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ANIMAL SCIENCE

Toxic effects of *Abrus precatorius* L. seeds on laboratory rats[◊]

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Abstract

In traditional medicine people uses the seeds of *Abrus precatorius* L. plant (commonly known as peony) for multiple purpose. The objective of this investigation was to study the toxic effects of the seeds from the *A. precatorius* in experimental rats. An experimental study of diagnostic type with analytical, explanatory, and descriptive phases was made. The study consisted of two phases: Phase 1: diagnostic phase where a survey was conducted with 30 people without sex distinction. Phase 2: experimental phase where 15 rats of the Sprague-Dawley race, from both sexes and 300g of weight, were used, distributed in 2 groups. Group 1 (control): it consisted of 3 rats to which distilled water was administered; Group 2 (experimental): constits 12 rats to which the watery extract of the peony seeds was administered. The 83% of the 30 surveyed people (Phase 1), knows the peony seed; 40% uses it; and 67% ignores that the seed is toxic. The pathological and anatomic findings revealed that the extract of peony seeds had a poisonous effect on the endothelial wall in the rats of Group 2. These findings could be correlated with the observed clinical manifestations.

Key words: Traditional medicine, *Abrus precatorius*, Toxoalbumin

Introduction

From time immemorial, the knowledge about plants and their medicinal values were existing, but only a little bit is known on their active principles, attributing them with nonexistent properties. All these knowledge have been transmitted from generations to generations assuming them as if they were certain, not knowing the ways to prepare them, toxic properties neither how to dose them to obtain a therapeutic effect. In extreme cases, they produce adverse reactions that in some cases, can cause very serious sequels and even, ultimately, death.

At the moment, there have been implemented health policies that have stimulated the use of medicinal plants as a therapeutic alternative. According to the World Health Organization (WHO), 80% of the people from developing countries trust on traditional medicine for primary medical and health care (García et al., 2001). Venezuela has a very rich medicinal flora and is widely used by populations that, in some occasions, do not know the properties of the plants (Perrinet al., 1977). Latin America has many communities that depend on herbal medicine and traditional healers for the care of their health (Siles et al., 2004).

Peony (*Abrus precatorius* L.) is a perennial climbing vine from the mountains of India that easily reaches 5 meters in length. It has ligneous stems in the base and herbaceous in its top. Its leaves are complex, displaying an even number of opposite leaflets. Its flowers are papilionaceous and are grouped in a cluster, with purple pink or reddish corollas, and also white in rare occasions. Fruits are flattened seedcases, which contain three to seven hard seeds, scarlet-red color and a peculiar, dark spot at one end (Sisa, 2009).

The seeds of the peony have been recognised for their toxicity since times immemorial. And so many poisonous plant materials including their seeds have been extensively used in folk medicine against a variety of illnesses and also for criminal purposes (Olsen, 2004; Martínez, 2003, 2006, 2008).

The presence of amino acids in the seeds of peony has been described and they could be responsible for their toxic and hem-agglutinating properties (Jurado, 1989; Ganem et al., 2000). Abrin, a highly toxic product from *A. precatorius* is

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shown to exhibit antitumor activity on Yoshida sarcoma (solid and ascites forms) in rats and a fibrosarcoma in mice (Subba Reddy, 1969). Moreover isoflavanquinones named abruquinones have been isolated from the roots of *A. precatorius* and exhibited with remarkable inhibitory effects on the platelet aggregation (Kuo et al., 1995).

It has been demonstrated that phytotoxins produced by the seeds of *A. precatorius* have high toxic activity and it was verified that the abrin can cause gastrointestinal problems, circulatory collapse, comma, and death, depending on the amount of completely chewed and ingested seeds (Villaseñor, 2004).

For the previous given reasons, the objective of this work was to determine the knowledge that people has about the seed of *A. precatorius* and to evaluate the poisonous effects of this seed in experimental rats.

Materials and Methods

Diagnosis phase

The investigation was made in two phases, a diagnosis phase that allowed obtaining the data of the traditional knowledge that people has about this plant toxicity, which are the basis this study. The second phase was a pure experimental part being the independent variable the administration of the peony seed to the experimental rats, and the dependent variables where the anatomic pathological findings that observed in the rats, after the administration of the referred seed.

In first stage, the sample was thirty (30) people without age or sex distinction, who were close to perfumery and plant sales located in Maracay, Municipality of Girardot, Aragua State, between May and July 2009, to determine their knowledge about the use of the seeds of *A. precatorius* to whom a survey was applied to know what information level they had on the use of the seeds of this plant.

Pharmacological experiments

In the second phase, 15 rats of the Sprague-Dowley stock were used for the experiments. They had a weight of 300g and 3 months old, and obtained from the Instituto Venezolano de Investigaciones Científicas (IVIC) and lodged in the animal station of the Institute of Biomedical Investigations of the University of Carabobo (BIOMED-UC). They were distributed as follows: a control group of three (3) rats signed 1 to 3 respectively, which were sacrificed for pathologic anatomy control and later comparison with the experimental group, and a second group of 12 rats, signed from 1 to 12, that constituted the experimental group. The watery extract of peony was administered through a nasogastric cannula, and subsequent to their death or sacrifice, the necropsy was performed and their organs properly preserved. The experiments are conducted in our lab under permitted ethical regulations and rules.

Rats from the control group and those that did not die during the experiment, were sacrificed by the method of cervical dislocation. Necropsy was performed to each animal and the dissected organs were placed in 10% formaldehyde water solution for preservation. Later, each piece was placed in a properly prepared slide for microscopic pathological anatomy evaluation. This phase was conducted in the Department of Pathological Anatomy, Faculty of Veterinary Sciences, of the Universidad Central de Venezuela at Aragua State.

Biological Material

The authentication of the plant was made in the herbarium "Victor M. Badillo" in the Institute of Agricultural Botany, Faculty of Agronomy, Universidad Central de Venezuela at Aragua State. 12 seeds from *A. precatorius* were taken and their weights were individually registered, verifying that the average weight of the seeds was 0.1g. The seeds were crushed to dust in a mortar. 0.1g of dust was weighed for each rat, equivalent to a seed. A watery extract was prepared by adding 7 mL of distilled water to each dust aliquot.

Results

The surveyed people, in the diagnosis phase, were asked if they knew the seed of peony (Figure 1).

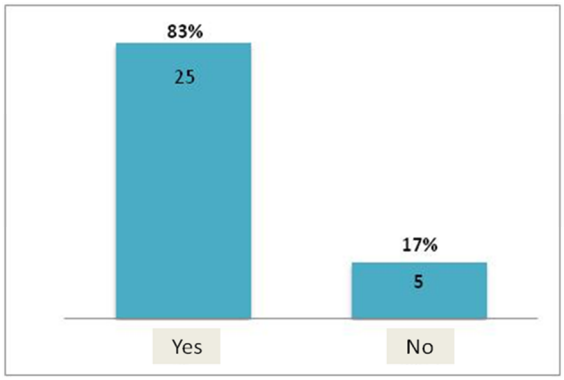

Figure 1. The survey results for - Do you know peony seed?

It can be observed that twenty five (25) people referred to know the seed of peony, which is 83% of the surveyed people.

The second question asked, if they ever heard about the use of seeds of peony. Twenty-six (26) people declared to know what the seed was used for. This represents 87% of the total surveyed

people, and four (4) people answered to ignore the use of those seeds, which represents 13% of the surveyed people.

The third question asked was, if they had ever used the seed, to which thirteen (13) people (43%) said they had used it, and 17 people (56%) referred they had not used it.

The fourth question enquired about the uses that surveyed people gave to the seed of peony (Figure 2).

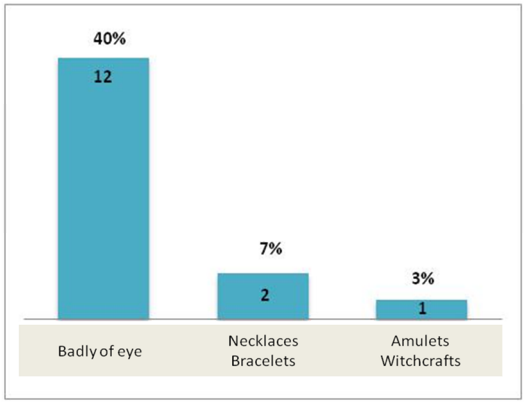

Figure 2. The survey results for – What are the uses of seed of peony?

Here it is observed that twelve (12) people use the seed against religious beliefs which represents 40% from the total, two (2) people (7%) use them as necklaces or bracelets, and finally one (1) person (3%) declared to use it as an amulet.

Question 5 enquired if the surveyed people knew the toxicity of these seeds (Figure 3).

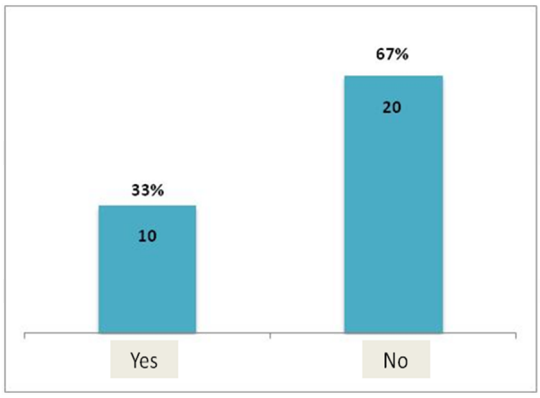

Figure 3. The survey results for – Have you ever heard that the seed of peony is toxic?

It was discovered that 20 people (67%) do not know the poisonous effects of peony seeds, whereas 10 people did know it, which represents 33%.

Results from Phase 2

Rats from the control group turned out to be in good conditions, with an approximate weight of 350g and without suggestive findings of being carrying some type of pathology. The results of the microscopic examinations are shown in Figure 4-8.

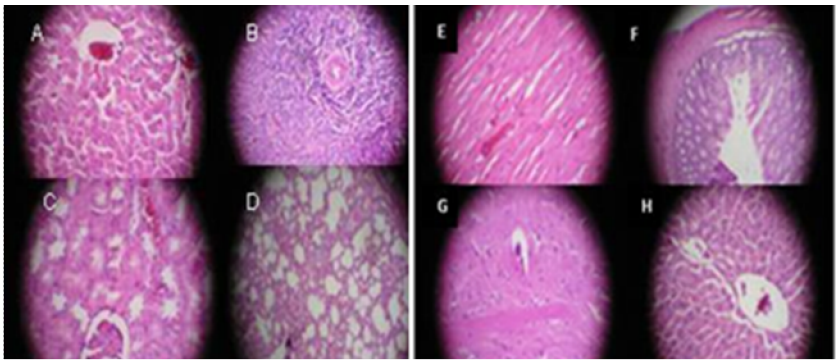

Figure 4. Histological sections in control rat group.

In the histological sections of the rat N°2 (control group) it was observed: A. Liver: fatty intra cytoplasmic degeneration of the hepatocyte. B. Spleen: presents no alterations. C. Kidney: increased Bowman space. D. Lungs: mixed focal polymorphoneutrophilic peribronchitis. E. Heart: showed no injuries. F. Small intestine: showed no alterations. G. Stomach: presented no alterations. H. Brain: presented no alterations.

In the experimental group the most severe injuries were observed in rats 2, 3, 11 and 12.

Rat N°2 of the experimental group died on the second day of the experiment. The necropsy was performed, in which it was microscopically observed

distension of thin loops and pulmonary hemorrhagic dots in multiple areas. The microscopic examination is described in Figure 5.

Like rat N°2 rat from the experimental group, rat N°3 rat died after 24 hours to have initiated the experiment. Microscopically it was observed distension of intestinal loops with microgranulations, congested lungs with hemorrhage in multiple areas and hepato splenomegaly. The microscopic findings can be observed in Figure 6.

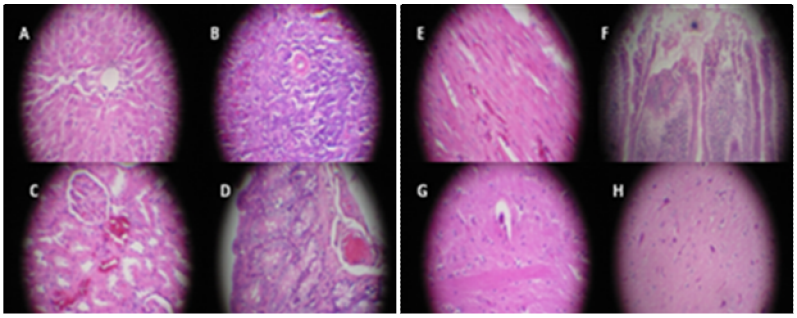

Figure 5. Histological sections from rat N°2 in the experimental group.

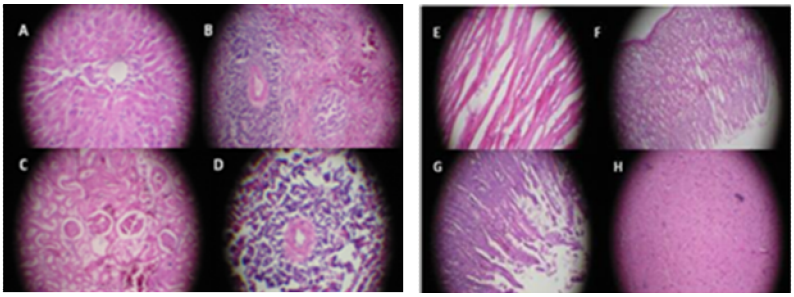

Figure 6. Histological sections from rat N°3 in the experimental group.

Histological sections of the rat N°3, from the experimental group, were observed: A. Liver: Noticeable congestion. Periacinar Necrosis. Slight focal hemorrhage. B. Spleen: severe lymphatic depletion, hemorrhage, and hemosiderosis. C. Kidney: It did not showed hemorrhage. Presence of hydropic degeneration. D. Lungs: Congestive, Diffuse edema. E. Heart: slight interstitial focal hemorrhage and myofibrillar edema. F. Small intestine: severe duodenal hemorrhagical enteritis. G. Large intestine: necrotic and hemorrhagical enteritis. H. Brain: severe brain edema.

Rat Nº 11 started to have severe respiratory difficulties, intercostal retractions, and loud expiratory grunt from the experimental group, after 72 hours under experimental conditions. There was evident the presence of an episode of moderated epistaxis. Soon after, the rat was sacrificed by cervical dislocation with an immediate brilliant strong bleeding through the nostrils. Necropsy showed severe pulmonary hemorrhage, liver with a accented lobular pattern and a severe hepatomegaly, friable kidney, splenicontraction, presence of gaseous colitis because the rat was hiporhexic, and brain con hemorrhagic aspect (Figure 7).

Observations of the histological sections from rat N°11 in the experimental group were: A. Liver: Severe fat degeneration and slight focal hemorrhage. B. Spleen: amyloidosis, thrombosis of the splenic artery, focal slight limphoid depletion. C. Kidney: glomerular synechiae, thrombosis, vascular rexis. D. Lungs: thrombosis and slight focal edema. E. Heart: intermyofibrillar edema. F. Submucous congestion, slight focal hemorrhagic enteritis. G. Large intestine: it did not show apparent injuries. H. Brain: severe edema. Espongilosis.

Rat N° 12 remained active till the fifth day reason why it was sacrificed for posterior necropsy. Microscopic findings were: delimited and slight pulmonary hemorrhage, hepatosplenomegaly.

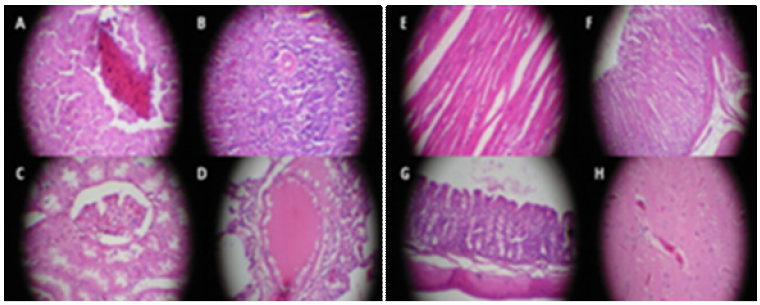

Figure 7. Histological sections from rat N°11 in the experimental group.

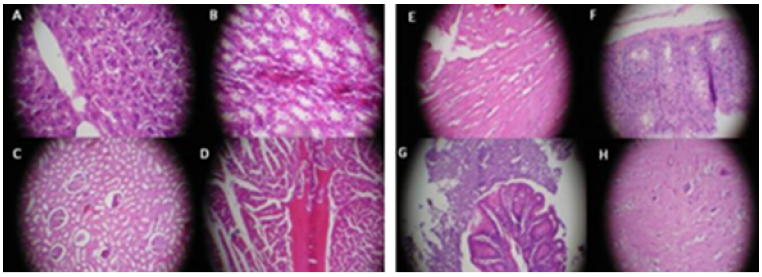

Figure 8. Histological sections from rat N°12 in the experimental group.

The histological sections from rat N°12 in the experimental group, presented: A. Liver: severe focal fat degeneration. Zonal severe hepatic necrosis. B. Spleen: amiloydosis, thrombosis of splenical arteriole. Necrosis. C. Kidney: severe interstitial hemorrhage, hydropic degeneration. D. Lungs: thrombosis, congestion and pulmonary hemorrhage. E. Heart: severe thrombosis. F. Slight focal hemorrhagical enteritis. G. Large intestine: It did not have apparent injuries. H. Brain: Slight neuroaxonal edema.

Discussion

The seed of *Abrus precatorius* L. is referred as protective amulet in the children in traditional knowledge. Having this in mind, a diagnostic phase was evaluated, which revealed that 40% of the surveyed population have used the seed as an amulet while 67% expressed not to know the toxicity of the seed and related lethality over ingestion of the peony seed. The obtained results in the second phase, demonstrated that the 12 rats that constituted the experimental group, presented poisoning which was manifested in the clinical studies and was corroborated by the posterior anatomic pathological analyses.

These analyses allowed observation of the of vascular hemodynamic changes present, clinically characterized by epistaxis, microscopically and histologically by edema, congestion and pulmonary hemorrhage, sub-epicardial hemorrhage, brain and splenic hemorrhage, brain edema and hemorrhagic gastroenteritis, with variable degrees of intensity. Vascular changes were observed by rhexis.

The rest of the rats from the experimental group showed similar injuries but less intense. The anatomic pathological study finally revealed that the macroscopic and histological findings show a poisonous effect of the studied substance on the endothelial wall of the rats in the experimental group.

Conclusion

The ethno botanical use of different parts of *Abrus precatorius* is common in Venezuela for the treatment of various bacteria-related diseases. The results substantiate that the ingestion of the watery extract of *A. precatorius* seeds had poisonous effects in experimental animals which were feed with it during the study. This demonstrates that the active principles present in the seed of *A. precatorius* are highly toxic and how the use of this seed is common in Venezuela, because it is a part of the cultural heritage. This implies that this cultural practice is putting in risk health and wellness of the infantile population. For this reason, it becomes mandatory to form the health personnel so that they could contribute to the diffusion of these poisonous effects at community levels, and ultimately to diminish the risk of poisonings by the ingestion of this seed.

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