TOXICOLOGICAL ASSESSMENT OF METHANOL EXTRACTS OF DELONIXREGIA SEEDS IN WEANER RABBITS

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Abstract
This study was carried out to determine the toxicity of extracts from Delonixregia seeds so that they could be used for ration formulation of rabbit diets. The pods of Delonixregia were collected from 30 trees at Ahmadu Bello University Zaria and soaked in body of water for the pods to split. The seeds were sun-dried and grounded in hammer mill. The toxicity was determined using extraction funnel, methanol and vegetable oil to administer the extract on rabbits. The toxicity and anti-nutritional factors of Delonixregia were determined. It was observed that there was no sign of drowsiness, depression and death on the nine (9) rabbits studied at 1000/100ml per body weight. The extract could not kill the test population by using median lethal dose LD(50) which is the index for acute toxicity. It was concluded that Delonixregia seeds are not toxic since the extract could not kill the test population – LD(50) which is the index for acute toxicity.

Keywords: Toxicological, Extracts, Death, Delonixregia seeds.
Background to the Study
Seeds often contain factors which are deleterious or indeed toxic to animals or man (Liener, 1996). There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition (Igile, 1996). The knowledge that these compounds elicit both toxic and advantageous biological response has given rise to several investigations in recent times as to their possible physiological implications in various biological systems (Igile, 1996). It is therefore essential that any potential food sources should be examined for toxicity. A general survey of anti-nutritional properties of Delonixregia seeds had been considered (Kaga, 2011). Plants and seeds in their natural state vary in potency and may contain multiple pharmacological substances which can cause under scribe side effects. Mengset et al, 2004.

Investigation of acute toxicity is the first step in the toxicological investigations of an unknown substance. Whether the chemicals in the seeds are very toxic, toxic, less toxic or not toxic at all. Anti-nutritional factors diminish animal productivity but may cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992). Substances which occur naturally in food manifest their toxicity especially when consumed with food in large or little doses. It is therefore essential that any food sources should be examined for its toxicity before incorporation into rabbits diets.

Objectives of the Study
1. To examine the toxic effect of Delonixregia seeds
2. To determine the median lethal dose of LD(50) of Delonixregia (Hook)

Materials and Methods
Plant seeds: the seeds were collected at Ahmadu Bello University Zaria, Bassawa and Palladan areas of SabonGari Local Government Area of Kaduna State. Large quantities of the pods were collected and soaked in a pool of water batch by batch for 3 days to ensure plitting of pods, since Delonixregia seeds are dispersed by explosive mechanisms. The seeds were sun-dried and grounded in hammer mill.

The toxicity of the seeds was determined using extraction funnel and methanol to remove the extract. Delonixregia seeds were oven dried and ground into fine powder. The powdered flour was put in an extraction funnel and methanol was added. This was allowed to stand for 24 hours for the extraction of the seed concentrate to take place (cool extraction). The concentrate was allowed to stand for several days until the methanol evaporated leaving only the seed concentrate. Care was taken to ensure that no trace of the methanol was found in the concentrate. 2.5grams of the extract was dissolved in 3ml of pure vegetable oil, which was sealed
before used. This was made up to 500g/mls. The rabbits were distributed into 3 rabbits each of almost the same weight and age and were administered with methanolic seed extract at doses 10, 100, 1000mg/kg body weight intraperitoneally, and observed for signs of toxicity and death for 24 hours. The mean lethal dose LD\(_{(50)}\) was determined using the method previously described by Lorke (1983) but modified by allowing the concentrate to stand for several days to allow the methanol to evaporate (Kaga, 2011).

**Statistical Analysis**
The data obtained in this work were subjected to statistical analysis using statistical programmes in Microsoft Excel and Statistical Package for the Social Sciences (SPSS 10.0 package). The statistical analysis carried out was mean and standard deviation, analysis of variance (ANOVA). Duncan's Multiple Range (DMR) test (Ogbeibu, 2005).

**Results and Discussions**
The acute toxicity of *Delonix regia* seeds is presented in table 1. The acute toxicity carried out on rabbits shows that there were no signs of drowsiness and depression. Similar results have been obtained by Vaishali et al., (2012) on the extract of the leaves of *Delonix regia* seeds (150-200g) administered to Wister albino rats and mice (20 – 25g) of either sex. According to these authors, it was safe up to a dose level of 5000mg/kg of body weight and no lethality or any toxic reactions were found up to the end of their study period. The absence of death in the 3 groups' of rabbits treated with *Delonix regia* seeds concentrate as high as 1000g/100ml of the extract indicated a high margin of safety (Lorke, 1983). The exposure of rabbits to these seeds aqueous extract did not change significantly the body weights, physical and behavioural characteristics of the affected rabbits which implies no adverse effect on the metabolic activities of these rabbits. A similar result was obtained by Isitua and Ibeh (2013) when extract of Moringa Oleifera and Caulis Bambusae leaves were given to rabbits.

The seeds of *Delonix regia* are safe for inclusion into rabbits and monogastric diets since 1000g/100ml of the concentrate could not kill 50% of the test population (LD\(_{(50)}\)). It was not possible to determine the LD\(_{(50)}\) of the extract because high volumes of it would have been administered and could result into death due to volume effect rather than a toxic effect of the extract. A similar result have been reported in aqueous extract of *Senna miller* pods given to rabbits. The observed toxic effect was due to higher doses or frequency of administration. (Ibrahim et al., 2012) This unaltered toxicological profile indicates that the extract was free of any side effect. These facts were confirmed by the results of the anti-nutritional factors Kaga (2011).
Awoniyi et al. (2000) observed that nutritional studies should not be limited to performance, carcass quality and nitrogen alone, but the effect on blood constituents is also very relevant. Furthermore, Biobaku (1993) in a study reported that during the process of digestion toxic substances present in raw legume grains usually associate with protein of the diets resulting in increased fecal nitrogen excretion and decreased protein digestion. It is therefore suggested that appropriate cooking temperature should be sort for seeds.

**Conclusion/Recommendations**

The result of this study indicates that *Delonix regia* seeds are not toxic since there was no depression, drowsiness and death recorded. The seeds are safe for inclusion into rabbit’s diets since 1000g/100ml of extract could not kill the test population (LD₅₀).

1. It is therefore, recommended that the seeds should be cooked for 60 minutes at 100°C to reduce all anti-nutritional factors in the seeds.
2. Effort should be geared towards finding other means of processing the seeds in order to render them more useful and useable as protein source for monogastric animals.
3. Farmers are encouraged to use *Delonix regia* for ration formulation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight of Rabbits (g)</th>
<th>Dose per 100ml</th>
<th>No. of Deaths</th>
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