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EFFECT OF THE ANTIPARASITIC DRUG "CLOSANTEL" ON FETAL DEVELOPMENT AND SOME BIOCHEMICAL PARAMETERS IN RABBITS

AMANY E. YOUSSEF

Animal Health Research Institute, Dokki, Giza.

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SUMMARY

The teratogenic effect of closantel subcutaneously injected in doses of 5 and 10 mg / kg. b. wt. during the period of organogenesis was studied in rabbits. Moreover its effect on some serum biochemical constituents was studied. Closantel decreased significantly fetal body weight and percentage of living foetuses. Dilatation of lateral ventricles of the brain, pericarditis, intrathoracic haemorrhage, hypoplasia of the lung, dilatation of renal pelvis, spinal bifida incomplete ossification of skull bones and absence of some sternbrae and phalanges were reported.

Closantel increased significantly the activities of Aspartate - aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). It also increased the concentration of serum total bilirubin, urea and creatinine but decreased the level of total protein.

INTODUCTION

Closantel (N- [5- chloro - 4 - [4- chlorophenyl cianomethyl] - 2- methyl phenyl] - 2- hydroxy - 3, 5, diiodobenzamide) is a long acting anthelmintic drug. It is effective against both mature and immature forms of Fasciola hepatica, F. gigantica. (Stromberg et al, 1984) and blood sucking nematodes (Guerrero, 1983). It also has anticestodal and antiarthropodal activity in sheep and cattle (Guerrero, 1984 and Brander et al, 1990).

Application of this drug in incorrect doses in animals especially ruminants may cause some biochemical and reproductive disorders (Paul et al; 1993). Therefore this work was initiated to investigate the possible teratogenic and biochemical effects resulted from closantel application in rabbit as an experimental animal.

MATERIALS AND METHODS

Drug

Closantel was obtained from United Veterinary Drugs Industrial Company Limited (UVEDCo), Jordan. It is present in a liquid form, packed in bottles for subcutaneous injection. Its concentration is 98.0% W.W. in reference to dry basis.

Animals

Thirty female Newzeland white rabbits having the same body weight. 2 - 3 kg were used. The animals were obtained from the Antiserum and Vaccine Institute, from Helwan, Egypt and kept under hygienic conditions provided with balanced ration and water add - libtium. All animals were kept under observation for 2 weeks before the start of the experiment for atmospheric and handling accommodation.

Effect of Closantel on fetal development

Each female in estrus was paired with a fertile male in a separate cage. Mating was observed and was considered as day zero of pregnancy. The pregnant rabbits were allocated equally into three groups. The first group was kept as a control whereas the other two groups were given the tested drug daily subcutaneously in doses of 5 and 10 mg / kg. b. wt. respectively during the period of organogensis (6th - 18th day of gestation). Pregnant rabbits were kept under observation until the 29th day of gestation at which they were sacrified.

External morphological examination

The total number of foetuses, live and dead fetuses, weight of foetuses and external malformation were recorded. Implantation sites and resorped foetuses were also recorded as described by (Cook and Fairweather, 1968). The foetuses were taken and divided into 2 halves, one for visceral and other half for skeletal examination.

Visceral examination

Foetuses scheduled for visceral examination were left in Bouin's solution for at least one week, with no change of the fixative then the foetuses were rinsed with cold water, sections were made using the technique described by Hays, (1986).

Skeletal examination

It was carried out according to the technique employed by Hayes, (1986).

Effect on some biochemical parameters in serum of rabbits

A blood sample was obtained from each rabbit during the period of organogensis, left to clot and the serum was separated for biochemical analysis. The activities of AST, ALT and ALP were determined by the method of (Reitman and Frankel, 1957 and Roy, 1970), Serum Total proteins, total bilirubin, urea and creatinine were estimated according to the method of (King and Watton, 1959, Monnet, 1963, Kaplan, 1965 and Husdan and Rapopart, 1968) respectively.

Statistical Analysis

The results were statistically analyzed by using one way ANOVA test according to (Snedecor and Cochran, 1982).

RESULTS

Teratogenic effect

External examination

External morphology of the obtained foetuses in control and closantel treated groups were recorded in table 1. No cases of implantation or resorption were noticed either in control or treated groups. Precentage of living foetuses was higher in control group (94.74% of total uterine implants) than treated groups (71.42 and 64.61% for the small and large dose respectively). The mean value of foetal weight was decreased significantly in treated groups. Some external anomalies were observed in rabbits foeti obtained from treated dams in the form of vestigeal tail (Fig. 1), Dwarf foetuses (Fig. 2) and deformed foetuses (Fig. 3). The percent of these anomalies were higher in foetuses from dams treated with 10 mg / kg. b. wt.

Visceral examination

The results of visceral examination of the foetuses were recorded in Table 2. The most prominent abnormalities in the examined foeti were dilatation of lateral ventricles of the brain (Fig. 4), pericarditis and intrathoracic haemorrhage (Fig. 5), hypoplasia of the lung, dilatation of renal pelvis (Fig. 6) and spinal bifida.

Skeletal examination

The results of skeletal examination of the foetuses were recorded in Table 3. Malformations (as incomplete ossification of skull) occurred in 14.28 and 35% of skulls of fetuses obtained from dams treated with closantel in the small and large dose respectively; this is compared to 1.61% in fetuses obtained from control dams (Fig 7). Absence of some ribs was observed in 10.71 and 12.5% of foetuses corresponding to small and high dose respectively. Moreover absence of some sternbrae was recorded in 7.14 and 22.37% of foetuses in small and high dose respectively (Fig. 8) and absence of some phalanges occurred in 10.71 and 12.5% of foetuses in small and high dose respectively.

Biochemical Analysis

Closantel caused significant (P>0.05) increase in the activities of AST, ALT and ALP and elevation in the level of total bilirubin, urea and creatinine, but the total protein were significantly (P>0.05) decreased Table 4.

GroupsDose mg/Kg b.wtTotal numbers of damsTotal numbers of
uterine implantsImplantation
SitesLiving
foetusesMean fetal weight/gmTail

Table (1): Morphological examination of rabbit foetuses obtained from dams subcutaneously injected with Closantel at doses of 5 and 10 mg/Kg b.wt. on days 6-18 of gestation as well as those obtained from control dams

Groups	Dose mg/kg b.wt	Total number of dams	Total number of uterine implants	Implantation Sites		Living foetuses		Mean fetal weight/gm	Tail deformity		Dwarf foetuses	
				number	%	number	%	number	number	%	number	%
Control		10	95	0	0	90	94.74	30.4±0.7	0	0	0	0
Closantel	5	10	42	0	0	30	71.43	28.01±0.42	1	2.38	1	2.38
	10	10	65	3	4.62	42	64.62	27.5±0.16	2	3.08	5	7.61

Values are expressed as mean ± S.E. (P>0.05)

Table (2): Visceral malformations in rabbit foetuses obtained from dams subcutaneously injected with closantel at doses of 5 and 10 mg /Kg b.wt. on days 6-18 of gestation, as well as those obtained from control dams

Groups	Dose mg/kg b.wt	Nnumber of foetuses	Brain		Heart		Lung		Kidney		Spinal cord	
			No.	%	No.	%	No.	%	No.	%	No.	%
Control		33	0	0	0	0	1	3.03	1	3.03	0	0
Treated	5	14	0	0	0	0	2	14.28	2	14.28	0	0
	10	22	2	9.99	2	9.99	4	18.18	4	18.18	2	9.99

Table (3): Number and percentage of skeletal malformations of rabbit foetuses obtained from control dams and those subcutaneously injected with Closantel at doses of 5 and 10 mg /Kg b.wt. on days 6-18 of gestation.

Groups	Dose mg/kg b.wt	Nnumber of foetuses	Malformation							
			Skull		Ribs		Sternum		Phalanges	
			No.	%	No.	%	No.	%	No.	%
Control		62	1	1.61	0	0	0	0	0	0
Treated	5	28	4	14.28	3	10.71	2	7.14	3	10.71
	10	40	14	35	5	12.5	4	22.37	5	12.50

Table (4): Serum biochemical values in control rabbits and those subcutaneously injected with closantel at doses of 5 and 10 mg/Kg .b. wt.

Groups / Parameters	Control	Closantel (mg/kg. b.wt)	
		5	10
Total protein (gm/L)	6.98 ± 0.25 a	6.77 ± 0.20 a	5.84 ± 0.17 b
AST (unit/L)	67.6 ± 3.77 c	88.9 ± 2.11 b	98.6 ± 3.18 a
ALT (unit/L)	49.8 ± 3.52 b	50.5 ± 4.05 b	74.3 ± 3.05 a
ALP (unit/L)	44.8 ± 2.23 c	69.7 ± 3.60 b	89.7 ± 1.90 a
Urea (mg/dl)	17.76 ± 5.17 c	18.89 ± 0.39 b	20.15 ± 0.32 a
Creatinin (mg/dl)	1.21 ± 0.38 c	2.24 ± 0.19 b	3.21 ± 0.13 a
Total bilirubin (mg/dl)	0.44 ± 0.13 c	1.43 ± 0.45 b	3.21± 0.13 a

Values are expressed as mean ± S.E. a, b and c values in the same row with no common superscripts differ significantly (P>0.05). Number of animals in each group = 10

AST = Aspartate Amino Transferase ALT = Alanine Amino Transferase

ALP = Alkaline Phosphatase

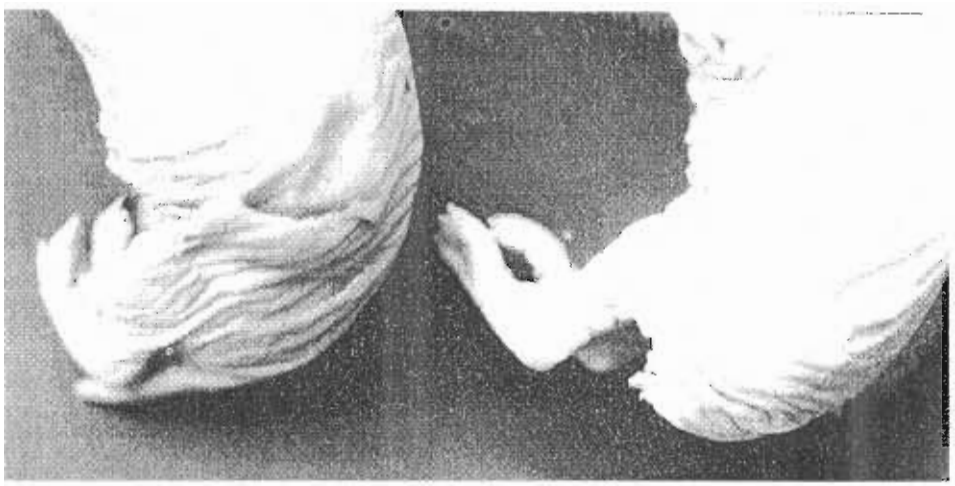

Fig. (1): Malformed rabbit foetus obtained from mother s/c injected with Closantel in a dose of 10 mg / Kg b.wt. (Right) showing vestigial tail as compared to control foetus (Left).

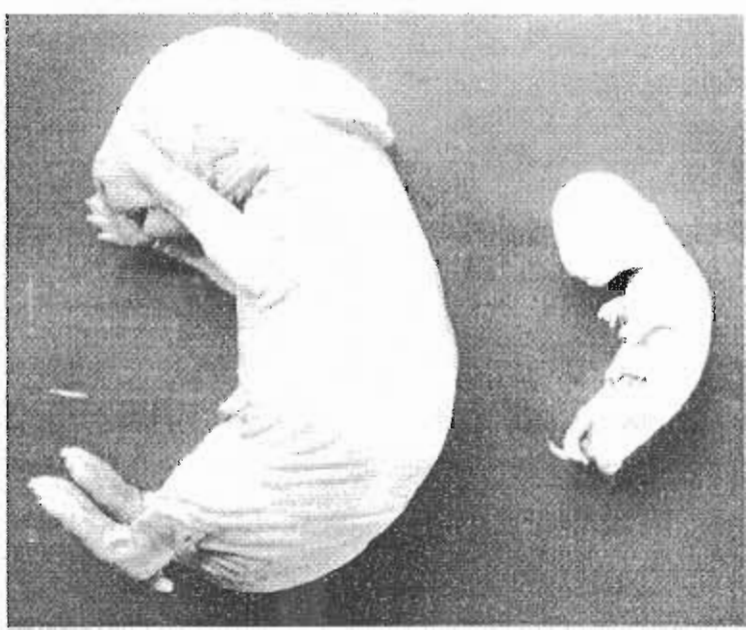

Fig. (2): Control foetus (Left) and dwarfed foetus obtained from mother s/c injected with Closantel in a dose of 10 mg / Kg b. wt. (Right).

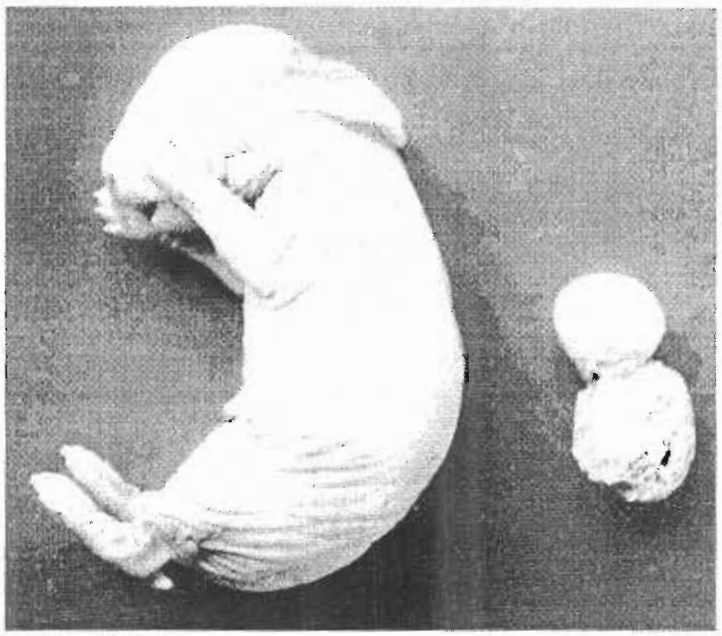

Fig. (3): Control foetus (Left) and deformed foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b. wt. (Right).

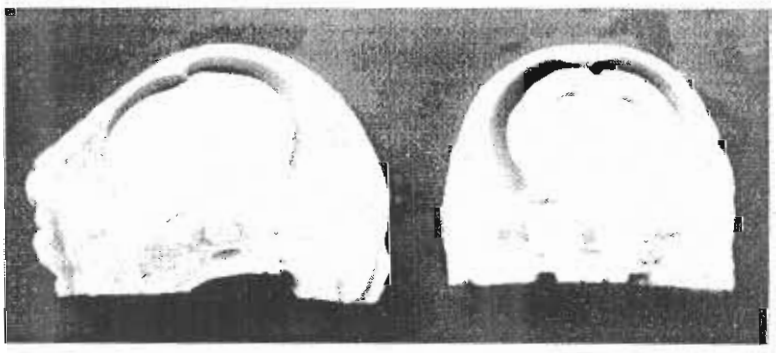

Fig. (4): Showing dilatation of lateral ventricles of the brain in rabbit foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b. wt. (Right) as compared to control one (Left)

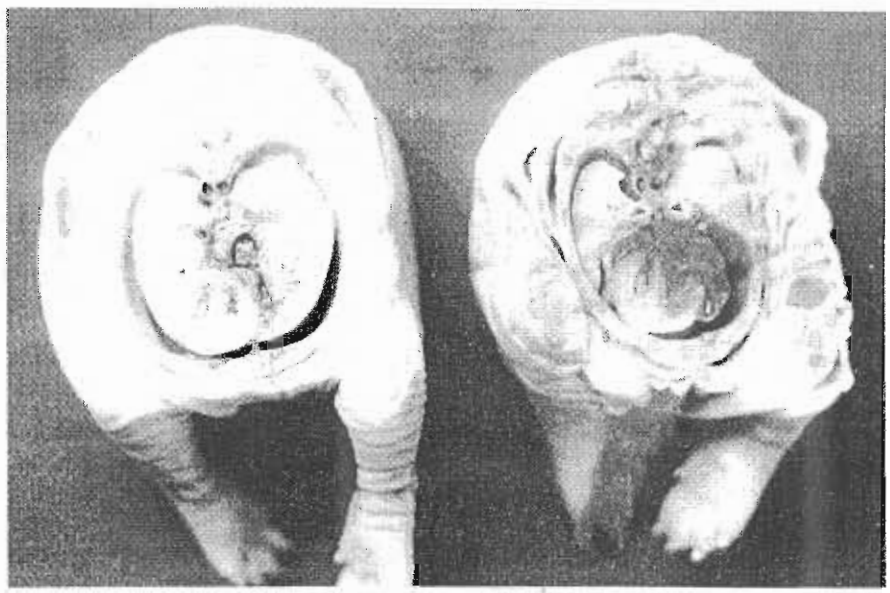

Fig. (5): Showing pericarditis and intrathoracic haemorrhages in rabbit foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b. wt. (Right) acompared to control one (Left).

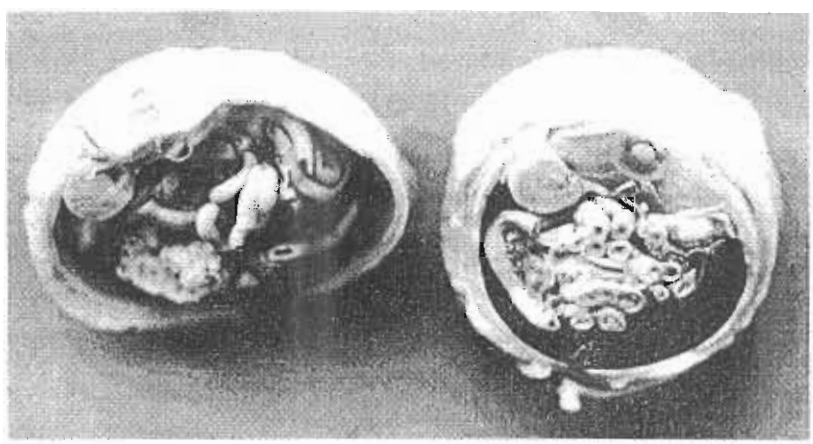

Fig. (6): Showing dilatation of the renal pelvis in rabbit foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b. wt. (Right) as compared to control one (Left).

Fig. (7): Incomplete ossification of the skull of rabbit foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b.wt. (Right) as compared to control one (Left).

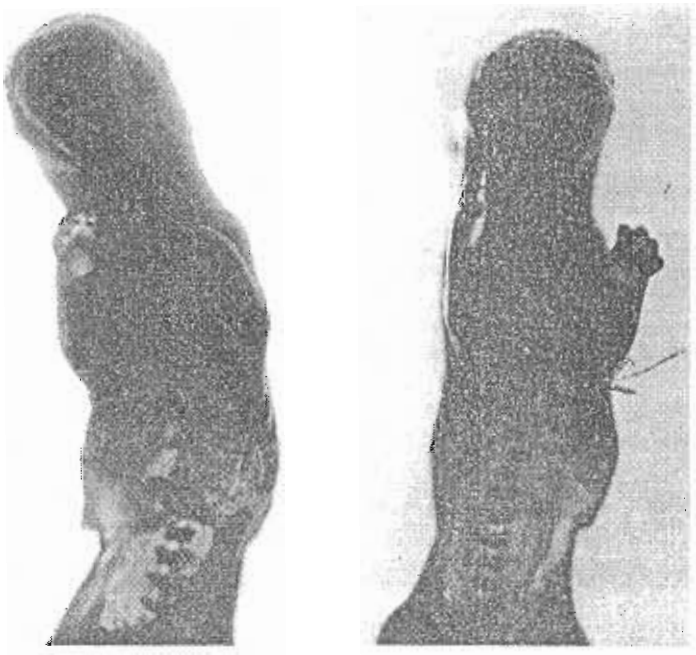

Fig . (8): Abcesnce of some sternbrae in rabbit foetus obtained from mother s/c injected with closantel in a dose of 10 mg / Kg b.wt. (Right), compared to control one (Left).

DISCUSSION

Closental, a salicylanilide derivative is a potent uncoupler of mitochondrial oxidative phosphorylation (Michials et al, 1987).

The drug has a long terminal plasma half-life of 14 days minimum. This long terminal half-life is probably due to the high (> 99%) plasma protein binding of the drug. Because of its long persistence in the body it is possible that putative efficacy against immature and 6-weeks-old flukes is due to drug remaining in the plasma until those flukes mature. Closental by intramascular and oral routes to sheep achieved an elimination half-life of about 3 weeks (Brander et al, 1990).

Regarding the effect of Closantel on fetal development resulted in decrease in weight of foeti, also some viscercal and skeletal anomalies were observed. The teratogenic effect of closantel may be attributed to that it has electropositive charges which enable the compound to pass through membrane of the placenta, this opinion was supported by (Harbison et al., 1975) and (Stevens and Harbison, 1974). They reported that compounds of large molecular weight (>600) as closantel and those with strong electropositive or electronegative charges can and do pass from the maternal to foetal blood stream.

Similar results were recorded by (Poul et al, 1993) who noticed that Rafoxanide (is drug related chemically to Closantel) decreased the number of pups per litter, induced high mortality in rats. Awad, (1995) also revealed that Niclosamide (another salicylanilide derivative) decreased litter size and gravid uteri weight and produced pronounced increase in abnormalities of foetuses and litters. Moreover Sackey et al, (1991) mentioned that abortions were observed in pregnant sheep and goats after administration of rafoxanide. However Lapteva et al, (1987) recorded that Fascoverm (Closantel hydrochloride) had no embryotoxic or teratogenic action when tested in mice by subcutaneous injection at a dose of 15 mg / Kg b. wt.

Moreover Cauteren et al, (1985) found that subcutaneous injection of Closantel at doses of 5 and 20 mg / Kg b. wt. in sheep produce no effect on fertility. This contradiction could be attributed to species differences.

Closantel significantly increased the activities of AST, ALT, ALP, and elevated the level of total bilirubin, urea and creatinine but decreased the concentration of total protein in serum. This maybe due to a hepatotoxic effect of closantel in rabbits.

These results agree with those reported by Ente: et al, (1995) who found that closantel intoxication in dogs caused an elevation of hepatic enzymes together with the excess of bilirubin in blood and urine confirmed the hepatotoxicosis.

On the other hand Cubbeddu et al, (1983) found that there is no evidence of hepatotoxic effect in sheep subcutaneously injected with 2 ml Closantel solution (concentration not stated) per 10 Kg b.wt.

Moreover, Costa et al, (1986) reported that closantel has no effect on blood enzymes values in cattle given single oral dose of 10, 25 ml/Kg. Although, the exact concentration of closantel used by those authors is not clear but it should be pointed out that they used sheep or cattle in their study while rabbits were used in this study.

CONCLUSION

The studied drug may be considered as teratogenic and cause some biochemical alternations in blood of rabbits Therefore it is highly indicated to avoid injection of closantel to pregnant females.

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