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TOXICITY OF EVISECT'S (THIOCYCLAM HYDROGEN OXALATE) TO NUBIAN GOATS

AMANI B.A.ABDELMAGED[1]*, MOHAMED O.S.A.[2], SAEED A.E.M.[3]
[1]Department of Veterinary Medicine, Pharmacology and Toxicology, College of Veterinary Science, Baheralghazal University, Sudan
[2]Department of Clinical Studies, College of Veterinary Medicine and Surgery, Sudan University of Science and Technology, Khartoum, P.O.Box 407, Sudan
[3]Department of Chemistry, College of Science, Sudan University of Science and Technology , Khartoum, P.O.Box 407, Sudan
*Corresponding Author: Email- aemsaeed@gmail.com Fax: +249 183 769363 Telephone: + 249 183 774843

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Abstract- The toxicity of Evisect was studied in Nubian goat kids at oral dose rates of 500, 250, 50 and 25 mg / kg / day b.w. The clinical signs were profuse frothy salivation, abdominal pain, tremors of face and jaws muscles, erected tail, staggering, upward kink of the neck, backward movements, convulsions, recumbent and death occurred within 15 min. and 13 days. The pathological findings include satolytosis and slight gliosis in the cerebrum and cerebellar tissues, pulmonary emphysema and oedema, heart flabbiness, scattered foci of inflammatory cells infiltrating the liver tissue and gastrointestinal tract and slight renal tubular dilatation and necrosis. The serum glucose concentration was significantly ($p < 0.05$) decreased.
Key words- Evisect"S", toxicity, Nubian goats

Introduction

Livestock wealth in the Sudan is considered to be a leading in Sudanese economy contributes about 20 to 25 % of the gross domestic product. The economy of Sudan is also depends on agriculture, as such several agricultural schemes were established. The increase in both human and animals herds populations necessitates availability of food and fodder. The boost in agriculture production is inseparable from usage of chemicals like fertilizers and pesticides. However, their toxic potential is still alarming particularly with negligence or indiscriminate use. In Sudan Abdellatif (1993) described pesticides pollution to be relatively in less degree and restricted to irrigated, mechanized and traditional agricultural areas. But in recent years as the result of expansion in cultivation, large quantities of diversified pesticides are in use. According to the Plant Protection Directorate and Department of Pesticides reports, more than 600 pesticides are registered in Sudan of which more than 400 are in use. Severe cases of poisoning by pesticides were reported in Sudan. For example, (Saad 1991) reported that, in 1991 in Barber area about 31 people died due to consumption of treated sorghum with endosulfan, which stored since 1983. Between 2001 and 2002, farmers in northern Ghana averaged 15 workdays lost each season due to acute poisoning (Pretty, 2005).
Analogues of nereistoxin have been known for decades. They generally are stomach poisons with some contact action and often show some systemic action. A major share of the development and use of these compounds has taken place in Japan. They are based on a natural toxin of the marine worm *Lumbriconereis heteropoda*. Of the many analogues synthesized only those that were metabolized back to the original nereistoxin after application were active. In this sense members of this class (Cartap, Bensultap and thiosultap-sodium) are *proinsecticides* in that they are applied in their manufactured form but are known to degrade to a specific active component. The members of this group tend to be selectively active on Colopteran and Lepidopteran insect pests (Ware and Whitacre, 2004). Due to its moderate toxicity, it offers a good level of safety for man. It does not affect cholinesterase activity .Its residues are rapidly degraded and do not persist in the environment (Tomlin 2000).Members of this class act as acetylcholine receptor agonists at low concentrations and as channel blockers at higher concentrations.
A little is known about the toxicity of Evisect insecticide. Therefore, the present study was designed to investigate and describe the acute and subchronic toxicity of this compound as casualties in animals due to inadvertent ingestion might be expected.This might be the first report to investigate the toxicity of Evisect in large animals, at least in this species.

Materials and Methods

Experimental design

Evisect is a systemic insecticide and has a common name (Thiocyclam Hydrogen Oxalate) with chemical name(N, N-dimethyl-1,2,3-trithian-5-ylaminehydrogenoxalate), of molecular formula ($C_7H_{13}NO_4S_3$) and discovered and developed in Sandoz Laboratories, Basle, Switzerland. It is colourless and odourless crystalline solid. Found in powder form at concentration of 50% and obtained from Agricultural Research Center (Wadi Medni).

Animals, dosing and blood sampling

Fifty, healthy male Nubian goat kids of 5 - 6 months old and weighing 10 - 12 kg, were purchased from Hilat Kuku Goats Market, Khartoum North. They were kept in standard pens at the College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology. Goats were fed on forage sorghum (*Sorghum vulgarae)* and provided water *ad libitum*. The animals were kept 16 days for adaptation and acclimatization during which bacterial diseases, worms and coccidiosis were cleared. The animals were then divided randomly into 5 groups each of ten. Each group was kept separately.

Each goat in each group (1, 2, 3 and 4) received doses of Evisect by drench at the rate of 500, 250, 50 and 25 mg / kg / day b.w. respectively. Drenching was continued daily until the animals died or sacrificed. Goats in group 5 were not drenched and used as controls.

Each experimental animal was subjected to blood sampling on days 1, 3, 7, 14, 21, 28, 35 and 45 - post dosing. Additional samples were taken from animals in moribund condition. A volume of 10 ml blood was collected from the jugular vein puncture using a disposable 10ml syringe with 18.5 gauge needle. Immediately, 1 ml of the collected blood sample was poured into a small clean 5ml vacutainer containing anticoagulant EDTA (ethylene diamine-tetra-acetic acid) and used for the haematological investigations. Another 1ml was poured into a small clean 5ml vacutainer containing fluoride oxalate as an anticoagulant and used immediately for glucose measurement. The remaining blood was kept to clot overnight, centrifuged at 3000 r.p.m. for 5 min. and sera were collected and kept at -20 °C for serobiochemical analysis.

Experimental animals were closely observed for clinical signs and behavioural changes. Dead or sacrificed animals underwent post mortem examinations immediately and lesions were recorded. Samples from the cerebellum, cerebrum, heart, lungs, liver, spleen, pancreas, kidneys, abomasum, omasum and small intestines were collected and fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin (H&E) for histopatholoical investigations.

Haematological investigations

EDTA – anticoagulated blood were investigated according to the methods described by Dacie and Lewis (1991) for haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells count (RBC), white blood cells count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) .

Serobiochemical Analysis

Collected sera were analyzed for the activities of aspartate aminotransferase (GOT,AST,E.C.2.6.1.1.), alanine aminotransferase(ALT,GPT, E.C.2.6.1.2,), alkaline phosphatase (ALP, E.C. 3.1.3.1.) and for the concentrations of total proteins, albumin , total bilirubin, and urea using commercial kits, (Plasmatec Laboratory Products Ltd., England), and absorbance was measured in a spectrophotometer (Unicam 8625 uv / vis Spectrometer – PU , England). The concentration of serum globulins was obtained by subtracting values of serum albumin from the total proteins. Glucose concentration was measured using enzymatic colorimetric kit. (GOD-PAP method. Fouz Diagnostics Laboratory) (FDL) and the concentration was read in spectrophotometer (Perkin -Elmer 2380, Germany). Serum concentration of sodium (Na), potassium (K), calcium (Ca), inorganic phosphate (PO_4), magnesium (Mg), copper (Cu) and iron (Fe) was determined using the method described by Allen (1989). Calibration curve was prepared for each element. The sample solution was aspirated along with the standard according to the specific cathode lamp used and they were read out in the atomic absorption spectrophotometer (Perkin – Elmer 2380, Germany) against the deionized water as blank.

Statistical method

The data was analyzed using Students t- test (Byrkit, 1987).

Results

Clinical signs

Goats in groups 1 and 2 showed immediately post dosing profuse frothy salivation, abdominal pain, tremors of face and jaws muscles, erected tail, staggering, upward kink of the neck, backward movements, convulsions, paralysis of the hind limbs. These animals became recumbent and died within 15 min after the first dose. Animals in group 3 immediately post dosing showed moderate same clinical signs which accompanied with bloat and diarrhoea. These signs disappeared gradually within 4 hours but there was depression and decrease in the appetite. These animals died between days 10-13. Goats in group 4 showed mild clinical signs two hours post dosing, which lasted after four hours, and they were sacrified with the control group on day 45.

Post mortem lesions

The congestion and haemorrhages were the dominating features in the different organs of the drenched groups .In groups 1 and 2 the lesions were more severe than in groups 3 and 4. The heart was flabby and hydropericardium was seen in groups 3 and 4.The pulmonary emphesyma and oedema were recorded in all

drenched animals but more severe in group 1.The liver and the kidneys showed scattered foci of fatty changes and /or necrosis. Haemorrhagic foci were seen on the internal surface of the small intestines. No apparent lesions were observed in the rumen, omasum, abomasum, spleen, cutaneous blood vessels and lymph nodes. There were no gross lesions in the control goats (group 5).

Histopathological findings

Some neurons were surrounded by glial cells in the cerebellum and cerebrum tissues in all dosed groups. Pulmonary emphysema and oedema were detected and some alveoli maintained oval or rounded shape. The interstitial tissue was thickened and the interstitial septa infiltrated with lymphocytes and neutrophils specially in groups 3 and 4 (Fig.1). Some of the cardiac muscle bundles underwent necrosis and infiltrated with lymphocytes and neutrophils, these bundles were separated from each other. The liver tissue showed widening of portal cords and the portal tracts showed slight hyperplasia. Slight to moderate hepatocytic degeneration and necrosis were observed either in the central zone or as scattered foci. Scattered foci of inflammatory cells infiltrating the liver tissue were recorded. However, in the kidneys, the glomeruli were shrinked demonstrating widening of Bowmans space where the tuft lobulated, disappeared, or infiltrated with lymphocytes. Also there was slight renal tubular dilatation and necrosis particularly the medulla (Fig.2). The pancreas was congested and there was mild degeneration of some pancreatic acini. The abomasum and the small intestines showed slight hypercellularity of *lamina propria*. The intestinal lumen contained shreds of epithelial cells, inflammatory cells and RBC. Foci of haemorrhages were also seen in the submucosa and *lamina propria*. No lesions were observed in the spleen and the omasum. No histopathological findings were seen in group 5.

Haematological and serobiochemical findings

No samples were collected from goats in groups 1 and 2 because the animals died immediately post dosing. No significant difference was observed in the values of Hb, PCV, RBC count, WBC count, MCH, MCV and MCHC. No significant difference was recorded in the serum concentrations of total proteins, albumin , globulins ,urea, total biliburin, Na, K, Mg, Ca, P , Cu and Fe and in the activities of AST , ALP and ALT .The serum glucose concentration was significantly decreased ($P<0.01$).

Discussion

The present study investigated the toxicity of the Evisect in Nubian goat kids. The study was descriptive. Results showed that single oral dose of 250 mg / kg and more was toxic and fatal within 5 - 15 min. Clinical signs consisted of profuse frothy salivation, abdominal pain, tremors, erected tail, staggering and upward kink of the neck. Doses of 50 mg / kg / day was also toxic and fatal when drenched daily and death occurred within 13 days. Doses of 25 mg /kg / day caused mild toxicity.

George and David (2004) mentioned that Evisect act as acetylcholine receptor agonists at low concentrations and as channel blockers at higher concentrations. IRAC (2005), classified nereistoxin analogues thiocyclam as nicotinic acetylcholine receptor-agonists/antagonists. Meloin and William (1993) mentioned that cholinergic synapses are described as functionally related to nicotinic activity or muscarinic activity. Hence, the synapses at the ganglion cells of the entire autonomic nervous systems, as well as the somatic myoneural junctions were known to be excited by nicotine where it was in low concentrations, depolarizing the post synaptic or effector membrane. In higher concentrations it blocked the same site by preventing repolarization. Nicotine – induced blockade when sodium channels become non-responsive to depolarizing currents and the cholinoceptors themselves fail to respond to acetylcholine. Therefore, the cause of death can be attributed to cardiovascular and respiratory failure.

Sandoz Agro Division (1984) reported that the acute oral LD_{50} in male rats equal to 540 mg / kg while the results of the present study reported that the LD_{50} was 250 mg/kg and can be attributed to age and / or species differences.

The results of the present study showed that there were no significant changes in the Hb, PCV, WBC count, RBC count and RBC indices which may point to that Evisect has no effect in the haemopoeisis. Congestion in the vital organs can be attributed to the catecholamines which secreted due to stimulation of the cortical medulla of the supra adrenal gland. Pertram and Anthony (1993) indicated that α-adrenergic receptors of the vascular smooth muscles in the skin and splanchnic vessels were contracted by the release of catecholamines. Although there were no changes in urea, and in the electrolytes concentrations we believe that there was glomerulonephritis at early stage which confirmed by the histopathological findings.Coles (1986) mentioned that, absence of abnormal increase in UN during very early stages of acute renal shutdown, as time must elapse for a sufficient quantity of urea to accumulate to be considered abnormal and in chronic renal diseases, more renal parenchyma may be destroyed at a relatively slow rate permit remaining viable nephrons to undergo structural and functional compensation. Results of the present study showed that Evisect caused hepatic damage despite that there were no changes in the activities of AST, ALT and ALP because findings such as slight to moderate hepatocytic degeneration and necrosis with scattered inflammatory foci were observed. This means that the liver is still capable to function.

The slight decrease in serum glucose concentration might be attributed to the nicotinic effect agonist /antagonist of Evisect on the adrenal gland. Kenneth *etal* (2003) mentioned that glucocorticoids antagonized the effect of insulin and catecholamine activity, hence, altered blood glucose concentration by several mechanisms, of which β-adrenergic stimulus of

pancreatic β-cells increase insulin release. In addition, the present study reported damage on the acinar cells of the pancreas which might involve the beta cells of the islets of Langerhens which secrete insulin.

The diarrhea, the abdominal pain and the bloat might be attributed to the irritating action of the Evisect on the mucosa of gastrointestinal tract or as a result of neuronal effect.

The present study concludes that Evisect is fatal at single oral dose of 250 / mg / kg b.w., while doses of 50 / mg / kg / day are toxic and fatal when repeated for 13 days.

Further studies are needed to investigate the teratogenic and carcinogenic effects of Evisect in large animals. In addition, the pharmacology of the Evisect is also needed to be studied deeply in the different body organs especially of large animals.

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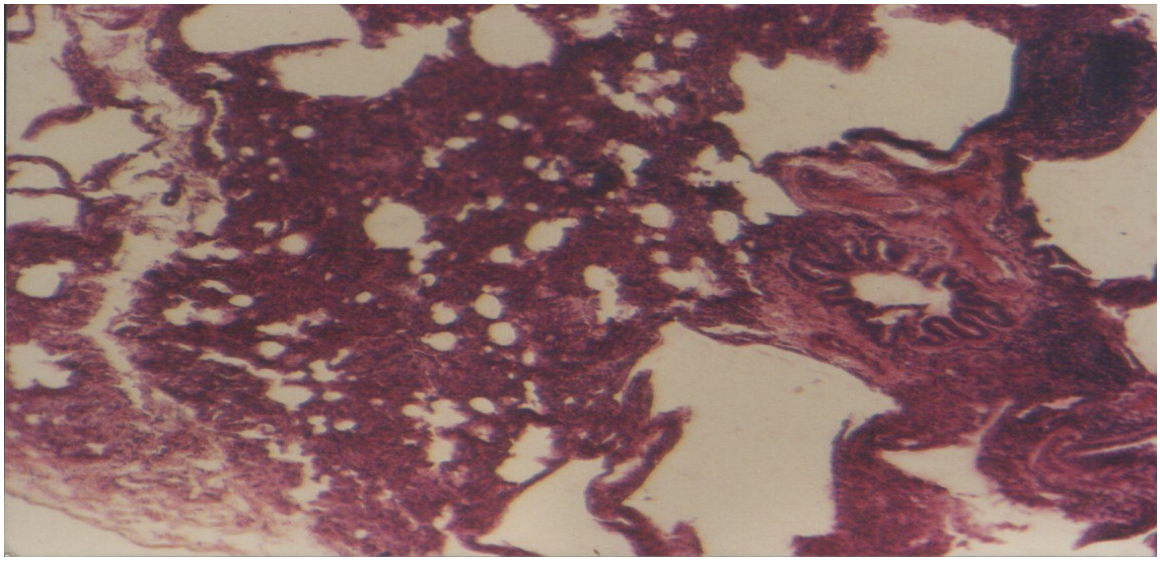

Fig. 1- The Lung of a goat in group (3) dosed with 50 mg/kg/day of Evisect and died on day 12 postdosing showed area of interstitial pneumonia. (H & E × 100)

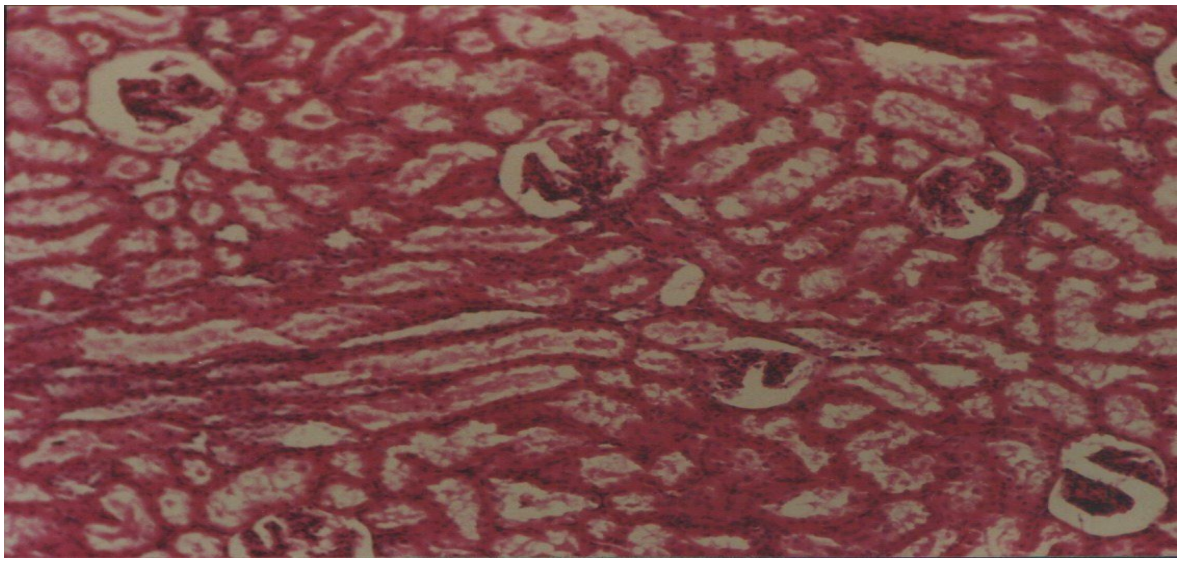

Fig. 2- The kidney of a goat in group (3) dosed with 50 mg/kg /day of Evisect, and died on day 12 postdosing, showed shrinking and lobulation of glomerular tufts, widening of Bowman's space and slight tubular dilatation. (H & E × 100)