Original Article

Histological and biochemical studies of *Tamarindus indica* pulp extract on the cerebral cortex in prenatal ethanol exposure in Wistar rats

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Abstract

Introduction: Ethanol consumption during pregnancy has been shown to jeopardize the health of the mother and the embryo. **Aim:** The aim of the present study was to evaluate the histological and biochemical changes associated with the administration of Tamarindus indica pulp extract (TIPE) on the cerebral cortex in prenatal ethanol exposed Wistar rats. **Methodology:** Twenty four (24) pregnant rats were divided into 7 groups. Group 1 received 1ml of distilled water, Group 2 received 200mg/kg of body weight (bw) of TIPE only, Group 3 received 300mg/kg bw of Vitamin E only, Group 4 received 0.1ml of olive oil only, Group 5 received 2ml (30%v/v) of ethanol only, Group 6 received 2ml (30% v/v) of ethanol and 200mg/kg bw of TIPE while Group 7 received 2ml (30% v/v) of ethanol and 300mg/kg bw of Vitamin E. All administrations were via gastric intubation and lasted from prenatal day (PD) 7 to 14. The dams were allowed to litter and the brain tissues of the pups were collected for biochemical and histological studies. **Results:** The result of oxidative stress studies showed significant decrease in the mean levels of catalase and glutathione concentration in Groups 5, 6 and 7 respectively when compared with the Control ($P < 0.05$), while the mean concentration of malondialdehyde showed significant increase in Group 5 when compared to the Control ($P < 0.05$) and there was no significant difference in the mean level of superoxide dismutase in all the Groups. The result of sialic acid assay showed significant decrease in the mean level of free, bound and total sialic acid contents in Group 5 when compared to the Control ($P < 0.001$). The histological studies of the cerebral cortex showed normal architecture in Groups 1, 2, 3 and 4, while Group 5 showed degenerative changes with light staining of Nissl substances when compared to the Control and Groups 6 and 7 showed mild degenerative changes when compared to the Control. **Conclusion:** Treatment with TIPE has been shown to have potential protective effect on the Cerebral cortex of Wistar rats during prenatal ethanol exposure.

Key words: Cerebral cortex, ethanol, prenatal, *Tamarindus indica*

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INTRODUCTION

Ethanol consumption during pregnancy may lead to the delivery of children with fetal alcohol syndrome (FAS) (Maier and West, 2001). Features of FAS includes; growth deficiency, microcephaly, and central nervous system (CNS) dysfunction (May and Gossage 2001; Onu *et al.,* 2014). Fetal alcohol spectrum disorders describe a collection of permanent birth defects associated with prenatal ethanol exposure (Astley, 2004). The most sensitive signs of prenatal ethanol exposure are changes in CNS function (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 (Ahveninen *et al.,* 2000; Sampson *et al*., 2000; Musa *et al.*, 2012). Ethanol is capable of generating free radicals, thereby affecting the antioxidant defensive mechanisms in humans and experimental animals (Guidot and Duncan, 2002). Despite the association of prenatal ethanol exposure with injuries of multiple organs and tissues (Fillmore, 2003), alcohol abuse remains a pressing issue especially among pregnant women (Bowie, 2004). Assuming that oxidative stress is one of the major routes of ethanol-induced damage, it is reasonable to supplement antioxidants, in an effort to attenuate this damage (Ornoy and Ergaz, 2010).

Tamarindus indicia belongs to the family Leguminosae and subfamily Caesalpiniaceae, and is the third largest family of flowering plants with a total of 727 genera and 19,327 species (Lewis *et al.,* 2005). *T. indica* has been used in the treatment of many diseases such as fever, dysentery, jaundice, gonococci, and gastrointestinal disorders (Kheraro and Adam, 1974; Kobayashi *et al.,* 1996; Ferrara, 2005). All extracts of *Tarmarindus indica* exhibited good antioxidant activity (Siddhuraju, 2007). Vitamin E is a potent antioxidants capable of reducing oxidative damage by augmenting the function of endogenous free radical scavengers such as superoxide dismutase, catalase (CAT), and glutathione (GSH) peroxidase (Whitehead and Keller, 2003; Son *et al.*, 2004; Ayo *et al*., 2006; Suteu *et al.*, 2007).

Despite the association of prenatal ethanol exposure with multiple organ and tissue injuries, alcohol abuse remains a pressing issue especially among pregnant women (Fillmore, 2003; Bowie, 2004). Oxidative stress is one of the major routes of ethanol-induced damage to organs and tissues, and as such, it is then reasonable to study the effect of different antioxidants in an effort to reduce the damage caused by the oxidative agents such as ethanol (Ornoy and Ergaz, 2010). The aim of this study was to evaluate the histological and biochemical changes due to *T. indica* pulp extract (TIPE) on the cerebral cortex in prenatal ethanol exposure in Wistar rats and its comparison with Vitamin E, thereby providing gestational intervention in the protection of the developing brain against the damaging effect of ethanol during prenatal exposure.

MATERIALS AND METHODS

Vitamin E and Ethanol Preparation

Capsules of Vitamin E manufactured by Gujarat Liquid Pharmacaps Pvt. India were purchased from Farmek Pharmacy Samaru Zaria, Kaduna State. A suspension containing 67 mg of the Vitamin E in 0.1 ml of the suspension was prepared by adding olive oil. Vitamin E was protected from direct contact with air and sunlight to avoid degradation by storing them in a dark and airtight jar.

Absolute ethanol manufactured by BDH Chemical Ltd. Poole England was obtained from Grace Chemicals Sokoto Road Zaria, Kaduna State. A stock solution of 30% v/v of ethanol was prepared by diluting 30 ml of absolute ethanol with 70 ml of distilled water.

Plant Material

T. indica pulp was purchased from Samaru Market, Zaria, Kaduna State, and was authenticated, with a Voucher number of 602 in the Herbarium of Biological Sciences Department, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna State. The extraction of the plant was carried out by maceration method in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria as outlined by Jindal *et al.,* (2011).

Experimental Animals

Twenty-eight adult nonpregnant female and 14 adult males Wistar rats were obtained from the animal house of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. They were fed with pelletized growers feed by Vital Feed, Jos, Nigeria before and during the experiment. Daily examination of a vaginal smear of each female rat was carried out to determine the estrous cycle as outlined by Marcondes *et al.,* (2002). Mating was induced by housing females with a male in the ratio of 2:1 overnight. The presence of vaginal plug the following morning, marked the 0 day of gestation.

Experimental Design

Out of the 28 nonpregnant female rats, 24 female rats were confirmed pregnant and were grouped into seven groups. Group 1 - made of four pregnant rats served as the Control Group and received 1 ml of distilled water only. Group 2 - made of three pregnant rats were given 200 mg/kg body weight (bw) of TIPE only. Group 3 - made of three pregnant rats were given 300 mg/kg bw of Vitamin E only. Group 4 - made of four pregnant rats

were given 0.1 ml of olive oil only. Group 5 - made of four pregnant rats were given 2 ml of 30% v/v of ethanol only. Group 6 - made of three pregnant rats were given 2 ml 30% v/v of ethanol and 300 mg/kg bw of Vitamin E and Group 7 - made of three pregnant rats were given 2 ml 30% v/v of ethanol and 200 mg/kg bw of TIPE.

All administrations were done orally by gastric intubation from the 7th to 14th day of gestation. On the day of gestation, the pups were humanely sacrificed using hypothermic method, and the brain of the pups was opened through a mid-sagittal suture and the cerebral cortices of the brains were removed from each group for oxidative stress, sialic acid, and histological studies.

Histological Studies

The cortical brain tissues were routinely processed as outlined by Cullen *et al.,* (2013) and stained using Cresyl Violet stain and photomicrographs were taken at ×400 using MD900 Amscope digital camera.

Homogenization of the Brain Tissue

Ten grams of the cortical brain tissues were weighed and then homogenized in 100 ml of phosphate buffer, with at a pH of 7.2. The homogenates were centrifuged at a speed of 1500 × *g* for 5 min. The supernatants were collected and used for sialic acid and oxidative stress assays.

Oxidative Stress Studies

CAT activity was determined using the method described by Sinha, (1972) and the absorbance was read at 570 nm. A standard curve was made by plotting the absorbance obtained at various levels of the assay. CAT activity was then obtained from the graph of the standard curve.

The activity of superoxide dismutase (SOD) was determined as outlined by Fridovich, (1989), the absorbance was measured every 30 s up to 150 s at 480 nm from where the SOD activity was calculated.

Malondialdehyde (MDA) as evidenced by the formation of thiobarbituric acid reacting substances, was determined as outlined by Niehaus and Samuelson, (1968), the absorbance of the pink supernatant was then measured against a reference blank using a spectrophotometer at 535 nm.

Reduced GSH concentration was determined as outlined by Ellman, (1959) and the absorbance was read at 412 nm.

Sialic Acid Assay

Sialic acid assay was carried out as outlined by Aminolf, (1961). The sample (500 μl) was pipetted into a test tube, and 500 μl of distilled water was pipetted into a different test tube as the blank. Two hundred and fifty microliter of periodate reagent (25 mM periodate in 0.125 N H_2SO_4 pH 1.2) was added to the test tubes. The tubes were then incubated at 37°C for 30 min in a water bath. The excess periodate was reduced with 200 μl of sodium arsenite reagent (2% of a solution of sodium arsenite in 0.5 N-HCl). As soon as the yellow color of the iodine disappears in 1–2 min, 2000 μl of thiobarbituric acid reagent (0.1 M solution of 2-thiobarbituric acid in distilled water was adjusted to pH 9.0 with NaOH), was added and test tube was covered with aluminum foil. The test tube was incubated in a boiling water bath for 7.5 min. The colored solution was then cooled and shaken with 5000 μl of acid butanol (acetic acid in n-butanol in a 19:1 ratio). The test tube was centrifuged at 1500 ×*g* for 5 min to facilitate rapid separation of the two phases. The color intensity in the butanol layer was then read at 549 nm after standardizing the machine with the blank, and the readings were recorded.

Statistical Analysis

Data were presented as mean ± standard deviation. For establishing significant differences, data were analyzed by one-way analysis of variance, followed by Least Significant Difference *post hoc* test. Values were considered statistically significant when $P \leq 0.05$.

RESULTS

Oxidative Stress Studies

The results of the oxidative stress studies showed significant reduction and an increase in some oxidative stress parameter as presented in Table 1. The mean levels of CAT and GSH concentration showed significant reduction in Groups 5 and 7 and Groups 5 and 6 respectively when compared with the Control ($P < 0.05$), while the mean concentration of MDA showed significant increase in Group 5 when compared to the Control ($P < 0.05$). The result showed that there was no significant difference in the mean levels of superoxide dismutase in all the treatment groups when compared to the Control as shown in Table 1.

The Sialic Acid Studies

The result of the sialic acid studies showed a significant decrease in the mean concentration of free, bound and total sialic acid in Group 5 when compared to the Control ($P \leq 0.001$) as shown in Table 2.

Histological Studies

Groups 1, 2, 3, and 4 showed normal histological sections with intact pyramidal cell bodies and neuroglia cells [Figure 1a-d respectively]. Group 5 showed evidence of degeneration such as vacuolation and chromatolysis with light staining of Nissl substance [Figure 1e]. On the other hand, Groups 6 and 7 showed less degenerative changes with moderate staining of Nissl

Table 1: Effect of *Tamarindus indica* pulp extract and Vitamin E on oxidative stress parameters during prenatal ethanol exposure

Groups	Treatment	CAT (U/mg protein)	MDA (μmol/mg protein)	SOD (U/ml)	GSH (μg/ml)
1	Control group	158.58±4.84	6.17±3.98	41.67±8.33	23.21±1.56
2	TIPE (200 mg/kg)	123.26±4.89	7.33±4.94	75.00±16.67	29.68±3.94
3	Vitamin E (300 mg/kg)	164.84±81.42	14.75±3.05	62.50±4.17	16.39±1.07
4	Olive oil (0.1 ml)	118.32±8.05	12.89±3.46	45.83±12.50	25.53±6.38
5	Ethanol (30% v/v)	46.25±2.10*	30.78±9.59*	50.00±16.67	4.47±2.10*
6	Ethanol and Vitamin E	73.91±4.15	23.59±4.56	87.50±4.17	5.74±0.22*
7	Ethanol and TIPE	49.19±3.16*	20.33±4.44	62.29±5.53	16.60±1.49

Values are expressed as mean±SEM, *$P<0.05$. CAT - Catalase, SOD - Superoxide dismutase, MDA - Malondialdehyde, TIPE - *Tamarindus indica* pulp extract, SEM - Standard error of mean, GSH - Glutathione

Table 2: Effect of *Tamarindus indica* pulp extract and Vitamin E on sialic acid content in the brain tissues of pups during prenatal ethanol exposure

Groups	Treatment	Free sialic acid (mmol/ml)	Bounded sialic acid (mmol/ml)	Total sialic acid (mmol/ml)
1	Control	1.24±0.15	0.05±0.01	1.29±0.16
2	TIPE (200 mg/kg bw)	0.71±0.07	0.03±0	0.73±0.07
3	Vitamin E (300 mg/kg bw)	0.79±0.09	0.03±0	0.83±0.09
4	Olive oil (0.1 ml)	1.32±0.40	0.05±0.02	1.37±0.42
5	Ethanol (30% v/v)	0.19±0.03*	0.01±0*	0.20±0.03*
6	Ethanol and Vitamin E	0.31±0.04	0.02±0	0.34±0.04
7	Ethanol and TIPE	0.56±0.02	0.02±0.01*	0.58±0.02

Values are expressed as mean±SEM, *Represents significance ($P<0.001$). TIPE - *Tamarindus indica* pulp extract, SEM - Standard error of mean, bw - Body weight

substances [Figure 1f and g, respectively] when compared to the Control Group [Figure 1a].

DISCUSSION

Prenatal exposure to ethanol from prenatal day 7–14 was observed to be associated with significant reduction in the mean concentration of CAT and increase in MDA concentration when compared to the Control, thereby buttressing the fact that prenatal ethanol exposure is associated with the induction of oxidative stress with consequent tissue oxidative damage, especially of developing CNS (Goodlett and Horn, 2001). The mean level of superoxide dismutase did not show significance change in all the treatment groups. The mean level of reduced GSH showed significant reduction when compared to the Control. This observation was in agreement with the work of Henderson *et al.,* (1995), who observed a decrease in reduced GSH concentration as a result of prenatal exposure to ethanol. Wu and Cederbaum (2003) reported that ethanol was observed to deplete GSH level in the liver, presumably by reducing the expression or activity of the enzyme needed for GSH production. Ethanol consumption causes generation of free radicals, decrease in the antioxidant level, and subsequent potentiation of oxidative stress (Das and Vasudevan, 2005). Treatment with standard antioxidant, Vitamin E was observed to be associated with the protection in the mean CAT concentration. Treatments with Vitamin E and TIPE were observed to be protective during prenatal exposure to ethanol as values obtained did not show a significant difference when compare to the Control Group. These findings were in agreement with the report that antioxidants protect against oxidative damage (Delanty and Dichter, 2000). As an antioxidant, Vitamin E scavenges peroxyl radicals and stops other free radicals such as singlet oxygen, superoxide, and hydroxyl radicals (Shirpoor *et al.*, 2014). Ferrara, (2005) also reported the antioxidant activities of TIPE.

The result of the sialic acid assay showed that prenatal ethanol exposure was associated with significant decrease in the free, bound and total sialic acid content when compared to the Control. These findings were in agreement with findings of Tanaka *et al.,* (1998) who reported that total sialic acid content can be a potent indicator of oxidative stress in the brain tissues, even in ethanol-induced generation of free radical (Cederbaum *et al*., 1974). The decrease in the total sialic acid content could be a result of an increase in sialidase activity, which is involved in the cleaving of sialic acid (Wilson *et al*., 2011). Azuine *et al.,* (2006) had demonstrated that chronic ethanol administration increases sialidase activity in the myelin sheath and synaptosome membrane fraction. Treatments with Vitamin E and TIPE were both protective during prenatal ethanol exposure, and this observation supports the opinion which considered Vitamin E as a potent antioxidant (Gallo *et al*., 2010), with *T. indica* also showing a strong antioxidant activity (Osawa *et al.,* 1994).

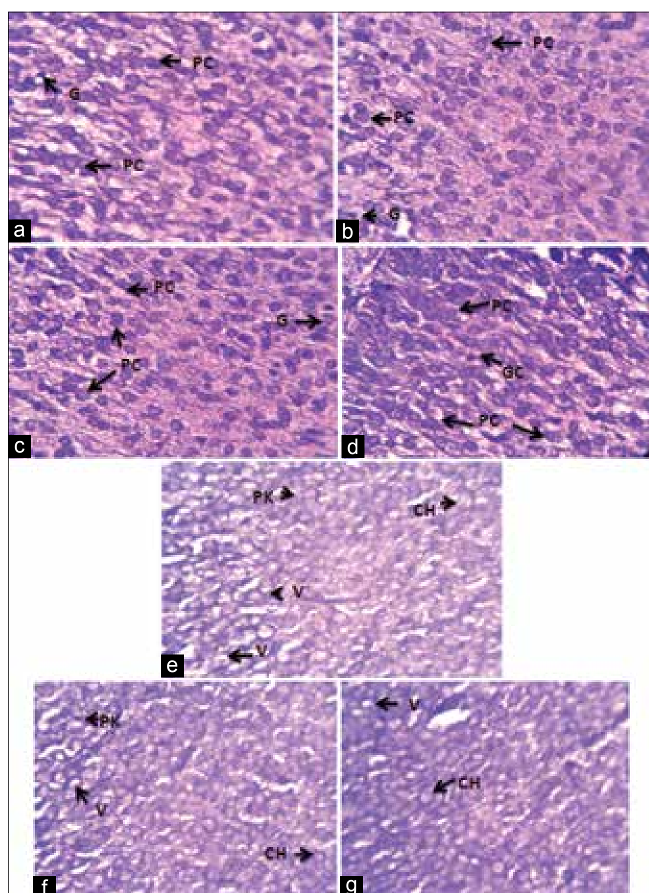

Figure 1: Sections of cerebral cortex of pups in Group 1 (a), Group 2 (b), Group 3 (c), and Group 4 (d), showing pyramidal cell body and glia cell. Group 5 (e) showed evidence of degeneration; vacuolation, and pyknosis with light staining of Nissl substance, while Groups 6 and 7 (f and g) showed evidence of mild degeneration; vacuolation, and pyknosis with moderate staining of Nissl substance (Cresyl Violet, ×400). G - Glia cell, PC - Pyramidal cell, V - Vacuolation, PK – pyknosis, CH - Chromatolysis

The result of the histological studies showed degenerative changes such as pyknosis, vacuolation, and chromatolysis in groups exposed to ethanol. This observation was in line with the findings of Iqbal *et al.*, (2004) and Allam and Abdul-Hamid, (2013), who reported similar pathological changes as a result of prenatal ethanol exposure. High intensity of Nissl substances in neurons is associated with high metabolic activity of these neurons (Steven and Lowe, 1997). Normal neurons showed intense staining of Nissl substance in the present studies. These observations are in line with the findings of Steven and Lowe (1997) who reported that the newborns of humans have high metabolic activity. The ethanol treated group showed light staining of Nissl substances suggesting the interference with metabolic activity in the affected neuronal cells. This observation was in agreement with the findings of Hu *et al.,* (1995) and Heaton *et al.*, (2000) who reported that alcohol causes a disturbance in metabolism, protein deficiency, and consequent cell dysfunction. Treatment with Vitamin E and TIPE were both observed to show lesser neurodegeneration; this could be due to the protective potential of Vitamin E and TIPE. Zhu *et al.*, (2007), had reported that Vitamin E administration prevents oxidative stress and tissue damage caused by ethanol consumption in the brain through its antioxidant properties as well as non-antioxidant dependent activities (Shirpoor *et al.*, 2014).

CONCLUSION

From the present studies, it can, therefore, be concluded that prenatal ethanol exposure was associated with tissue damage. The administration of TIPE and Vitamin E during prenatal ethanol exposure was protective based on the oxidative stress, sialic acid, and the histological studies, as such further studies should be carried out on plant constituent that could protect the developing brain even in prenatal ethanol exposure.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Ahveninen J., Escera C., Polo M.D., Grau C., Jääskeläinen I.P. (2000). Acute and chronic effects of alcohol on preattentive auditory processing as reflected by mismatch negativity. Audiol Neurotol 5 (6):303-11.
2. Allam A., Abdul-Hamid M. (2013). Effect of ethanol ingestion in the pregnant albino rats on the development of pyramidal neurons. Life Sci J 10 (4):241-7.
3. Aminolf D. (1961). Method for quantitative estimation of N-acetylneuraminic acid and application of hydrolysates of sialomucoid. Biochem J 81 (2):384-92.
4. Astley S.J. (2004). Fetal alcohol syndrome prevention in Washington State: Evidence of success. Paediatr Perinat Epidemiol 18 (5):344-51.
5. Ayo J.O., Minka N.S., Mamman M. (2006). Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. J Vet Sci 7 (2):127-31.
6. Azuine M.A., Patel S.J., Lakshman M.R. (2006). Effects of chronic ethanol administration on the activities and relative synthetic rates of myelin and synaptosomal plasma membrane-associated sialidase in the rat brain. Neurochem Int 48 (1):67-74.
7. Bowie B.H. (2004). Interventions to improve interactions between drug abusing mothers and their drug-exposed infants: A review of the research literature. J Addict Nurs 15 (4):15.
8. Cederbaum A.I., Lieber C.S., Rubin E. (1974). Effects of chronic ethanol treatment of mitochondrial functions damage to coupling site I. Arch Biochem Biophys 165 (2):560-9.
9. Clarren S.K. (1982). The diagnosis and treatment of fetal alcohol syndrome. Compr Ther 8 (10):41-6.
10. Cullen C.L., Burne T.H., Lavidis N.A., Moritz K.M. (2013). Low dose prenatal ethanol exposure induces anxiety-like behaviour and alters dendritic morphology in the basolateral amygdala of rat offspring. PLoS One 8 (1):e54924.
11. Das S.K., Vasudevan D.M. (2005). Effect of ethanol on liver antioxidant defense systems: A dose dependent study. Indian J Clin Biochem 20 (1):80-4.
12. Delanty N., Dichter M.A. (2000). Antioxidant therapy in neurologic disease. Arch Neurol 57 (9):1265-70.

13. Ellman G.L. (1959). Tissue sulphydryl groups. Arch Biochem Biophys 82 (1):70-7.
14. Ferrara L. (2005). Antioxidant activity of *Tamarindusindica* L. Ingredient Alimentary 4 (6):13-5.
15. Fillmore M.T. (2003). Drug abuse as a problem of impaired control: Current approaches and findings. Behav Cogn Neurosci Rev 2 (3):179-97.
16. Fridovich I. (1989). Superoxide dismutase: An adaptation to a pragmatic gas. J Biol Chem 264 (14):7761-4.
17. Gallo C., Renzi P., Loizzo S., Loizzo A., Piacente S., Festa M, *et al*. (2010). Potential therapeutic effects of Vitamin E and C on Placental oxidative stress induced by nicotine: An *in vitro* evidence. Open Biochem J 4:77-82.
18. Goodlett C.R., Horn K.H. (2001). Mechanisms of alcohol-induced damage to the developing nervous system. Alcohol Res Health 25 (3):175-84.
19. Guidot D.M., Duncan J. (2002). Chronic ethanol ingestion increases susceptibility to acute lung injury of oxidative stress and tissue remodeling. Chest J 122:309-14.
20. Heaton M.B., Mitchell J.J., Paiva M. (2000). Amelioration of ethanolinduced neurotoxicity in the neonatal rat central nervous system by antioxidant therapy. Alcohol Clin Exp Res 24 (4):512-8.
21. Henderson G.I., Devi B.G., Perez A., Schenker S. (1995). In utero ethanol exposure elicits oxidative stress in the rat fetus. Alcohol Clin Exp Res 19 (3):714-20.
22. Hu I., Singh S.P., Snyder A.K. (1995). Effects of ethanol on glucose transporter expression in cultured hippocampal neurons. Alcohol Clin Exp Res 19 (6):1398-402.
23. Iqbal U., Dringenberg H.C., Brien J.F., Reynolds J.N. (2004). Chronic prenatal ethanol exposure alters hippocampal GABAA receptors and impairs spatial learning in the guinea pig. Behav Brain Res 150:117-25.
24. Jindal V., Dhingra D., Sharma S., Parle M., Harna R.K. (2011). Hypolipidemic and weight reducing activity of the ethanolic extract of *Tamarindus indica* fruit pulp in cafeteria diet- and sulpiride-induced obese rats. J Pharmacol Pharmacother 2 (2):80-4.
25. Kheraro J.; Adam J.G. (1974). The traditional Senegalese pharmacopoeia, Medicinal and Poisonous Plants. Vigotet Freres (Eds), Paris. Pg.1011
26. Kobayashi A., Adenan M.L., Kajiyama S.I., Kanzaki H., Kawazu K. (1996). A cytotoxic principle of *Tamarindus indica*, di-n-butyl malate and the structure-activity relashionship of its analogues. J Biosci 51 (3-4):233-42.
27. Lewis G, Schrire B, Mackinder B, Lock M (2005). Legumes of the World. Royal Botnaic Gardens, Kew.
28. Maier S.E., West J.R. (2001). Regional differences in cell loss associated with binge-like alcohol exposure during the first two trimester's equivalent in the rat. Alcohol 23 (1):49-57.
29. Marcondes F.K., Bianchi F.J., Tanno A.P. (2002). Determination of the estrous cycle phases of rats: Some helpful considerations. Braz J Biol 62 (4A):609-14.
30. May P.A., Gossage J.P. (2001). Estimating the prevalence of fetal alcohol syndrome. A summary. Alcohol Res Health J 25 (3):159-67.
31. Musa A.S., Ibrahim S., Umana U.E., Adebisi S.S., Hamman W.O. (2012). Pathological lesions in the lungs of neonatal wistar rats from dams administered ethanol during gestation. Asian J Med Sci 4 (1):4-7.
32. Niehaus W.G., Samuelson B. (1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 6 (1):126-30.
33. Onu J.E., Oke B.O., Ojegbe P.C., Oyewale J.O. (2014). Morphological alteration of seminiferous tubule of the testes of wistar rats offspring exposed to alcohol during pregnancy and/or lactation. Int. J. Biol. Chem. Sci 8 (1); 1-7.
34. Ornoy A., Ergaz Z. (2010). Alcohol abuse in pregnant women: Effects on the fetus and newborn, mode of action and maternal treatment. Int J Environ Res Public Health 7 (2):364-79.
35. Osawa T., Tsuda T. Watanabe M., Oshima K., Yamamoto A. (1994). Antioxidative components isolated from the seeds of tamarind (*Tamarindus indica* L.). J Agric Food Chem 42:2671-4.
36. Sampson P.D., Streissguth A.P., Bookstein F.L., Barr H. (2000). On categorizations in analyses of alcohol teratogenesis. Environ Health Perspect 108:421-8.
37. Shirpoor A., Norouzi L., Khadem-Ansari M.H., Ilkhanizadeh B., Karimipour M. (2014). The protective effect of Vitamin E on morphological and biochemical alteration induced by pre and postnatal ethanol administration in the testis of male rat offspring: A three months follow-up study. J Reprod Infertil 15 (3):134-41.
38. Siddhuraju P. (2007). Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT Food Sci Technol 40:982-90.
39. Sinha A.K. (1972). Colorimetric assay of catalase. Anal Biochem 47 (2):389-99.
40. Son E.W., Mo S.J., Rhee D.K., Pyo S. (2004). Vitamin C blocks TNF-alpha-induced Nfkappa B activation and ICAM-1 expression in human neuroblastoma cells. Arch Pharmacol Res 27 (10):1073-9.
41. Stevens A., Lowe J. (1997). Human Histology. 2nd ed. Grafos, SA: Arte Sobre Papel, Madrid.
42. Suteu R., Altuntas I., Buyukvanli B., Akturk O., Koylu H., Delibas N. (2007). The effects of diazozin on lipid peroxidation and antioxidant enzymes in rat erythrocytes: Role of Vitamins E and C. Toxicol Ind Health 23 (1):13-7.
43. Tanaka K., Tokumar U.S., Kojo S. (1998). Changes in the level of sialic acid in plasma, brain and liver of inherently scorbutic rats during Vitamin C and E deficiencies. J Biosci Biotechnol Biochem 62 (8):1592-3.
44. Whitehead C.C., Keller T. (2003). An update on ascorbic acid in poultry. Worlds Poult Sci J 59:161-184.
45. Wilson J.I., Emonido O.F., Akpulu S.P., Igbigbi P.S. (2011). Effect of ethanol and sialidase activities on the developing kidney of wistar rats. J Cell Anim Biol 5 (10):200-5.
46. Wu D., Cederbaum A.I. (2003). Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 27 (4):277-84.
47. Zhu Q., Emanuele M.A., LaPaglia N., Kovacs E.J., Emanuele N.V. (2007). Vitamin E prevents ethanol-induced inflammatory, hormonal, and cytotoxic changes in reproductive tissues. Endocrine 32 (1):59-68.