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Litter Size, Sex Ratio and Some Liver Biomarkers in Sprague-Dawley Rats Recovering From Exposure to Ethanol Extract of *Lepidagathis alopecuroides*

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Abstract: This study was aimed at assessing reproductive recovery of Sprague-Dawley rat after cessation of treatment with ethanol extract of *Lepidagathis alopecuroides* (Vahl). Thirty sexually mature male Sprague-Dawley rats were previously divided into six groups (A-F). Groups B-F administered ethanol extract of *Lepidagathis alopecuroides* orally at a daily dose of 50, 100, 150, 200 and 250 mg/kg body weight, respectively, for 35 days were tested for fertility following 35 days recovery period. Each male was kept with two mature females for mating purposes and observed. Upon delivery the sex, litter sizes and weight of pups were taken. Results showed significant ($p<0.01$) increase in litter size in the 35 days recovery groups. The mean birth weight and the weaning weights of the pups were comparable ($p>0.05$) to the control group. There was small but non-significant ($p>0.05$) increase in Sex ratio in the recovery group and no morphological abnormalities were observed in the pups. Liver function Transaminases (Alanine Transaminase ALT, Aspartate Transaminase AST) elevated during the treatment period reduced to control levels. Phosphatases (Alkaline Phosphatase ALP, Acid Phosphatase) assessed after the recovery period were also reduced to control values 35days after cessation of treatment. Similar reversion to control values was observed in serum total protein, albumin, creatinine, urea and total bilirubin. This investigation reveals that the toxic and reproductive inhibitory effect of *Lepidagathis alopecuroides* is reversible in mammals after cessation of the treatment. Chronic use of the extract is not recommended. However, caution in the use of the plant as an herbal medicine is advocated.

Keywords: Birth weight, litter size, plant extract, rat, sex ratio, weaning weight

INTRODUCTION

It is generally expected based on the chromosome theory of sex determination that there will be equal proportions of the two sexes in vertebrates. However, a wealth of experimental reports indicates small but significant deviations from exact equality in several species including rodents (Rosenfeld and Roberts, 2004; Trivers and Willard, 1973). Evidence from such reports shows that the sex ratio at birth (secondary ratio) differs from that at fertilization (primary sex ratio) due to differential mortality of embryos. Inequality in sex ratio is generally attributed to several factors including selective fertilization and selective mortality of embryos resulting from environmental or genetic influences (Krackow, 1995). Environmental factors capable of distorting the sex ratio in rodents include nutrition of the mother (Clutton-Brock and Iason, 1986). In rats, a maternal diet high in sodium and potassium but low in calcium has been reported to affect the sex ratio of offspring (Bird and Contreras, 1986) interestingly; hamsters dosed with caffeine have significant skewing of the sex ratio toward females which Weathersbee *et al*. (1975) speculatively attributed to inhibition of cAMP phosphodiesterase activity.

Females stressed in some manner tend to produce fewer males than non-stressed females (Lane and Hyde, 1973). Housing pregnant females under crowded conditions reduces sex ratio (Krackow, 1997) even as feed high in fat content ameliorates this effect (Dama *et al*., 2011) Parity has been observed to influence sex ratio of pups born to golden hamsters (Huck *et al*., 1988) Mating at first postpartum estrus tended to produce more males as litter size and sex ratio tend to increase until the third litter and then declined in subsequent litters.

Several plant extracts have been reported to influence litter size and birth weight in rodents including *Phyllantus amarus* (Etta, 2008) *Lepidium meyenii* (Ruiz-Luna *et al*., 2005), *Cajanus cajan* (Olayaki *et al*., 2009), *Ficus platyphylla* (Ugwah-Oguejiofor *et al*., 2011) *Lepidagathis alopecuroides* has already been described (Obomanu *et al*., 2006). Earlier reports have documented the anti-spermatogenic activities of *Lepidagathis alopecuroides* in *Clarias*

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gariepinus (Orlu and Gabriel, 2011a), spermicidal activity in *Clarias gariepinus* (Orlu and Ogbalu, 2011), Partial spermatogenic inhibitory effect in Sprague-Dawley rats (Orlu and Ogbalu, 2012), effect on Fulton's condition factors in *Clarias gariepinus* (Gabriel *et al*., 2010) and effect on some biochemical parameters on the liver and plasma of *Clarias gariepinus* (Orlu and Gabriel, 2011b) The aim of this investigation was to assess the reproductive recovery, if any ,of the male Sprague-Dawley rat following administration and withdrawal of ethanol extract of *Lepidagathis alopecuroides* and to evaluate the effect of the extract on hepato-specific enzymes and other biochemical parameters.

In order to eliminate the effect of competing environmental variables from impacting on litter size and sex ratio, the female rats used in this investigation were of the same species with the males. All females were of the same age, weight, parity, fed the same diet, were housed in pairs per male and fertilization was by natural mating.

MATERIALS AND METHODS

Location of experiment: This investigation was carried out in the Postgraduate Laboratory of the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt; located at latitude 4°48′14″ N and longitude 6°59′12″ E.

Animals and management: Thirty male Sprague-Dawley rats aged four months old and with a mean weight of 250.25±10.16 g were obtained from the Biochemistry Department of the University of Port Harcourt, Rivers State. All rats were housed individually in plastic cages with wire mesh cover in the Postgraduate Laboratory under standard conditions (12 hL:12 hD, room temperature 26±2ºC, relative humidity 54%). The animals had access to cool clean water and standard rat pellet *ad libitum* and were acclimated for 14 days before the commencement of the experiment. The male rats previously administered orally ethanol extract of *Lepidagathis alopecuroides* at daily dose of 50, 100, 150, 200, 250 mg/kg body weight for 35 days were kept for recovery after cessation of the treatment. Females of the same species and age weighing between 180-200 g were randomly selected and divided into six groups (A-F). They were paired with male in the recovery groups (1 male: 2 females) for mating purposes .All experiments were conducted according to the institutional animal care protocols at the Rivers State University of Science and Technology, Nigeria and followed approved guidelines for the ethical treatment of laboratory animals.

Upon delivery the litter size was determined and the pups weighed and checked for deformities.

Other reproductive parameters assessed included:

Sex ratio: Determined as the number of male pups divided by the number of females in the litter.

Litter size: Evaluated as the total number of pups in the litter, dead or alive.

Weaning weight: Determined as body weight of pups alive at day 21 Mean birth weight and mean weaning weight was also calculated.

Biochemical analysis: The male rats were sacrificed after 35 days recovery for the evaluation of liver function. Blood samples were collected individually by carotid bleeding into sterile tubes and the serum separated at 2500 g for 10 min. A sample of the liver was homogenized in 5 mL of physiological saline and the homogenate centrifuged at 3000 rpm for 5 min. The supernatant was removed for biochemical analysis and determination of Alanine amino Transferase (ALT) (Bergmeyer, 1986b) and Aspartate amino Transferase (AST) (Bergmeyer *et al*., 1986a) in serum and liver. Alkaline Phosphates (ALP) (McComb and Bowers, 1972), total bilirubin, (Doumas *et al*., 1973), Urea (Marsh *et al*., 1965), total protein (Strickland *et al*., 1961) Albumin (Doumas *et al*., 1971).

Statistical analysis: Data were subjected to analysis of variance and the Students't-test using the software XLSTAT 2011. All data in tables and figures are presented as the mean±S.D. and the significance level was set at $p<0.05$.

RESULTS

The result of the fertility test showed that there was significant change in the litter size of female rats mated to male rats treated with ethanol extract of *Lepidagathis alopecuroides* at concentrations of 100 mg/kg ($p<0.05$) and 150 mg/kg ($p<0.01$) while the females mated to males treated with higher concentrations did not deliver any litter (Table 1). The extract appeared to exert no effect on the sex ratio as ratios of the treated rats were comparable to those of the control.

On withdrawal of the treatment, followed by 35 days recovery period, fertility of the male rats was restored as seen in Table 2. Litter was obtained from dams mated to males at all concentrations. There was no significant difference ($p>0.05$) between litter size of dams mated to the treated and the control rats.

There was no significant difference ($p>0.05$) in birth weight of pups in the treated and control rats (Table 3). The weaning weight was also not adversely affected by treatment with the extract.

There was no litter in the 200 and 250 mg/kg treated group hence birth weight and weaning weight could not be assessed.

There was no significant change in the birth weight and weaning weight of pups from females mated to

male rats recovering from exposure to *L. alopecuroides* and the control group (Table 4).

The relative concentrations of some biochemical markers in the liver and serum of Sprague-Dawley rats exposed to ethanol extract of *Lepidagathis alopecuroides* are shown in Fig. 1(a-h). Marginal and non-significant decrease of both Serum Total Protein (STP) and Liver Total (LTP) (Fig.1a) was observed after 35 days exposure to ethanol extract of *L. alopecuroides*. Similar non-significant decrease occurred in Serum Total Albumin (STA) (Fig.1b) while the trend in reduction of the concentration

Table 1: Effect of ethanol extract of *Lepidagathis alopecuroides* on litter size and sex ratio in sprague-dawley rats

Conc. of *L. alopecuroides* (mg/kg)	Litter size		Sex ratio	
	Control	Treatment	Control	Treatment
50	8.8±1.26	8.6±1.28	0.51±0.01	0.52±0.02
100	8.6±1.44	6.8±1.48*	0.52±0.02	0.54±0.02
150	8.4±1.64	2.2±1.46**	0.51±0.02	0.54±0.02
200	8.4±1.86	NIL	0.51±0.02	NIL
250	8.6±1.86	NIL	0.51±0.02	NIL

Value are Mean±S.D.; *: Values significant p<0.05; **: Values significant p<0.01

Table 2: Reproductive recovery of sprague-dawley rat (litter size and sex ratio) after 35 days withdrawal of treatment

Concentration *of Lepidagathis alopecuroides* (mg/kg)	Litter size		Sex ratio	
	Control	Treatment	Control	Treatment
50	8.8±1.68	8.4±1.76	0.51±0.02	0.51±0.02
100	8.4±1.68	8.4±1.44	0.51±0.02	0.51±0.04
150	8.8±1.68	8.4±1.42	0.51±0.01	0.52±0.03
200	8.4±1.68	6.2±1.98	0.51±0.02	0.52±0.02
250	8.4±1.68	6.4±1.98	0.51±0.01	0.51±0.02

Table 3: Effect of ethanol extract of *Lepidagathis alopecuroides* on birth weight and weaning weight of pups of sprague-dawley rat

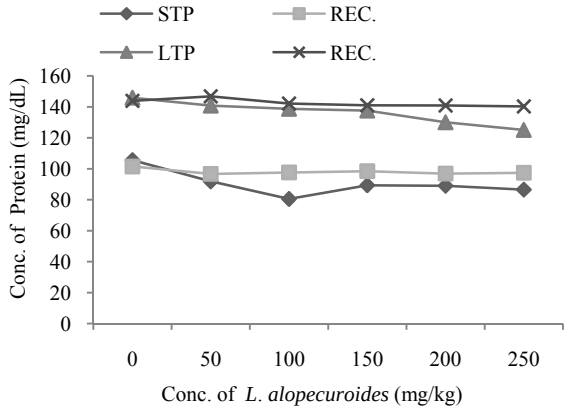
Conc. of *L. alopecuroides* (mg/kg)	Birth weight (g)		Weaning weight (g)	
	Control	Treatment	Control	Treatment
50	5.25±0.50	5.45±0.50	110.50±1.98	108.50±1.96
100	5.78±0.48	5.25±0.65	108.82±1.89	108.08±1.86
150	5.76±0.68	5.24±0.45	110.65±1.68	106.46±1.64
200	5.84±0.55	NIL	108.67±1.55	NIL
250	5.32±0.58	NIL	110.21±1.52	NIL

Values are mean±S.D.

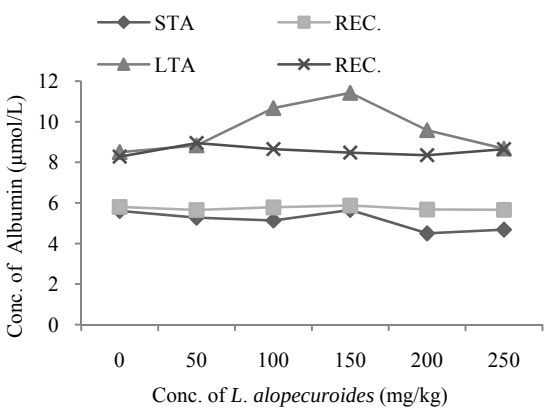
Table 4: Birth weight and weaning weight of 35 days recovery group in sprague-dawley rats

Conc. *of L.alopecuroides* (mg/kg)	Birth weight (g)		Weaning weight (g)	
	Control	Treatment	Control	Treatment
50	5.45±0.45	5.22±0.62	110.88±1.85	109.85±2.01
100	5.48±0.22	5.02±0.32	108.86±1.62	106.25±1.96
150	5.45±0.25	5.12±0.60	108.68±1.96	108.62±1.94
200	5.45±0.42	4.98±0.76	108.75±1.88	106.92±2.15
250	5.42±0.65	4.92±0.76	106.86±1.98	104.28±2.22

Values are mean±S.D.



(a)



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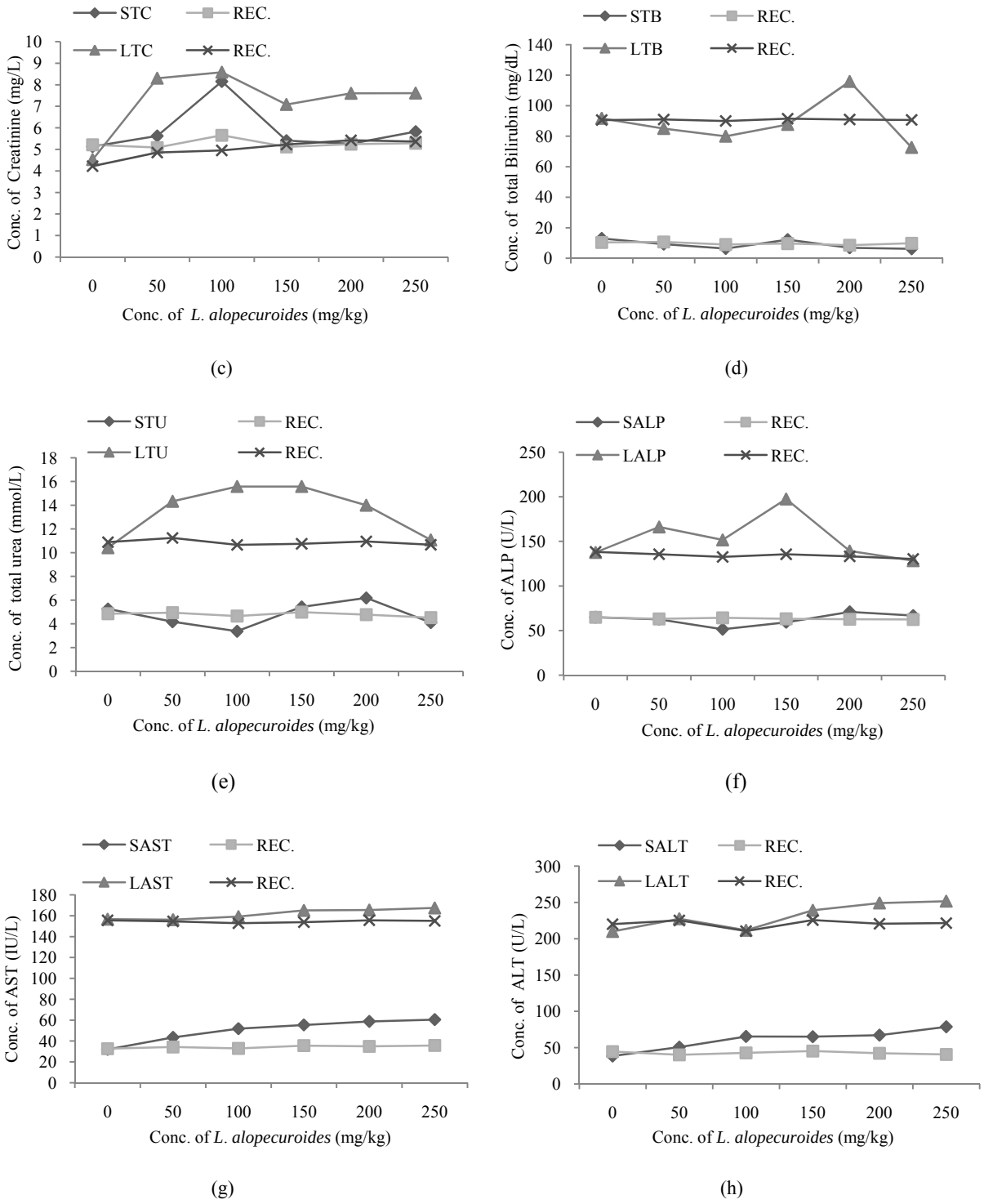

Fig.1: Relative activity of some biochemical parameters in the plasma and liver of male sprague-dawley rats 35 days after treatment with ethanol extracts of *Lepidagathis alopecuroides*
REC.: Recovery group for both serum and liver parameters; S: Serum parameters; L: Liver parameters

of total protein and albumin appeared to be concentration-dependent; there was no statistical difference between the treated and control rats. On the contrary, significant increase was observed in Creatinine (TC) (Fig. 1c), Bilirubin (TB) (Fig. 1d) and Urea (TU) (Fig. 1e)

Also significantly elevated in serum and liver were Aspartate amino Transferase (AST) (Fig. 1g) and

Alanine amino Transfetrase (ALT) (Fig. 1h). However, 35 days after withdrawal of the treatment the levels of the amino Transferases (AST, ALT), metabolites (TP, TC, TB, TA and TU) in the serum and the liver, reduced and were comparable to those of the control animals (Fig. 1a REC-h REC).

DISCUSSION

The result of the fertility test of female Sprague-Dawley rats mated to males treated with high doses of ethanol extract of *Lepidagathis alopecuroides* showed reduction in litter size and complete lack of fertilization at doses of 200-250 mg/kg body. This is an indication that *Lepidagathis alopecuroides* oral administration to male rats interfered with the spermatogenic process exhibited the potential to reduce sperm production and thus reduced fertilizing ability and fertility of the test animals. This result is in agreement with earlier studies which reported that oral administration of ethanol extracts of *Lepidagathis alopecuroides* inhibited spermatogenesis in gravid male *Clarias gariepinus* (Orlu and Gabriel, 2011a) and male Sprague-Dawley rat (Orlu and Ogbalu, 2012). Moreover, it is also in line with the report on spermicidal activities and reduction in sperm count and motility in *Clarias gariepinus* following exposure of gravid males to sublethal concentrations of aqueous extract of this plant (Orlu and Ogbalu, 2011). The finding is also in agreement with Etta (2008) on reduction of litter size and birth weight by intraperitoneal administration of *Phyllanthus amarus* extract at 200 mg/kg bodyweight. The result is, however, in contrast of Ugwah-Oguejiofor *et al*. (2011) who reported that *Ficus platyphylla* increased litter size in female *Rattus norvegicus* thus promoting fertility in the species. Also at variance with present findings is the report on increased litter size but reduced birth weight of *Cajanus cajan* (Olayaki *et al*., 2009) as well as, increased litter size in normal adult female mice by *Lepidium meyenii* (Ruiz-Luna *et al*., 2005)

The mean number of males to females in a litter (sex ratio) was not significantly affected by administration of the extract. This may indicate that there was neither selective fertilization nor selective mortality of male embryos. Analysis of the birth weight and weaning weight of the pups showed no significant change between those of the mothers mated to treated males and the control. Thus it would appear that the ethanol extract of *Lepidagathis alopecuroides* had no adverse effect on body weight of the litter.

Evaluation of recovery by fertility test, 35 days after cessation of treatment showed that the animals in the recovery group regained fertility and produced litter. The ability of the treated rats to recover is indicative of the possibility that anti-spermatogenic and inhibitory effect of the extract is reversible and the treated rat regained fertility after one full cycle of spermatogenesis. This observation is in agreement with Faridha *et al*. (2006) and Lohiya *et al*. (1999) who reported recovery of fertility by mice 70 days after exposure to aflatoxin B1 for 45 days and Rabbits treated with chloroform extract of *Carica papaya* seed respectively.

Assessment of recovery with respect to biochemical parameters showed that all values of the parameters analyzed in serum and liver readjusted to control levels and were statistically comparable.

It was, therefore, concluded that the anti-spermatogenic and inhibitory effect as well as the spermicidal and hepatotoxic effects of *Lepidagathis alopecuroides* extract are reversible within 35 days of withdrawal of treatment in rats. However, caution in the use of the plant as an herbal medicine is advocated.

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