of erythrocytes.

This is an indication that the extract could affect erythropoiesis in animals. The observed decrease in RBC count, Hb and PCV may therefore be assumed to be associated with retarded haemopoiesis, destruction and shrinkage of RBC. Also, the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues could be affected following the extract administration since RBC and Hb are very important in transporting respiratory gases. These results suggest that chronic administration of mistletoe extract may induce some level of anaemia.

Increase in white blood cells (WBC) was also observed in rats after administration of mistletoe, although the increase was not statistically significant. The crucial role of WBC in defending the body against infection and tissue damage is well known. This supports previous reports that mistletoe and some commonly prescribed medicinal plants contain agents that stimulate the production of leucocytes. This suggests that the extract may have immune boosting effect on the animals. Such effects may also be due to increase in vascular permeability. Administration of the mistletoe extract appears to exhibit stimulatory effect on the effectors cells of the immune system. Immune boosters are usually recommended to strengthen and harmonize degenerative systems and assist the immune system to fight invading agents such as bacteria and viruses.

There were no statistically significant changes in MCV, MCH and MCHC values in rat administered mistletoe from the three host plants when compared with control. It could be concluded from this study that although mistletoe from the three host plants had no significant effect on most of the hematological parameters in rats but the administration of the extract caused reductions in PCV, RBC, platelets, and hemoglobin in rats. This suggests that the use of this extract especially at high doses could suppress the haemopoietic system.

Conclusion

It is therefore advisable that the use of this extract in herbal medicine should be with some cautious measures to avoid the risk of anaemia in patients treated with the extract. Also, the increase in the WBC count observed in rats administered mistletoe from the three host plants suggests that mistletoe extract contains agents that could stimulate the production of leucocytes, therefore the plant extracts could serve as immune booster.

References


