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Estimation of Heavy Metals in Rats Fed with Graded Levels of Indometacin (Indocid R) Meal

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Abstract: Lead, cadmium, arsenic and zinc were quantified using a spectrophotometry method in the head, intestine, hair, liver, heart and feacal samples of albino rats of different sexes administered indocid drugs in August 2006. The results revealed that the mean live weight of the samples were between 89.1 and 98.1 g. Significant differences were observed among the treatments in terms of mean final body weight, mean body weight gain, total feed intake and average daily water intake. Lead and arsenic occurred in head, intestine, liver, hair, feacal and indocid samples at concentrations of ND and 0.02-0.07, ND and 0.01-0.05, ND and 0.01-0.02, 0.02-0.05 and 0.01-0.03, ND-0.04 and 0.01-0.05 and ND and 0.03 mg kg^{-1}, respectively. Cadmium was not detected in all the samples. From the results obtained in this work, there were no significant differences ($p<0.05$) concerning heavy metals in the control, test and indocid samples. However, it is necessary to monitor the heavy metal contents of rats before human consumption.

Key words: Heavy metals, rats, indocid, feed water, bait, organs

INTRODUCTION

Rats originated from Central Asia. They are domesticated in the 17th century and the process has continued to the present, resulting in many breeds that are docile and excellent pet quality. They are found in all kinds of habitats and nearly all land masses of the world, an enduring tribute to their adaptability and their long-time association with people. They tend to be omnivorous, but exhibit tremendous number opportunism in their feeding habits when living in and around human dwellings (Harkness and Wagner, 1988).

Rats can be a problem in urban, suburban and rural areas. The problems include:

- They eat food and contaminate it with urine and excrement
- They gnaw into materials such as paper, books wood etc which they use as nest materials. Occasionally they bite people and may kill small animals.
- They or the parasites they carry spread many diseases.
- They can damage ornamental plants, vegetables etc.
- Rats are socially unacceptable because they have an incredible ability to survive (Hovanic *et al.,* 2006).

Rats problems have been managed in various ways which include; exclusion, eliminating food sources, use of rat repellents, humane trapping and application of rodenticide baits.

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Indocid (indometacin) belongs to a group of medicines called Non-steroidal Anti-inflammatory Drugs (NSAIDs). It works by blocking the production of a chemical (prostaglandin) which the body produces in response to injury or certain diseases. This prostaglandin would otherwise go on to cause swelling, pain and inflammation. Indocid is used for abdominal pain associated with menstrual periods, muscle pain, pain, pain following surgery, inflammation pain and acute gout. The drug can not be used for patients who are allegic to aspirin, peptic ulcers, piles/hemorrhoids and ulcer caused by non-steroidal anti- inflammatory drugs (Net Doctor, 2004).

Heavy metals are important environmental pollutants, but may also be natural components of man and animals (Abulude, 2006). It is necessary to identify the migration, distribution and possible accumulation of heavy metals in rats (organs). Several reports have been given by professional pest control operators and researchers (Harkness and Wagner, 1988; Net Doctor, 2004; Hovanic *et al*., 2006), but none of them have reported on indocid as a rodenticide or trap.

The aim of this study was to provide for the first time, detailed information on rats administered few dosage of indocid by examining the content of certain heavy metals (Zn, Pb, Cd and As) and their distribution in the rats' organs, including their hair and faecal samples. Based on the findings, recommendation would be given whether the rats' organs can be consumed.

MATERIALS AND METHODS

The wistar rats used for this analysis were obtained from a local shop in Akure, Ondo State, Nigeria in August 2006. They were housed and fed with grower mash in the Chemistry Laboratory of Federal College of Agriculture, Akure. The indocid capsules used in this experiment were purchased in a pharmacy shop also in Akure.

Experimental Design

The experiment was designed by separating the experimental animals into three groups (A-C). Group A- control, fed without indocid. Group B- fed with feed and 0.1 g indocid. Group C- fed with feed and 0.2 g indocid. On the first day, the rats were fasted for 4 h and later fed these experimental diets. Subsequent days until death, 20 g of feed and 20 mL^{-1} of water were served ad-libitum without indocid. At the end of each day, left over water and feed were measured to calculate the average water and feed intake. Each day, feacal samples were collected, processed to remove dirts, sun dried for 6h, ground with pestle and mortal, sieved (2 mm mesh) and kept prior to analysis.

At the end of the experiment the dead animals were dissected, the organs weighed and prepared for heavy metal analysis.

Heavy Metal Estimation

Each of the ground sample (0.5 g) were wet ashed in 20 mL^{-1} of 2 mL HCl, heated on hot plate until volume reduced to about 1 cm^{-3}, filtered, and made up to 50 cm^{-3} with 2 mL HCl. Zn, Pb, As and Cd were determined in each solution using a Pye Unicam SP 9 spectrophotometer (Abulude *et al*., 2006a).

Statistical Analysis

Data were analyzed using SPSS for window 10.0 package. T-test was used for comparison between control and test values. ANOVA was used to assess differences between groups.

RESULTS AND DISCUSSION

The mean live weights of the samples were between 89.1 and 98.1 g. The head, intestine, liver and feacal samples were 10.8-13.7, 4.2-8.5, 2.4-2.7 and 9.0-10.2 g, respectively (Table 1). The anatomical weights in this study were below the values recorded for cattle egret (Abulude *et al*., 2005). The differences were due to the physiological compositions of the different species.

Table 2 depicts the performance evaluation of the experimental animals. Significant differences ($p<0.05$) were observed among the treatments in terms of mean final body weight, mean body weight gain/loss and total feed intake. Average water intake is depicted in Table 3. Average daily weight gain and feed intake for the test and control ranged from 2.0-2.4 and 94-120 g rat, respectively. Days of survival of the control was highest (14 days) while tests rats survived for only 6days.

The indocid samples killed the rats by thinning the blood until the animals hemorrhaged internally. The rats suffered some dizziness and then died from loss of blood. The loss of blood and dehydration in the rats caused the reductions in feed and water intake which eventually caused loss in weight. In contrast, control rats were more voracious feeders and consumed proportionately more food. According to Harkness and Wagner (1988), food consumption of rats and mice varies with the quality of the food(s) offered, the age, health and breeding status of its individual, the environment temperature and the time of day.

Water intake was significant ($p<0.05$) among the treatments and varied according to the levels of drugs administered. The rat fed without indocid drank more water unlike those administered with drugs. From 1st day to the last day, water intake decreased with increase in the level of indocid for the test samples, whereas the control's water intake increases as number of days and weight increased.

The rats organs, hair, indocid and feacal samples were subjected to heavy metal (Zn, Cd, As and Pb) analysis (Table 4). Pb values for the control ranged between ND and 0.03 mg kg^{-1} and the Pb (test) ranged between ND and 0.05 mg kg^{-1}. For the control, maximum levels were recorded in hair and heart, whereas in test it was recoded in hair.

Table 1: Mean anatomical weight of the rat samples (weight in g)

Parts	A[a]	B	C
Head	13.7	10.8	11.2
Intestine	8.5	5.5	4.2
Liver	2.7	2.5	2.4
Heart	0.5	0.5	0.5
Liver weight	98.8	97.1	89.1
Feacal	10.2	9.7	9.0

[a]A (control)-rat fed without indocid, B-rat fed with 0.1 g indocid, C-rat fed with 0.2 g indocid

Table 2: Performance evaluation of experimental animals

Performance	A Control	B 0.1 g	C 0.2 g
Mean initial live weight (g/rat)	98.8	97.1	89.1
Mean fuel body weight (g/rat)	111.5	84.7	80.3
Mean body weight gain (g/rat)	12.7	-	-
Mean body weight loss (g/rat)	-	12.4	8.8
Av. Daily weight gain (g/rat)	2.0	-	-
Av. Daily weight loss (g/rat)	-	2.0	1.5
Total feed intake (g/rat)	120.0	103.9	94.4
Av. Daily feed intake	20.0	17.3	15.7
Mean days of survival (days/rat)	14.0	6.0	6.0

[a]A (control)-rat fed without indocid, B-rat fed with 0.1 g indocid, C-rat fed with 0.2 g indocid

Table 3: Average water intake of rats (mL/day)

Days	A Control	B 0.1 g	C 0.2 g
1	10	10	10
2	12	8	8
3	13	8	6
4	13	4	2
5	15	-	-
6	18	-	-

Table 4: Heavy metal composition (mg kg^{-1}) of rat and indocid used for the analysis (Mean±SEM)

	Organs	Code	Pb	Cd	As	As
A	Control					
	Head	0_1	ND	ND	0.07±0.006	2.7±0.015
	Intestine	0_2	ND	ND	0.05±0.005	2.8±0.016
	Hair	0_3	ND	ND	0.03±0.005	6.1±0.005
	Liver	0_4	ND	ND	0.01±0.002	2.7±0.005
	Heart	0_5	0.03±0.005	ND	0.01±0.001	2.7±0.015
	Faecal	0_6	ND	ND	0.01±0.002	14.7±0.055
B	0.1 g					
	Head	I_1	<0.01	ND	0.03±0.005	ND
	Intestine	I_2	<0.01	ND	0.04±0.003	ND
	Hair	I_3	<0.03	ND	0.03±0.005	8.3±0.045
	Liver	I_4	<0.01	ND	0.02±0.004	2.7±0.015
	Heart	I_5	<0.01	ND	0.03±0.005	3.0±0.025
	Faecal	I_6	<0.04	ND	0.05±0.004	2.7±0.025
C	0.2 g					
	Head	12_1	<0.01	ND	0.02±0.005	2.8±0.015
	Intestine	12_2	<0.01	ND	0.01±0.005	2.9±0.005
	Hair	12_3	<0.05	ND	0.01±0.005	2.6±0.025
	Liver	12_4	<0.01	ND	0.01±0.005	5.7±0.025
	Heart	12_5	<0.01	ND	0.02±0.005	2.7±0.015
	Faecal	12_6	<0.04	ND	0.01±0.002	6.4±0.035
	Indocid	A_{14}	ND	ND	0.03±0.002	2.9±0.015

ND-Not Detected, [a]A (control)-rat fed without indocid, B-rat fed with 0.1 g indocid, C-rat fed with 0.2 g indocid

Monitoring of the observed samples revealed the highest mean value of Zn in the control feacal sample (14.7 mg kg^{-1}) followed by hair (6.1 mg kg^{-1}). In the test samples 8.3 and 6.4 mg kg^{-1} were recorded for liver and feacal samples groups B and C, respectively.

The highest level of As was obtained in control (Head, 0.07 mg kg^{-1}), ND was recorded in most parts of the rats. In all the samples parts ND was recoded. For the indocid samples, 2.7 and 0.03 mg kg^{-1} were recorded for Zn and As respectively whereas ND were noted for Cd and Pb.

Significant ($p<0.05$) differences observed among the treatments in terms of body weight, weight gain/loss, feed intake, daily water intake were reflections of variations in the feeds quality and effects of indocid administered rates. Results of food intake of the control and test were not similar. Control samples recorded weight gain whereas test produces weight loss. The reason was obvious, this was as a result of inclusion of indocid into the feed of the test. Water intake of the control in this study agreed with the results of Nworgu *et al.* (2003) whose reports were increase in intake for the first 2weeks of feeding cockerel chicks.

The Pb concentration in some of the control and test samples was higher than ND recorded in the indocid sample used for this study. Present values were lower than those reported for different animal organs and environment, Korenekova *et al.* (2002) recorded (0.009-0.325 mg kg^{-1}) for cattle, Omitoyin *et al.* (2003) reported 1.67-5.0 mg kg^{-1} for *Clarias gariepinus*. Ajani *et al.* (2003) recoded ND-2.83 mg kg^{-1} for *Tilapia zill* and Sogut and Yalcin (2005) reported 1.09-6.11 mg kg^{-1} for *Mytillus galloprovincialis*. Since the values for the control and test were higher than the indocid sample, it is then correct to deduce that there were no significant influences of indocid on the Pb level of the control and test samples. The results point to the fact that Pb could have been ingested from the environment through air (fine dust particles) water and feed.

Cd was not detected in the entire organ and the indocid sample. It is gratifying to note because heavy metals poising particularly Pb and Cd have been reported to give rise to quite a number of chemical syndromes for example Cd accumulation is associated with hypertension, osteomalacia and itai-itai diseases. Pb poising has been found to be associated with permanent brain damage, behavioral disorders and impaired hearing (Abulude *et al*., 2006b). It is advisable to do constant monitoring for the purpose of avoiding the distribution of foodstuffs that could pose a risk to human health if consumed. Cd accumulates in the kidneys and the liver of cattles because its rate of elimination from these organs is relatively low (Korenekova *et al*., 2002). This is due to the binding of Cd to metallothionein in these tissues (Garcia-Fernande *et al*., 1996).

Zn values of the present study showed that values of the feacal sample in control was relative higher than that recoded for hair, moreover these values were higher than in liver. The values obtained by us were not in agreement with values observed for liver muscle of cattle in Sweden and Poland (Lopez-Alonso *et al*., 2000). In *Tilapia zilli* and *C. gariepinus* gill and intestines were they contained the highest Zn accumulation (Ajani *et al*., 2003; Omitoyin *et al*., 2003). The high Zn content observed in this study could be from the Zn content of the container used as the receiver for the feacal samples. Since the indocid samples Zn content was low. The general pattern of the accumulation of Zn in this study was reported to be in the following order. Feacal>liver>hair>head.

Rats, like mice have been consumed and used extensively in biomedical research in Africa and they are numerous in Nigeria. Most of the tremendous number of breeds and strains currently in existence has resulted from intensive inbreeding efforts by research laboratories over the years. The heavy metals in the study occurred in the organs, hair and feacal samples of rats in physiological concentrations and do not pose any threat to the potential consumers. However, liver, head, intestine and heart with Zn content were rich sources of this element for man. EUROPEAN COMMISSION (2001) has given the tolerance levels of Pb and Cd to be between 0.1-1.0 mg kg^{-1} for slaughtered animals. The Pb and Cd contents observed in this study were within these limits.

There were significant differences ($p<0.05$) between the control, test and indocid. The data of As content of the organs seen to indicate a weak as threat to the rats by the indocid and environment. As arsenic is a very poisonous compound, animals need it in small amounts to metabolize protein, amino acids and taurine. Adults need about 12-15 μg day^{-1}. There is no known human deficiency of arsenic (Wardlaw, 1999). Generally, Present results indicated that the organs of the test and control rats were considered safe from As toxicity intake. Head of the control sample can make the greatest contribution to the dietary As intake.

CONCLUSIONS

The results indicated that rats cannot tolerate up to 0.1 g of indocid. The drug administered caused reductions in feed and water intake, which led to toxicity including anemia, weight loss, decreased growth and eventually death. In contrast, water and feed intake increased progressively in control samples. Based on rats organs, hair, feacal and indocid heavy metal data, it can be concluded that indocid did not have significant effects on the rat. Additionally, this part of the country can be seen to be an area of low heavy metal contamination.

The meat samples can be recommended for consumption by man and animal.

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