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The effect of deisopropylngaione on the renal function in sheep

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Abstract

Following intoxication of sheep with 150 mg/kg body weight of Deisopropylngaione [DIN] intra-peritoneally, there was impairment of the renal function as depicted by decreases in the glomerular filtration rate [GFR], renal plasma flow [RPF] and tubular transport maximum for glucose [TMG] which were essentially pre-renal in origin. The mean feed intake, free water intake and urine output also decreased following intoxication of sheep with DIN. There were increases in the plasma aspertate transferase [AST], alkaline phosphatase [ALP] and plasma creatinine post exposure to DIN. The mean values for plasma sodium, potassium and chloride concentrations showed no significant differences following intoxication with deisopropylngaione in sheep.

Key words: *deisopropylngaione*, intoxication, pre-renal impairment

Introduction

Following poisoning of sheep by the plant *Myoporum deserti*, Johnstone and Alien (1944) observed from histopathology slides considerable congestion of both kidney cortex and medulla with evidence of some toxic damage to the glomerulus and epithelial cells of the convoluted tubules of the kidneys. Riek and Wright (1946) dosed sheep with the oil obtained from *Myoporum acuminatum* another member of the family *Myoporacae*, and described widespread congestion of both kidney cortex and medullary layers together .with degenerative changes in the epithelial cells of the convoluted tubules from histopathology slides.
This paper considered the effect of deisopropylngaione, one of the twelve essential oils in the plant *Myoporum deserti* [figure 1] on the renal function in the sheep.

Materials and methods

The Deisopropylngaione [DIN] was obtained from the leaves of *Myoporum deserti* by steam distillation. A total of twelve female merino lambs weighing between 10-15kg and aged 9-15 months were used in this experiment. Seven lambs were employed in the renal clearance studies while five were used to establish a dose rate sufficient to cause changes in the renal function. They were kept in fiberglass metabolism cages in an air-conditioned room maintained at 22 to 25°C and relative humidity of 55%. Two periods -conditioning and treatment were recognizable during the experiment. During the conditioning period each lamb was accurately weighed to 0.lkg body weight, ear tagged, dewormed with Fenbendazole bolus at 250mg/body weight and their urinary bladder were catheterized using 12G x 5ml balloon Bardex silicone elastomere coated Foley catheter. This is to ensure

that the urethra were patent. Each lamb was fed 16g dry matter per kg body weight of Lucerne chaff daily from a single source throughout the experimental period. Aliquot samples from this feed batch were subjected to proximate analysis for moisture content, crude protein, crude fibre, total ash, nitrogen free extract; sodium, potassium and chloride ions determinations AOAC (1990). Electrolyte free water was offered ad libitum. Pre treatment blood and urine samples were collected for blood chemistry and urinalysis.

Blood Chemistry:

10 ml blood sample drawn from jugular vein was put in 125i.u. lithium heparin and spun down on Bachman TJ-6 centrifuge at a speed of 3,000 rounds per minute for 5 minutes. The plasma so obtained was withdrawn into a clean plastic tube using two stop Eppendorf pipette for the following determinations: (a) albumin and (b) creatinine using Harleco-Ultrachem bromocresol green reaction method on I.L. Multistat III machine; (c) Blood urea concentration, and (d) aspertate transferase using Boehringer Mannheim reagent on I.L. Multistat machine, (e) bilirubin using Corning colorimeter model 252. In addition to above sodium and potassium ions were determined using the flame emission method on photometer AHS (AUST) equipment; Chloride ion by calorimetric titration method on radiometer CMT-10 chloride titrator, and total protein by Harleco-Ultrachem biuret reaction method on I.L. Multistat III machine.

Urine analysis:

100ml of freshly voided urine put in clean plastics cups were examined for the following: (a) Osmolality using the vapour pressure osmometer from Wescot Inc. (b) specific gravity by refractometry on T.S. [total solid] meter (c) pH using Beckman expanded . scales pH meter. In addition, using the N-Multistix, bile salt, urobilinogen, protein ketones, glucose and bilirubin were assessed from the urine samples.

The clearance techniques:

Renal clearance CI and CII representing pre and post exposure to the toxic dose of DIN were carried out on the 5^{th} day (CI) and between day the 13th and 14^{th} day (CII) from commencement of the experiment. Prior to the day of CI, each lamb was clipped to the skin an area 20cm x 20cm on the ventral aspect of the neck region to expose the jugular veins. A thumb tourniquet was applied at the jugular groove to establish jugular vein patency and degree of prominence. The urethral patency was also checked by passing a 12G x 5cc balloon Bardex silicone elastomere coated Foley's catheter. On the 5^{th} day [CI], each experimental lamb was weighed and drenched with 25ml/kg body weight distilled water using a clear polyvinyl stomach tube. This is to ensure that a urine flow greater than 2 ml/minute is obtained. They were thereafter returned to the fiberglass metabolism cages and restrained with a head halter. The delivery end of the Foleys catheter was inserted into 500ml plastic bottle which was secured between the lambs two hind legs by means of a gauze bandage. The left and right jugular veins were cannulated using a 15G Teflon Dwellcath cannulae. A baseline (Zero) 10ml blood and urine samples were collected. A drip set was hooked onto a 1 liter 5% dextrose bottle and connected to the cannulae. 0.5 uCi/kg body weight of sodium iothalamate iondine 125(I^{125}) was added to the 1 liter 5% dextrose via the rubber stopper of the bottle. A fast 100ml from the bottle was administered to the lamb as priming dose. This was followed immediately by 8mg/kg body weight of a 20%

aminophippurate sodium via the injection site of the delivery tube. To the remaining 900ml of 5% dextrose which contained iothalamate iodine was added 2mg/ml of 20% aminohippurate sodium via the injection from the side of the rubber stopper of the bottle. The sustaining infusion i.e. the 900ml 5% dextrose in sodium iothalamte and 2mg/ml of 20% aminohippurate sodium was given at 18 drops per quarter of a minute. Collection of blood samples from the second cannula started one hour later. With a 10ml plastic syringe, 10ml of blood was drawn via the vacant cannula at the mid-point of every 20 minute urine collection. 8ml was transferred into a 10ml lithium heparin coated plastic tubes and mixed gently. The remaining 2ml was put into 5ml plastic tube containing fluoride oxalate powder, mixed gently for glucose determination. The 8ml blood samples were centrifuged and plasma withdrawn. Six consecutive blood and urine samples were collected in this manner. Plasma sample for para-amino hippurate [PAH] creatinine and sodium iothalamate were not diluted while urine samples were diluted as follows: iothalamate 0; creatinine 1:10 and PAH 1:100. On the twelfth day from commencement of the experiment, the right paralumbar fossa of each lamb was prepared as for aseptic surgery. With an 18G x linch non-toxic pyrogen-free needle and a 5ml Luxblox interchangeable glass syringe experimental lambs received 150 mg/kg body weight deisopropylngaione [DIN] intraperitoneally via the prepared site. Post exposure clearance (CII) was carried out forty-eight hours post dosing with DIN. The GFR, RPF, and the TMG were determined same as in CI as contained in the clearance technique outlined above. On completion of CII, final blood and urine samples were collected for plasma biochemistry and urinalysis; followed by euthanasia using 325 mg/ml/kg body weight of pentobarbitone sodium intravenously.

Analytical methods for clearances.

The clearance of iothalamate iodine 125 (CIOT), creatinine (CCR), and tubular transport maximum for glucose CTMG) were measured by the methods adapted from Smith (1956). Radioactivity of iothalamate I^{125} in plasma and urine samples was counted on Packard Autogamma scintillation spectrometer 5230. Four separate counts were made and their averages were taken. P-aminohippuric acid concentrations in both plasma and urine samples were determined by the method of Harvey and Brothers (1962) on a first generation semi automatic analyzer.

Plasma urine creatinine concentrations were determined by Jaffe Reaction using Harleco-Ultrchem kit on I.L. Multistat III machine. Blood and urine glucose were assessed by the Glucose Hexokinase U.V. method using Boehringer-Mannheim reagent kit No. 124346 on I.L Multistat III machine.

Results

The renal clearance:

The changes in the mean values for the clearances of sodium iothalamate iodine 125 (Ciot) representing the glomerular filtration rate (GFR), the p-aminohippuric acid (CPAH) representing the renal plasma flow (RPF), the tubular transport maximum for glucose (TMG) and the filtration fraction percentages (FF% = GFR/RPF) were outlined in Table 1.

Table 1: Mean Values of the Glomerular Filtration Rate [GFRj, Renal Plasma Flow [RPF] Filtration Fraction Percent [FFP] and Tubular Transport Maximum for Glucose [TMG] Before and 48hurs Following Intoxication with 150 mgkg Weight Deisopropylngione in Sheep.

Sheep No.	Body weight (Kg)	Urine Flow (ml/min)	GFR(ClOT) (ml/min/kg Body weight)	RPF (ml/min/kg Body weight)	FF (%)	TmG (umol/min/kg Body weight)
121	12.9 12.5	3.9 2.6	2.81 1.53	15.41 2.01	18.42 76.74	25 9
53	15.8 15.8	4.8 1.6	2.35 1.57	13.54 1.22	17.47 127.65	27 19
128	14.4 14.4	3.6 3.5	2.82 2.39	15.47 15.56	18.17 18.52	22 27
122	13.7 13.2	3.7 3.1	2.44 2.32	17.77 14.71	13.75 15.86	36 32
124	15.1 15.4	5.0 4.0	2.00 1.96	14.31 14.11	14.08 12.19	19 13
126*'	14.9	3.9	2.57	14.36	17.99	28
856	15.2 15.6	4.0 3.4	2.91 1.44	13.02 2.15	22.62 85.00	40 25

Top row values represent pre dosing [Before] and bottom row values represent post dosing [After].

Post exposure to DIN resulted in significant decreases in urine flow in sheep 53 from 4.8ml/min pre dosing to 1.6ml/min post dosing; and in sheep 121 form 3.9ml/min to 2.6ml/min. Significant decreases were also seen in the RPF where in sheep 121 it dropped from 15.4ml/min/kg body weight pre dosing to 2ml/min/kg body weight post exposure to DIN. In sheep 53, RPF decreased from 13.5ml/min/kg body weight to 1.2ml/min/kg body weight post dosing. Consequently, the filtration fraction increased from 18.4% pre exposure to 76.7% in sheep 121; while in sheep 55 FF% went up from 17.4% to 127.7%. There were no significant changes for the tubular transport maximum for glucose. Sheep 126 was lost soon after intoxication with DIN and hence there was no post dosing clearance for her. Post mortem examination revealed extensive pulmonary oedema. In sheep 856 the GFR decreased from 2.9ml/min/kg body weight to 1.4 ml/min/kg body weight post exposure; the

RPF dropped from 13ml/min/kg body weight pre dosing to 2.2ml/min/kg body weight post exposure while the filtration fraction increased from 22.6% to 85% post dosing.

Table 2: Mean values of feed intake, free water intake, urine output analysis prior to and 48 hours following intoxication of sheep with 150 nig/kg body weight *Deisopropylngalone.*

Sheep No.	Feed intake [g/kg body weight]	Free Water intake (ml/body wt./day)	Urine Output (ml/kg)	Urine Analysis			
				Electrolytes mm/kg/day			
				SG	Na^+	K^+	Cl-
121	18.76 18.76	75.6 44.8	44.9 14.9	1.019 1.022	10 9	186 108	143 121
53	18.66 18.75	59.2 28.5	18.4 12.6	1.033 1.029	40 36	280 288	289 280
128	18.80 18.27	55.4 48.7	27.4 23.3	1.025 1.017	22 37	235 411	160 72
122	18.80 18.80	104.1 98.5	91.1 43.5	1.009 1.016	8 3	96 97	68 49
124	18.60 18.40	65.5 60.7	34.1 38.3	1.029 1.024	22 64	214 258	208 207
126	18.40	89.1	53.2	1.009	36	145	208
856	18.60 18.80	74.6 32.4	61.0 44.4	1.035 1.033	25 22	175 170	150 135

Top row values represent pre dosing [Before] and bottom row values represent post dosing [After]. Key: S.G - Specific Gravity Na^+ - Sodium ion

K - Potassium ion

Cl- - Chloride ion

Table 2 outlined the daily mean values of feed and water intakes and urine analysis before and after single 150 mg/kg of DIN to sheep. In sheep 128 the feed intake decreased from 18.8g/kg body weight to 11.3g/kg post dosing. Likewise, there was decrease in feed intake in

Table 3. Mean values of the plasma biochemistry prior to and 48 hours following administration of 150 mg/kg body weight deisopropylngaine to sheep.

Sheep No	BUN [mmol/L]	Creatinine [j.mol/L]	AST U/L	ALK.PHOS. U/L	Urine Analysis				
					Electrolytes mmol/kg/day			Protein [g/L]	
					Na^+	K^+	cr	Total	Albumin
121	9.35 9.70	70 100	28 456	53 87	138 151	4.9 3.1	96 97	59 51	34 21
53	9.03 9.65	92 121	29 435	18 32	142 155	4.5 4.1	134 131	60 52	34 33
128	8.6 6.3	73 80	30 128	50 57	139 143	5.0 4.7	89 95	61 65	34 33
122	8.7 4.1	138 355	43 965	13 49	143 152	3.8 4.0	106 107	59 52	38 30
124	11.2 9.1	95 78	31 591	17 53	142 141	4.7 4.2	104 105	64 62	35 33
126	10.5	81	46	56	142	4.2	94	65	36
856"	8.8 9.6	95 280	55 65	23 30	143 144	4.3 4.6	104 109	54 46	34 28

Top row values represents pre dosing [Before] and bottom row values represents post dosing [After].
Key: BUN - Blood Urea Nitrogen
AST - Aspertate Transferase
ALK.PHOS. - Alkaline Phosphatase

sheep 124 from 18.6g/kg to 8.4g/kg. Consequently, the free water intake for sheep 121 and 122 decreased significantly from 75.6ml/kg body weight to 44.8ml/kg and from 104.1ml/kg to 98.5ml/kg post dosing respectively. Urine outputs were also affected especially in sheep 121 and 122. In sheep 121 urine output decreased from 44.9ml/kg body weight to 14.9ml/kg post exposure to DIN and in sheep 122 from 91.1ml/kg to 43.5ml/kg body weight. In sheep 856 free water in take decreased from 74.6ml/bw/day pre dosing to 32.4ml/bw/day post dosing, and the urine output decreased from 61 ml/ml/kg to 44.4ml/kg post dosing. There were no significant changes in the urine specific gravity. However, there were slight changes in the urine electrolytes. For instance in sheep 128 urine sodium ion (Na^+) increased from 22mmol/kg/day to 37mmol/kg/day post exposure to DIN; and in sheep 124 from 22mmol/kg/day to 64mmol/kg/day. The chloride ion (Cl^-w) decreased from

160mmol/kg/day to 72mmol/kg/day in sheep 128; while in sheep 122 Cl^- decreased from 68.5mmol/kg/day to 49mmol/kg/day post exposure to DIN.

There were changes in the blood urea nitrogen, creatinine, aspertate transferase and alkaline phosphatase. For instance, in sheep 121, creatinine increased from 70umol/L pre dosing to l00umol/L post dosing and in sheep 122 from 137.5umol/L to 354umol/L. In sheep 121 the aspertate transferase (AST) increased from 27.5μ/L pre dosing to 456μ/L post dosing while in sheep 128 the AST went up from 30μ/L to 128μ/L; 42.8μ/L to 964μ/L in sheep 122; and in sheep 124 the AST shot up from 31μ/L pre dosing to 591.7U/L post dosing. Significant changes were also recorded for the serum alkaline phosphatase (ALP). In sheep 121 ALP rose from 53μ/L to 87μ/L; from 12.7μ/L to 48.5μ/L in sheep 122 and 17μ/L to 52.7μ/L in sheep 124.

Discussion

In comparing measurements of renal function before intoxication of sheep with deisopropylngaione (DIN) it was assumed that in the normal sheep each kidney contained approximately the same number of nephrons that contribute equally to the total function. The decreases in the urine flow and renal plasma flow coupled with the drop in the glomerular filtration rate following intoxication of sheep with DIN could have been precipitated by non-renal conditions encountered in the course of the experiment such as increase in the plasma aspertate transferase and alkaline phosphatase both indicating liver dysfunction. Lowenstein et al (1961) demonstrated a systematic decrease in GFR, RPF and TMG with increase in age. Young lambs (9-15 months) were used in this experiment with the expectation that the microsomal mixed function oxidase system is at its optimum Functioning capacity. Hence the decreases in the GFR seen in this experiment could not have been due to age but probably due to intoxication by DIN. Horster and Levy (1970) demonstrated a decrease in the RPF and rise in FF% during the neonatal period in rat in which renal function is disturbed by non-renal diseases which in turn resulted to pre-renal failure. Hepatic cirrhosis ranks high among non-renal diseases and it is characterized by profound metabolic disturbances leading to hypoproteinemia and subsequent formation of oedema, Seawright and Hrdlicka (1978). It was stated in the result portion of this paper that pulmonary oedema may have contributed to the death of sheep 856 from post mortem examination following intoxication by DIN. It can therefore be inferred that the increases in AST and ALP (Liver function tests, Benjamin, 1970) seen following intoxication with DIN are indeed as a result of liver injury in the sheep so intoxicated. When compared with the specific kidney lesions and decreased renal function in the absence of non-renal diseases or conditions seen in mercuric chloride poisoning in sheep Berling (1963) and Berling and Ulberg (1963) we conclude that the reduction in the renal function based on decreases in the GFR, RPF and urine flow seen in lamb*: intoxicated by DIN was in the main pre renal in origin

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