

Effect of Combined Oral Administration of *Jatropha curcas* Seeds and *Hibiscus trionum* Dry Shoots in Nubian Male Goats

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ملخص البحث

أجريت هذه التجارب لدراسة تأثير نبات حبة الملوك (*Jatropha curcas*) مخلوطة مع نبات ويكة الخلاء (*Hibiscus trionum*) الجافة علي ذكور الماعز النوبية. في البداية طحنت بذور نبات حبة الملوك وكذلك نبات ويكة الخلاء الجافة ثم خلط النباتات بنسب متفاوتة: ٧٥% من ويكة الخلاء مع ٢٥% بذور حبة الملوك و٥٠% من ويكة الخلاء مع ٥٠% من بذور حبة الملوك، ٣٣,٣% من ويكة الخلاء مع ٦٦,٦% بذور حبة الملوك و٢٥% من ويكة الخلاء مع ٧٥% من بذور حبة الملوك. هذه الخلطات ذوبت في الماء كل على حده ثم أعطيت في شكل جرعات يومية ثابتة وهي ٢ جرام لكل كيلو جرام من الوزن الحي للمجموعات ٤،٣،٢ و٥ علي الترتيب أما ماعز المجموعة الأولى فقد استخدمت لمقياس للمقابلة والتحقق. أهم العلامات المرضية لهذا التسمم هو الإسهال الشديد والأنين وسرعة التنفس والجفاف وفقدان الوعي وعدم الحركة. حدث الموت في الماعز بين اليوم الثاني والخامس بعد تناول الجرعات. الآفات المرضية الواضحة هي نزف كثيف علي الأسطح المخاطية للجهاز الهضمي والرئوي والكبد والرئة والقلب والكلي مع قصور في وظائف الكبد وزيادة في تركيز إنزيم الـ AST ونقص في تركيز البروتين والكالسيوم في مصل الدم. إتضح بالمقارنة مع دراسات سابقة أن التأثيرات السمية للنباتين معاً اشد علي ذكور الماعز من التفاعلات السمية لكل نبات.

Summary

Studies were carried out in Nubian male goats fed with a mixture of *Jatropha curcas* seeds and *Hibiscus trionum* dry shoot in fixed proportion 75% *Hibiscus* and 25% *Jatropha* seeds; 50% *Hibiscus* and 50% *Jatropha* seeds, 33.3% *Hibiscus* and 66.6% *Jatropha* seeds and 25% *Hibiscus* with 75% *Jatropha* seeds. The mixtures of the two plants were dissolved in water and given by drench at a fixed dose level of 2 g/kg/day to each animal in group (groups 2, 3,4 and 5). Goats of group 1 were kept as undosed controls.

Profused diarrhoea, rapid respiration, abdominal pain, salivation, moaning, anorexia, reduced water intake, ataxia and recumbency were the prominent clinical signs of toxicity. Death occurred 2 to 5 days following feeding of the mixture. The main pathological changes were echymotic diffuse haemorrhage and congestion in the rumen, reticulum, omasum, abomasum, liver, heart, lungs and kidneys, catarrhal and/or haemorrhagic enteritis, ulcers in the small intestine and pulmonary cyanosis were also prominent.

There was a significant increase ($P > 0.05$) in the activity of the enzyme AST and a significant decrease ($P > 0.05$) in the concentration of total proteins and calcium in sera of dosed goats.

No significant ($P < 0.05$) changes in the concentration of bilirubin, magnesium and in the activity of the enzyme ALT. It was concluded that the combined feeding of the two plants is more toxic and lethal to Nubian male goats than feeding each plant alone.

Introduction

Hibiscus trionum (Malvaceae) shoots and *Jatropha curcas* (Euphorbiaceae) seeds were each found to be toxic to goats (Onsa et al., 2001; Onsa et al, 2007). The combined toxic effects of the two plants in domesticated animals have been overlooked.

Previous studies have shown an increased toxicity of the combination of *Citrullus colocynthis* and *Rhaza strica* in Najdi sheep (Adam et al., 2000) and an increased toxicity of the combination of *Capsium frutescens* and *Citrullus colocynthis* in rats (AlQarawi and Adam, 2003).

The seeds of *J. curcas* are used as purgatives, anthelmintics and molluscicides (Amin et al, 1972; Dafalla and Amin, 1976) and contain a purging oil, tannins, terpenes, coxallumins, crotin and curicin as predominant constituents (Watt and Breyer-Brand, 1962;). However, neither the predominant constituents nor the therapeutic values have precisely been determined for *H. trionum* shoots. The present investigation was designed to study the effect of combined oral dosing of *J. curcas* seeds and the dry shoots of *H. crinum* on nubian male goats.

Material and Methods

Twenty, 8-9-month-old Nubian male goats were used. They were penned, fed on *Lucerne* and watered *ad libitum*.

Dosing:

Sun-dried *Hibiscus trionum* shoots and *J. curcas* seeds were powdered and dissolved in water, and the variable mixture was given by drench at a fixed dose level of 2g/kg/ day to 16 goats (groups 2, 3, 4 and 5). They were mixed together at 75% *Hibiscus* and 25% *Jatropha* and given to 4 goats (group 2), 50% *Hibiscus* and 50% *Jatropha* to 4 goats (group 3) and 33.3% *Hibiscus* and 66.6% *Jatropha* to 4 goats (group 4) and 25% *Hibiscus* and 75% *Jatropha* to 4 goat (group 5). The 4 goats in group 1 were kept as undosed controls.

Blood Samples:

All goats were bled by the Jugular vein on three occasions before dosing and at two appropriate intervals after dosing for haematological studies and biochemical analysis of sera constituents.

Haematological Methods:

Blood samples were examined for haemoglobin concentration (Hb%), red blood cells (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) by standard methods as described by Schalm (1975).

Biochemical Methods:

Blood samples were allowed to clot and sera were separated and stored at -20°C until used. Sera were analyzed for the activities of the enzymes Aspartate aminotransferase (AST) and alanine amino transferase (ALT) according to the method of Reitman and Frankel (1957), concentration of total proteins was determined using kits (Randax laboratories, UK), total bilirubin by the method of Dangerfield and Finalyson (1953) and calcium and magnesium according to Frankel and Reitman (1963).

Pathological Examination:

Necropsy was performed on all goats immediately after death or slaughter to identify gross lesions. Portions from the liver, kidneys, rumen, reticulum, omasum, abomasum small intestine, lungs and heart were fixed in 10% neutral buffered formalin, processed, embedded in paraffin wax, sectioned at 4 to 6 μm thick and stained with haemotoxylin and Eosin (H and E) for histopathological examination.

Statistical Analysis:

Data was subjected to ANOVA using the general linear procedure of SAS institute (1994).

Results

Details of the Nubian male goats fed with the mixture of *J. curcas* seeds and *H. trionum* shoots are shown in table 1.

Clinical Signs:

The prominent clinical signs manifested by all goats of groups 2, 3, 4 and 5 were in the form of profused diarrhoea, rapid respiration, abdominal pain, salivation, moaning, anorexia, reduced water-intake, ataxia and recumbency. Control goats (group 1) showed no clinical signs.

Gross Pathology:

Necropsy findings are summarized in Table 2. There was diffuse or ecchymotic haemorrhage on the mucosa of the rumen, reticulum, omasum and abomasum. Catarrhal or haemorrhagic enteritis, abomasitis and ulcers in the small intestine were also observed. There were congestion and haemorrhage in the lungs, livers and kidneys. Patchy pulmonary cyanosis and emphysema were prominent in all dosed animals.

Histopathology:

There was congestion of the sub-epithelial blood vessels of the rumen, reticulum, omasum and abomasum. Catarrhal or haemorrhagic abomasitis and enteritis. Haemorrhages in the splenic red pulp, congestion of the pulmonary alveolar capillaries and haemorrhages in many alveoli were also seen in all dosed goats. The liver showed cytoplasmic fatty vacuolations and hepato-cellular necrosis in the centrilobular areas. The renal glomeruli were shrunken and/or necrotized and the epithelial cells of the tubules were degenerated and/or necrotized.

Biochemical Findings:

There were no significant changes ($P < 0.5$) in the activity of the enzyme ALT or the concentration of bilirubin and magnesium in the serum of any goat, however, there was a significant decrease in the concentration of total protein and calcium ($P > 0.05$) (Table 3). The activity of the enzyme AST was significantly increased at the time of death ($P < 0.05$) compared to those of the control goats.

Haematological Findings:

The values of Hb%, total RBCs count, PCV and total WBC count were significantly increased ($P < 0.05$) in all dosed groups (Table 3). Leucocytosis encountered was due to increased neutrophils. There were no significant haematological changes ($P > 0.05$) in the control goats (group 1).

Table 1: Effect of the variable mixture of *Jatropha curcas* seeds and *Hibiscus trionum* shoots drench daily to male Nubian Male Goats.

Group	Goat kid number	Age (month)	Weight (kg)	Daily dose/L.b.wt / (g/kg)	Total daily dose (kg)	Death (day)
1 (Control)	1	8	9	Nil	Nil	-
	2	9	10	"	"	-
	3	9	8.5	"	"	-
	4	10	9	"	"	-
2	5	9	8.5	2	0.051	3
	6	10	9	"	0.036	2
	7	11	9	"	0.036	2
	8	9	9	"	0.036	3
3	9	11	9	2	0.036	2
	10	10	12	"	0.048	2
	11	9	10	"	0.030	2
	12	10	9	"	0.036	2
4	13	8	9.5	2	0.076	4
	14	9	10	"	0.120	6
	15	9	12	"	0.120	6
	16	8	9.5	"	0.76	4
5	17	10	7.5	2	0.015	1
	18	9	9	"	0.036	2
	19	11	7.5	"	0.030	2
	20	9	9	"	0.036	2

Table 2: Gross Pathology in Nubian male Goats daily and orally drenched variable mixture of *Jatropaha curcas* seeds and *Hibiscus trionum*.

Organ	Finding	Degree of pathological changes in animals of each group				
		1	2	3	4	5
Rumen	Haemorrhage	-	++	++	+++	+++
Reticulum	Haemorrhage	-	++	++	+++	+++
Omasum	Haemorrhage	-	++	++	+++	+++
Abomasum	Congestion or haemorrhage	-	++	++	++	++
	Abomasitis	-	++	++	++	+++
Intestine	haemorrhagic enteritis	-	++	++	++	++
		-	++	++	++	+++
Liver	Congestion	-	++	+++	+++	+++
	Fatty changes and necrosis	-	++	++	++	++
Kidneys	Congestion and haemorrhage	-	++	++	++	++
Heart	Congestion and haemorrhage	-	++	++	++	++
Lung	Congestion, haemorrhage and Emphysema	-	+++	+++	+++	+++
		-	++	++	+++	+++

+ to +++ = denote degree of change; - = No change; group 1= control; Group 2= 75% hibiscus + 25% *Jatropha*; group 3= 50% *Hibiscus* +50% *Jatropha*; group 4 = 33.3% *Hibiscus* + 66.6% *Jatropha*; group 5= 25% *Hibiscus* +75 *Jatropha*.

Table 3: Sero-biochemical and haematological changes of the Nubian male goats orally drenched variable mixture of *Hibiscus trionum* and *Jatropha curcas* seed.

Parameters	Pre-dosing values	Control groups 1	Group 2	Group 3	Group 4	Group 5
AST (IU)	23.8±0.9 ^c	24.5±0.6 ^c	61±0.7 ^a	65.2±0.6 ^a	68.5±0.9 ^a	68.8±0.9 ^a
ALT(IU)	16.5±0.9 ^a	15.8±0.8 ^a	16.8±0.5 ^a	16.5±0.9 ^a	16.8±0.4 ^a	16.5±0.7 ^a
Total protein (g/dl)	7.2±0.7 ^a	7.3±0.8 ^a	6.5±0.4 ^b	6±0.6 ^b	5.8±0.8	5.6±0.7
Bilirubin (mg/dl)	0.28±0.5 ^a	0.3±0.7 ^a	0.4±0.3 ^a	0.38±0.7 ^a	0.3±0.6 ^a	0.31±0.6
Calcium (mg/dl)	2.2±0.8 ^a	2.5±0.6 ^a	1.6±0.8 ^b	1.2±0.7 ^b	0.98±0.4 ^c	0.85±0.7 ^c
Magnesium (mg/dl)	1.3±0.7 ^a	1.6±0.4 ^a	1.4±0.6 ^a	1.58±0.5 ^a	1.3±0.7 ^a	1.41±0.5 ^a
Hb (g/dl)	8.6±1.8 ^c	9.2±0.4 ^c	11.5±1.5 ^b	12.8±1.2 ^b	13.2±0.5 ^a	14.5±0.8 ^a
RBC (x10 ⁶ mm ³)	10.6±0.6 ^c	10.5±0.9 ^c	13.5±0.8 ^a	14.3±0.8 ^a	15.2±0.8 ^a	16.2±0.7 ^a
PCV%	32.5±1.5 ^b	33.5±0.6 ^b	40.2±1.6 ^a	40.9±0.9 ^a	41.5±1.2 ^a	42.5±0.9 ^a
MCV(m3)	30.2±0.5 ^a	31.2±0.4 ^a	29.7±0.7 ^b	28.6±0.5 ^b	26.6±0.6 ^c	26.3±0.8 ^c
MCHC%	26.8±1.8 ^b	27.7±0.4 ^b	28.6±0.6 ^b	31.3±1.12 ^a	31.7±0.8 ^a	33.7±0.5 ^a
WBC (x10 ³ mm ³)	10.6±1.5 ^b	11.8±1.5 ^b	12.5±2.3 ^a	13±0.9 ^a	12.5±0.6 ^a	13.4±1.2 ^a

Values are expressed as mean ± S.E.; means within the same row with no common letter (a-c) differ significantly ($P < 0.05$).

Discussion

Previous studies on separate dosing of goats with *H. trionum* shoots or *J. curcas* seeds have shown that the main pathological changes are congestion and haemorrhages in the gastrointestinal tract, liver, heart, kidneys and lungs, which correlate with the biochemical and clinical changes (Onsa *et al.*, 2001; 2007). In the present study, the effect of combined oral dosing of *J. curcas* seeds and *H. trionum* shoots cause rapid death of goats (2-6 day); a shorter periods than those caused by individual plants (Hibiscus or Jatropha). There were marked haemorrhages and tissue damage in all goats that were given the combination of *Hibiscus* shoots and *Jatropha* seeds at different concentrations.

Our results have revealed that the dosed goats suffer from a widespread and severe haemorrhage of the vital organs. The mechanisms that produce haemorrhages in the dosed animals are not known, but it has been suggested that there are some active constituents in *J. curcas* seeds and *H. trionum* shoots that have a direct toxic effect on blood vessels causing alternation in the permeability and extravasation of blood (Ahmed and Adam, 1979; Abdel Gadir *et al.*, 2003; Onsa *et al.*, 2007). The significant increase in the activity of the enzyme AST and the significant decrease in the concentration of total proteins reflect hepatic dysfunction (Ford, 1965; Gopinath and Ford, 1969; Adam, 1974; Onsa *et al.*, 2007).

The absence of bilirubinaemia in these experimental goats may be attributed to the absence of damage to periportal hepatocytes, a suggestion that has previously been advocated (Ford *et al.*, 1972; Adam *et al.*, 1973; Ali and Adam, 1978). The significant increase in Hb%, RBCs and PCV values were probably caused by haemo-concentration arising from fluid loss from the alimentary tract as suggested by Onsa *et al.* (2001) and Adel Gadir *et al.* (2003). The severe damage to vital organs associated with hepatic insufficiency could explain the loss of condition and ataxia. Nervous signs arising from hepatic malfunction were also observed in small ruminants fed with *Acanthospermum hispidum* (Ali and Adam, 1978) and *Crotalaria saltiana* (Barri, 1980). Similar manifestations also were observed in male rats given carbon tetrachloride (Elhaj, 2001).

In conclusion, feeding a mixture of *Jatropha curcas* seeds and *Hibiscus trionum* dry shoots to Nubian male goats are more toxic and lethal than feeding a single plant. The toxicity of the mixture of the two plants is characterized by a wide spread haemorrhage, congestion and severe entero-hepto-nephrotoxicity.

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References

- Abdel Gadir, W. S.; Onsa, T. O.; Ali, M.; El Badawi, S. M. A. and Adam, S. E. I. (2003). *Small Rum. Res.*, **48**:61-67.
- Adam, S. E. I. (1974). *Toxicol.*, **2**:67-76.
- Adam, S. E. I., Alfarhan, A. H. and AlYahya, M. A. (2000). *Am. J. Clin. Med.*, **28**:385-390.
- Adam, S. E. I.; Tartour, G.; Obeid, H. M. and Idris, O. F. (1973). *J. comp. Pathol.*, **83**:531-536.
- Ahmed, O. M. M. and Adam, S. E. I. (1979a). *Res. Vet. Sci.*, **27**:89-96.
- Ali, B. and Adam, S. E. I. (1978). *J. Comp. Pathol.*, **88**:533-544.
- Al-Quarawi, A. A. and Adam, S. E. I. (2003). *Phytotherapy Res.*, **17**:29-97.
- Amin, M. A.; Dafalla, A. A. and Abdel Moniem, O. (1972). *Trans. Roy. Soc. Trop. Med. Hyg.*, **66**:805-812.
- Barri, M. E. S. (1980). The effects of *Citrullus colocynthis* and *Crotalaria saltiana* on claves, goats and mice. M.V.Sc. Thesis, University of Khartoum, Sudan.
- Dafalla, A. A. and Amin, M. A. (1976). *East Afri. J. Med. Res.*, **3**: 185-195.
- Dangerfield, W. G. and Finalyson, R. (1953). *J. Clin. Pathol.*, **6**: 173-177.
- Elhaj, R. A. M. (2001). Hepatoprotective activity of two Sudanese plants on experimental liver damage in laboratory animals M.V.Sc. thesis, University of Khartoum, Khartoum, Sudan.
- Ford, E. J. H. (1965). *Vet. Rec.*, **77**: 1507-1519.
- Ford, E. J. H.; Adam, S. E. I. and Gopinath, C. (1972). *J. Comp. Pathol.*, **82**: 355-364.
- Frankel, S. and Reitman, S. (1963). *Clinical Laboratory Method and Diagnosis*. 6th edn. C. V. Mosly Company, St. Louis, USA, Pp. 106.
- Gopinath, C. and Ford, E. J. H. (1969). *J. Pathol.*, **99**:75-85.
- Reitman, S. and Frankel, J. (1957). *Am. J. Clin. Pathol.*, **28**:56-63.
- Schalm, O. W. (1975). *Veterinary Haematology*, 4th edn. Philadelphia, USA. 198p.
- Onsa, Thoria O.; Adam, S. E. I.; Hussain, A. M. (2007). *Sudan J. Vet. Res.*, **22**: 55-61.
- Onsa, Thoria O.; Adam, S. E. I.; Nassar, Sahar, M. A. and Hussain, A. M. (2001). *Sudan J. Vet. Res.*, **17**:109-115.
- Watt, J. M. and Breyer-Brand, J. K. N. G. (1962) *Medicinal and poisonous plants of Southern and Eastern Africa*. 2ndedn. Livingstone, Edinburgh, UK, Pp. 119.