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EFFECT OF PRENATAL ETHANOL EXPOSURE AND EXTRACT OF TAMARINDUS INDICA PULP ON THE CEREBRAL CORTEX OF WISTAR RATS

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ABSTRACT

Aim: The aim of the study was to evaluate the effect of ethanolic pulp extract of Tamarindus indica (EPTI) on the cerebral cortex in prenatal ethanol exposure in Wistar rats.

Method: Twenty four pregnant rats were divided into 7 groups. The Control received 1ml of water, Groups 2 and 3 received 200mg/kg and 300mg/kg body weight (bw) of EPTI and Vitamin E respectively, Group 4 received 0.1ml of olive oil, Group 5 received 30%v/v of ethanol, Groups 6 and 7 received 30% v/v of ethanol and 200mg/kg bw of EPTI; and 30% v/v of ethanol and 300mg/kg bw of Vitamin E respectively. All administrations were via oral route, from prenatal day 7 to 14. At littering the brain tissues of the pups were collected for histological studies while some pups were used for Neurobehavioral studies.

Results: The result of elevated plus maze test showed significant increase in the time spent in the closed arm, rearing and grooming in Group 5 compared to the Control ($p<0.05$). Histological studies showed normal architecture in Groups 1, 2, 3 and 4, Group 5, 6 and 7 showed degenerative changes compared to the Control.

Conclusion: Treatments with EPTI and Vitamin E have shown some potential protective effects on the Cerebral cortex of Wistar rats during prenatal ethanol exposure.

Key words: Cerebrum, Ethanol, Prenatal, Tamarindus indica

INTRODUCTION

Alcoholism and its related complication is one of the most common medical disorders (Musa et al., 2012). Maternal alcohol consumption during pregnancy may lead to the delivery of offsprings who may be diagnosed with fetal alcohol syndrome (FAS) (Maier and West, 2001). FAS are characterized by growth deficiency, microcephally and central nervous system dysfunction (May and Gossage 2001; Onu et al., 2014). Changes in central nervous system function may be the most sensitive signs of prenatal alcohol exposure (Clarren, 1982). The decrease in mental capacity and delayed maturation following prenatal ethanol exposure are associated with alteration in number and structures of neurons throughout the cerebral cortex and other parts of the brain (Musa et al., 2012; Ahveninen et al., 2000; Sampson et al., 2000). Ethanol is capable of generating free radicals, thereby impairing the antioxidant defensive mechanisms in humans and experimental animals (Guidot and Duncan 2002). Many ethanol-induced adverse effects can be prevented or attenuated by antioxidants (Shirpoor et al., 2014). Tamarindus indicia, belongs to the Dicotyledonous family Leguminosae and Sub family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19,327 species (Lewis et al., 2005). Tamarindus indica has been used in the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Kobayashi et al.,

1996; Ferrara, 2005). All extracts of Tarmarindus indica exhibited good antioxidant activity (Siddhuraju 2007). The aim of the study was to evaluate the effect of EPTI on the cerebral cortex during prenatal ethanol exposure in Wistar rats.

MATERIALS AND METHODS

Vitamin E and Ethanol Preparation

Capsules of Vitamin E from GLPL; Gujarat Liquid Pharmacaps Pvt. were cut open and emptied into a clean container. Olive oil was added to prepare a suspension containing 67 mg of the Vitamin E in 0.1 ml of the suspension. Vitamin E was protected from direct contact with air and sunlight to avoid degradation by stocking in a dark and air-tight jar. Absolute alcohol from Anala7 R analytical reagent; BDH Chemical Ltd Poole England were obtained and a stock solution of 30% v/v was prepared by diluting 30ml of absolute alcohol with 70ml of distilled water.

Plant Material

Tamarindus indica pulp was obtained from Samaru Market, Zaria, Kaduna State, and authenticated, with a voucher number of 602 in the Herbarium of the Department of Biological Science, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna State. The extraction of the plant was carried out by maceration as outlined by Jindal et al., (2011).

Experimental Design

Twenty four pregnant timed rats were grouped into seven on the seventh day of gestation. Group 1: Made of 4 pregnant rats served as the Control Group and received 1ml of distilled water only. Group 2: Made of 3 pregnant rats were given 200mg/kg bw of EPTI only. Group 3: Made of 3 pregnant rats were given 300mg/kg bw of Vitamin E only. Group 4: Made of 4 pregnant rats were given 0.1ml of olive oil only. Group 5: Made of 4 pregnant rats were given 2ml of 30% v/v of ethanol only. Group 6: Made of 3 pregnant rats were given 2ml 30% v/v of ethanol and 300mg/kg bw of vitamin E. Group 7: Made of 3 pregnant rats were given 2ml 30% v/v of ethanol and 200mg/kg bw of ethanolic extract of Tamarindus indica pulp. All administrations were done orally by gastric intubation for seven days from the 7^{th} day to the 14^{th} day of gestation. On post gestation day zero, pups were sacrificed humanely from each group for histological studies of the cerebral cortex, while others were used for neuro-behavioural test using elevated plus maze test on post gestation week 8 according to the methods of Shrestha and Singh (2013).

Neurobehavioral Studies

The apparatus consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm) which were connected through a central platform (10 cm × 10 cm). The arms were arranged in a cross shape with the two open arms facing each other and two closed arms facing each other. The maze was kept 45 cm above the floor. The test rat was placed at the center of the maze with its face directed towards one of the closed arms and observed for 5 minutes. The number of entries into closed arm and open arm, time spent in the open and closed arm, as well as number of rearing and grooming were observed. The floor and the walls of the open and closed arms were cleaned with 70% alcohol before each trial.

Histological Studies

The tissues were routinely processed as outlined by Culling (1981) and stained with H&E. Microphotographs were taken at ×400 using MD900 Amscope digital camera**.**

Statistical Analysis

Data were presented as mean ± SEM. For establishing significant differences, data were analyzed by one-way analysis of variance (ANOVA), followed by LSD post hoc test. Values were considered statistically significant when P value was ≤ 0.05).

RESULTS

Elevated Plus Maze Result

The result of the elevated plus maze test performed by the test rats on 8^{th} week of post-natal life (Table 1) showed that the least number of entry into the open arm (NEOA) (3.0 ± 2.1) and the highest number of entries into the closed arm (NECA), the time spent in the closed arm (TSCA), rearing and grooming are 11.5 ± 3.51, 271.2 ± 10.8, 23.5 ± 13.10 and 24.0 ± 15.55 respectively were observed in Group 5. TSCA, rearing and grooming observed in Group 5 were significantly high when compared to the values observed in the Control Group as shown in Table 1.

Histological Study

Groups 1, 2, 3 and 4 showed a normal histological section with intact pyramidal cell body and neuroglia cells. Group 5 treated with ethanol showed evidence of degeneration i.e. vacoulation and chromatolysis. On the other hand Group 6 and 7 showed less degenerative changes when compared to the Control group.

Table 1: Elevated plus maze result of rats from dams in the various treatment groups on the 8th week of postnatal life.

GROUP	Treatment	NEOA/5min	TSOA(sec)	NECA/5min	TSCA(sec)	Rearing/5min	Grooming/5min
		mean±SEM	mean±SEM	mean±SEM	mean±SEM	mean±SEM	mean±SEM
1	Control group	5.0 ± 2.58	38.0 ± 5.65	6.7 ± 1.50	154.0±158.39	13.7 ± 4.85	7.2 ± 1.25
2	Extract (200mg/kg)	5.5 ± 3.53	29.0 ± 10.9	7.0 ± 1.41	216.5 ± 80.05	13.5 ± 2.12	10.0 ± 4.08
3	Vitamin E (300mg/kg)	3.7 ± 1.70	36.0 ± 20.89	6.7 ± 1.50	236.0 ± 68.07	16.2 ± 4.64	11.2 ± 5.73
4	Olive oil (0.1ml)	6.0 ± 2.44	39.0 ± 24.52	8.6 ± 4.45	257.2 ± 25.06	13.7 ± 4.85	17.5 ± 12.81
5	Ethanol (30%v/v)	3.0 ± 2.16	32.7 ± 14.40	11.5 ± 3.51	271.2 ± 10.8*	23.5± 13.10*	24.0 ± 15.55*
6	Ethanol and vitamin E	3.7 ± 2.60	36.0 ± 20.89	9.0 ± 6.00	264.0 ± 20.89	16.2 ± 2.98	14.5 ± 6.35
7	Ethanol and extract	5.6 ± 4.98	42.8 ± 25.06	11.5 ± 3.41	267.2 ± 14.40	14.4 ± 4.56	16.2 ± 13.0

NEOA: Number of entries into open arm, TSOA: Time spent in the open arm, NECA: Number of entries into closed arm, TSCA: Time spent in the closed arm. * represents significance when compared to the Control ($p \leq 0.05$).

DISCUSSION

The elevated plus maze test which is a typical test widely employed to study potential anxiolytic and antidepressant compound in animal model (Acevado et al., 2014). Anxiety like behavior is measured as preference for the closed arm or less activity (entry or duration) in the open arm (Rodgers and Johnson, 1995), Grooming and Rearing are also associated with anxiety like behaviors in the elevated plus maze test (Espejo, 1997). The results of the elevated plus maze test showed a significant increase in TSCA, grooming and rearing in Group 5 when compared to the Control on the 8th week of post gestational life, indicating that prenatal ethanol exposure in this present study was associated with anxiety-like behavior. This observation is in line with the findings of Dursun et al., (2006), who found increased anxiety-like behavior in the EPM and open field tests, respectively, in young adult (PN80-85) offspring after prenatal exposure to high doses of ethanol (6 g/kg, GD7–20). Brocardo et al., (2012) found that exposure to high doses of ethanol during gestation (4.3 g/kg, GD1–22) and the early postnatal period (4 g/kg, PN4–10) resulted in significantly decreased open arm exploration in the EPM in both male and female young adult (PN60) offspring. Cullen et al., (2013) also observed that exposure of pregnant Dawley rat to ethanol (6%v/v) throughout gestation was associated with anxiety like behavior at 8 month and 15 month of post gestational life. Treatment with vitamin E and ethanol pulp extract of Tarmarindus indica did not show significant difference when compared to the Control Group. This observation could be associated with the protective effect accrued to antioxidants. The result of the histological studies showed that prenatal ethanol exposure was associated with neuro-degenerative changes such as pyknosis and vacoulation. These observations are in line with the findings of Iqbal et al., (2004) and Allam and Abdul-hamid (2013), who reported similar changes as a result of prenatal ethanol exposure. On the other hand treatments with ethanol pulp extract of Tarmarindus indica and Vitamin E showed some protection. This is in line with the finding of Zhu et al., (2007) who reported that Vitamin E administration prevented oxidative stress and tissue damage caused by ethanol consumption in the brain, with Tarmarindus indica also appearing on the scene. The observed effect may be due to their antioxidant properties as well as non-antioxidant dependent activities (Shirpoor et al., 2014).

CONCLUSION

Treatments with EPTI and Vitamin E have been shown to have potential protective effect on the cortex of Wistar rats during prenatal ethanol exposure.

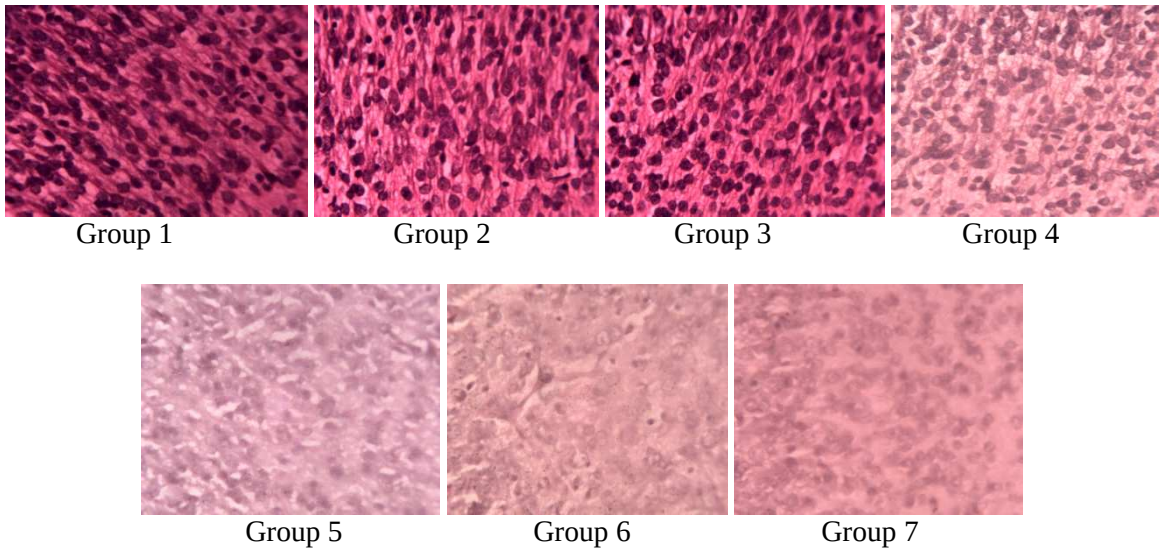

Plate 1: Sections of cerebral cortex of pups in Group 1, Group 2, Group 3 and Group 4, showing pyramidal cell body and glia cell. Groups 5, 6 and 7, showed evidence of degeneration; vacuolation and pyknosis (H and E; ×400).

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