

ORIGINAL ARTICLE

TAMARINDUS INDICA IMPROVED GROWTH RETARDATION ASSOCIATED WITH
PRENATAL ETHANOL EXPOSURE IN WISTAR RAT NEONATES

* Usman I.M.¹, Archibong V.B.², Etukudo E.M.¹, Elisa N.¹, Ayuba J.T.¹, Andrew E.T.¹, Adebisi A.³,
Mwabaleke J.¹ and Diaz E.F.¹

¹Department of Human Anatomy, Faculty of Biomedical Sciences, Kampala International University, Uganda

²Department of Human Anatomy, College of medicine and health sciences, University of Rwanda, Rwanda

³Department of Human Anatomy, College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT**Background:**

Complications associated with alcoholism such as enduring growth retardation, especially during pregnancy, are serious health issues calling for lots of attention.

Objectives:

The study evaluated the effect of *Tamarindus indica* during prenatal ethanol (ET) exposure on pregnancy outcome and morphometric features of Wistar rat pups.

Methodology:

Twenty-four (24) pregnant timed Wistar rats were randomly assigned into 6 groups (n=4); Group A received 2 ml (distilled water), Groups B and C were administered 200 mg/kg ethanol extract of *Tamarindus indica* pulp (EETI), and 300 mg/kg of Vitamin E respectively, Group D received 30% v/v (2 mg/kg) of ET, Group E received 30% v/v (2 mg/kg) of ET + 200 mg/kg EETI, while Group F was administered 30% v/v (2 mg/kg) of ET + 300 mg/kg of Vitamin E. All administrations were via the oral route and lasted for 7 days from prenatal day 7 to 14. On postnatal day (PoND) zero, physical observations were made and the total number of littered pups was counted. Morphometric studies involved the measurements of the crown-rump length (CRL), fore-limb length (FL), hind-limb length (HL), tail length (TL), and body weight (BW) of pups were made using digital vernier caliper and analytical balance.

Result:

Prenatal ET exposure interfered with the pregnancy outcome, CRL, FL, HL, TL, and BW measurement; while treatment with EETI and vitamin E was associated with marked improvement.

Conclusion:

The administration of EETI during prenatal ethanol exposure was associated with significant improvements CRL, FL, HL, TL, and BW pups. Therefore, more attention should be given to the important medicinal plant that could possibly reduce pregnancy complications associated with prenatal ethanol exposure.

Keywords: Ethanol exposure, Fetal alcohol syndrome, *Tamarindus indica*, Morphometric examination

***Corresponding Authors**

Ibe Michael USMAN; Department of Human Anatomy, Faculty of Biomedical Science, Kampala International University, Western Uganda; +256706666798; usmanim@kiu.ac.ug

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INTRODUCTION

Alcohol consumption during pregnancy is associated with fetal alcohol syndrome (FAS) [1]. FAS is characterized by restricted fetal growth and dysfunction of the nervous system [2-4]. The consequence of ethanol present in the prenatal environment is associated with long-term consequences such as enduring growth retardation [5]. Fetal ethanol exposure interferes with fetal growth by impairing placentation [6]. Ethanol has also been reported to interfere with the antioxidant defense system [7].

Fetal growth retardation could be assessed by measurement of Crown-rump length, Tail length, Limb length, and body weight [8]. Intrauterine ethanol exposure affects both fetal and post-gestation bone development [5], hence the need to look for efficient but available remedies.

The use of plant material in complementary and alternative medicine has continued to gain more acceptance over the years [9-11]. *Tamarindus indica* (TI) is used in the management of gonococci, fever, jaundice, and dysentery [12, 13]. TI fruit is an important source of all essential amino acids except tryptophan [14], therefore seeing to the tagging of TI as a very important and accessible source of protein [15]. Phytochemical analysis results of TI reported the presence of phenolic compounds like procyanidin, epicatechin, and tartaric acid [16], which are all known to possess antioxidant potential. Supplementation with antioxidant-rich natural substances can help reduce the risk of pregnancy-associated complications [17]. Previous studies on the effects of ethanolic pulp extract of TI have been reported to be protective during prenatal ethanol exposure [7, 18]. The study evaluated the effect of ethanol extract of TI pulp (EETI) during prenatal ethanol (ET) exposure on pregnancy outcome and morphometric features of Wistar rat

pups.

METHODS

Experimental Animal

Wistar rats were obtained and housed in the animal house of the Human Anatomy Department, Ahmadu Bello University, Zaria, Nigeria. The experimental animals were allowed unrestricted access to animal feed (Vital Feed Grower Mash, Nigeria Ltd) and clean drinking water. The animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Ethanol and Vitamin E preparation

Vitamin E Capsules (Gujarat liquid pharmacaps Pvt; Gujarat, India) were purchased from a reputable drug and chemical store respectively in Zaria, then prepared as outlined in a previous study by Usman et al. [7, 18].

Plant Material

TI pulp was collected around Zaria, and authenticated in the Herbarium Section of the Department of Botany, Faculty of Life Sciences, ABU-Zaria, Nigeria. The extraction of TI pulp extraction was done by maceration method as outlined in a previous study by Usman et al. [7, 18].

Experimental design and administration

Twenty-four (24) pregnant timed Wistar rats were randomly assigned into 6 groups (n=4); Group A received 2 ml (distilled water), Groups B and C were administered 200 mg/kg EETI and 300 mg/kg of Vitamin E respectively, Group D received 30%v/v (2 mg/kg) of ET, Group E received 30%v/v (2 mg/kg) of ET + 200 mg/kg EETI, while Group F was administered 30%v/v (2 mg/kg) of ET + 300 mg/kg of Vitamin E.

All administrations were via gastric intubation from prenatal day 7 to 14 (7 days). On postnatal day (PoND) zero, physical observation was done and the total number of littered pups was recorded according to their grouping. The body weight (BW) of the pups was measured using an analytical weighing balance on PoND 0, 14, 28, and 42.

The crown-rump length (CRL), fore limb length (FLL), hind limb length (HLL), and tail length (TL) of pups were made by analytical balance and digital vernier caliper as outlined by Jyoti et al., [19]. The CRL, FLL, HLL, and TL measurement done in the present study is as shown in Figure 1.

RESULTS

Pregnancy Outcome

Pregnant dams in all the groups littered on day 21 of pregnancy, except in Group 5 where only three out of the four pregnant dams littered (Table 1). ET exposure in the present study was associated with a lowered number of pups per group and a mean number of pups per dam. Treatment with EETI was associated with some improvement (Table 1). Morphologic observation of pups from dams in the various treatment groups showed that there were no observable morphologic defects on all the delivered pups in all the studied Groups.

Crown-rump, Tail, Hind-limb, and Fore-limb length measurement

There was no significant difference between CRL and TL among the study groups, however, the lowest values for CRL and TL were recorded in the groups exposed to ET, with slight improvement following treatment with EETI and vitamin E. Significantly lower HLL and FLL were recorded in the ET exposed group. Treatment with EETI and vitamin E was associated with marked improvement (Table 2).

Body Weight measure

The administration of ET was associated with a lowered body weight on PoND 0 and 14. Treatment with EETI was associated with marked improvement in the mean body weight of pups, with a significant difference reached only on PoND 42 when compared to the group treated with only ethanol ($p < 0.05$) (Table 3)

DISCUSSION

In the present study, there was no visible malformation in all the pups in all the treatment groups. In the ET exposed, 75% of the pregnant dams littered, with a significantly ($p < 0.05$) lowered number of pups per group and mean number of pups per dam. This observation is linked with the ability of ET to induce the resorption of pregnancy or abortion. This observation was in line with the finding of Gundogan [20] who reported a dose-dependent pregnancy loss in which the group given 8% ethanol showed the loss of 5 out of 10 (50%) dams. The group given 18% ethanol, showed a loss of 3 of 9 (33%) dams and the group given 37% showed a loss of 5 of 14 (36%) dams in ethanol exposure groups on prenatal day 18, with no loss in the cControl group. Treatment with the EETI and Vitamin E during prenatal ethanol exposure was observed to be protective of the number of pups per group and the mean number of pups per dam. This observation is supported by the fact that Vitamin E supplementation as an antioxidant may help reduce the risk of pregnancy complications [17]. Early failure of pregnancy is usually associated with lipid peroxidation with resultant damage to the syncytiotrophoblast of the placenta [21], thus resulting in the reduction of the number of pups per group and mean number of pups per dam

There was no significant difference CRL and TL among the study groups, however, the lowest values for CRL and TL were recorded in the groups exposed to ET, with slight improvement following treatment with EETI and vitamin E. Significantly lower HLL and FLL were recorded in the ET exposed group. Treatment with EETI and vitamin E was associated with marked improvement. This suggested that prenatal ethanol exposure was associated with intrauterine growth restriction [22], caused by the impairment of food intake and nutrient absorption resulting from ethanol consumption [8]. Intrauterine ethanol exposure affects both fetal and post-gestation bone development [5]. Fetal ethanol exposure interferes with fetal growth by impairing placentation [6].

The result of the mean body weight of rats in the various treatment groups on postnatal days 0, 14, 24, and 42 showed ethanol exposure was associated with the reduction in mean weights of pups and is in agreement with the findings of Shrestha and Singh [23]. Lowered birth weight as observed on postnatal day zero could also be linked with the ability of ethanol to cause intrauterine growth restriction, an effect linked with ethanol's ability to alter food intake and nutrient absorption [24]. The observed persistent effect of ethanol exposure on the weight of the pups on PoND 14, 24, and 42 may be associated with the fact, the effect of prenatal ethanol exposure may extend into adulthood [5]. The consequence of ethanol present in the prenatal environment is associated with long-term consequences such as enduring growth retardation [5]. The observed improvement following treatment with EETI may link the presence of very important phytochemicals such as phenolic compounds like procyanidin, epicatechin, and tartaric acid [16]. The result of the study though basic offers insight into the protective potential of TI during prenatal ET exposure.

CONCLUSION

The administration of EETI and vitamin E during prenatal ethanol exposure was associated with significant improvements in mean body weight gain, crown rump, fore-limb, hind limb, and tail length of pups. Therefore, more attention should be given to the important medicinal plant that could possibly reduce pregnancy complications associated with prenatal ethanol exposure.

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Table 1: Pregnancy outcome following EETI following prenatal ET exposure

Treatment	%DP	NDP	MNR
			mean±SEM
Control Group	100	31	7.8±0.25 ^a
EETI (200mg/kg)	100	31	7.7±0.33 ^{a,b}
Vitamin E (300mg/kg)	100	31	7.7±0.33 ^{a,b}
Ethanol (2 mg/kg, 30% v/v)	75	17	4.3±1.20 ^c
Ethanol (2 mg/kg, 30% v/v) + vitamin E (300mg/kg)	100	24	6.0±0.00 ^{b,c}
Ethanol (2 mg/kg, 30% v/v) + EETI (200mg/kg)	100	23	5.7±0.33 ^{b,c}

n=4, %DP: Percentage of pups that delivered per group, NDP: Number of pups per group, MNR: Mean number pups per rat, EETI: Ethanolic extract of Tamarindus indica pup. Values along the same column with different superscripts ^{a,b} and ^c are significantly different ($p \leq 0.05$).

Table 2: Morphometric measurements on post gestation day zero following EETI following prenatal ET exposure

Treatment	CRL (mm)	TL (mm)	HLL (mm)	FLL (mm)
Control Group	3.96±0.04 ^a	1.32±0.28 ^a	1.62±0.15 ^a	1.39±0.11 ^a
EETI (200mg/kg)	3.86±0.11 ^a	1.19±0.52 ^a	1.30±0.15 ^a	1.09±0.06 ^b
Vitamin E (300mg/kg)	3.66±0.06 ^a	1.28±0.44 ^a	1.20±0.13 ^b	1.03±0.07 ^b
Ethanol (2 mg/kg, 30% v/v)	3.62±0.06 ^a	1.05±0.43 ^a	0.94±0.10 ^b	0.96±0.04 ^b
Ethanol (2 mg/kg, 30% v/v) + vit E (300mg/kg)	3.58±0.10 ^a	1.30±0.73 ^a	1.12±0.14 ^b	0.77±0.14 ^b
Ethanol (2 mg/kg, 30% v/v) + EETI (200mg/kg)	3.58±0.10 ^a	1.07±0.20 ^a	0.99±0.10 ^b	1.01±0.05 ^b

n= 6; EETI: Ethanolic extract of Tamarindus indica pup, CRL: Crown rump lengths, TL: Tail length, HLL: Hind limb length, FLL: Fore limb length on post-natal day zero. Values along the same column with different superscripts ^a and ^b are significantly different ($p \leq 0.05$).

Table 3: Mean body weight on PoN day 0, 14, 28 and 42 following EETI following prenatal ET exposure

Treatment	BWP 0 (g)	BWP 14 (g)	BWP 28 (g)	BWP 42 (g)
Control Group	5.83±0.18 ^a	29.80±1.11 ^a	64.60±1.78 ^a	117 ± 2.86 ^a
EETI (200mg/kg)	5.87±0.12 ^a	27.80±2.61 ^a	71.20±1.77 ^a	111 ± 4.80 ^a
Vitamin E (300mg/kg)	5.50±0.19 ^a	37.20±1.32 ^b	66.60±3.74 ^a	90.5 ± 11.50 ^b
Ethanol (2 mg/kg, 30% v/v)	4.41±0.30 ^b	25.40±2.50 ^a	59.40±4.21 ^a	91.5± 10.00 ^b
Ethanol (2 mg/kg, 30% v/v) + vit E (300mg/kg)	4.78±0.20 ^b	24.40±2.64 ^a	59.20±2.48 ^a	94.6 ± 3.12 ^b
Ethanol (2 mg/kg, 30% v/v) + EETI (200mg/kg)	5.06±0.18 ^b	29.80±2.25 ^a	64.40±3.14 ^a	113.8 ± 5.9 ^a

Values are expressed as mean±SEM. BWP 0: Body weight on post gestation day zero, BWP 14: Body weight on post gestation day 14, BWP 28: Body weight on post gestation day 28, BWP 42: Body weight on post gestation day 42, EETI: Ethanolic extract of *Tamarindus indica* pup. n=6, except on the 42nd day where n=4. Values along the same column with different superscripts ^a and ^b are significantly different (p≤ 0.05).

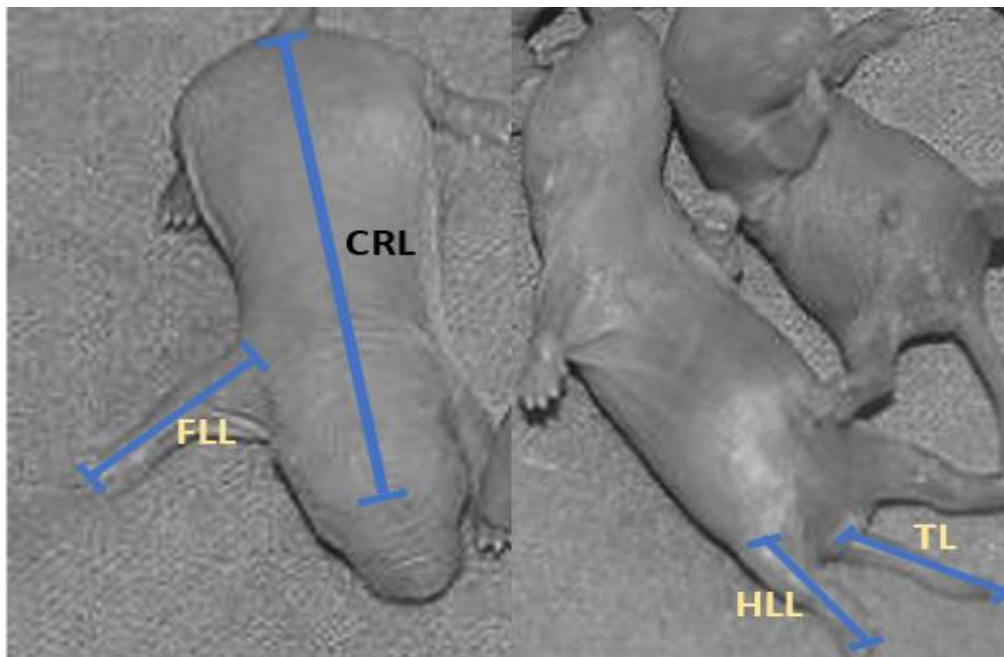


Figure 1: Crown-rump, Tail, Hind-limb, and Fore-limb length. CRL: Crown-rump length, TL: Tail length, HLL: Hind limb length, and FLL: Fore-limb length.