ORIGINAL ARTICLE

TERMINALIA CATAPPA SEED OIL (TCSO) MODULATES FERTILITY HORMONES DYSFUNCTION AND REDOX IMBALANCE IN MALE WISTAR ALBINO RATS EXPOSED TO CIGARETTE SMOKE

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ABSTRACT

The objective of the Study: *Terminalia Catappa* seed oil (TCSO) modulates fertility hormone dysfunction and redox imbalance in male Wistar albino rats exposed to cigarette smoke.
Methods: A total of 36 male Wistar albino rats were randomly distributed into 6 groups (A – F) of 6 rats each. Group A received normal saline and rat feed only. Group B – F were exposed to cigarette smoke for a period of 90 minutes. Group B was left untreated. Groups C - E were treated with 5.0, 2.5, and 7.5 ml/kg body weight (bw) of TCSO respectively. Group F was treated with 100 mg/kg bw of ascorbic acid. The animals were allowed free access to drinking water. Exposure to cigarette smoke was done by inhalation using the box method once daily while administrations of the seed oil and ascorbic acid were done by oral intubation twice daily for 28 weeks.
The serum male fertility hormones and oxidative stress markers were determined using standard biochemical procedures.
Results: There were marked elevated serum levels of follicle-stimulating hormone (FSH), malondialdehyde (MDA), reduced levels of luteinizing hormones (LH), testosterone, estradiol, reduced glutathione (GSH), decreased activities of catalase and superoxide dismutase (SOD) in Wistar albino rats exposed to cigarette smoke. Interestingly, the treatment of Wistar albino rats exposed to cigarette smoke with TCSO restored the hormonal dysfunction and redox imbalance.
Conclusion: The findings of this study show that TCSO was able to restore male hormonal dysfunction and redox imbalance in rats exposed to cigarette smoke and hence could be beneficial in the management of male hormonal and oxidative imbalance associated with cigarette smoking linked to male infertility

Keywords: *Terminalia Catappa*, Seed oil, Fertility hormones, Redox imbalance, Wistar male albino rats, Cigarette smoke

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INTRODUCTION

Cigarette smoking is one of the core causes of male infertility (1), and a previous study by Bundhun et al. (2) revealed that about 120, 000 young men between the ages of 30 to 50 years old in the United Kingdom are impotent as a result of this unhealthy habit. Kovac et al. (3) reported that about 50 % of male infertility cases among couples is progressively leading to depression and other psychological problems. Cigarette smoking has been linked to male infertility, resulting in lower sperm concentration, poorer sperm motility, and a smaller percentage of morphologically normal sperm (4, 5). Sansone et al. (6) reported that tobacco smoking is remarkably common in most first-world countries; despite a progressive decline in the United States (US), showed a prevalence of more than 30% in subjects of reproductive age-a disturbing perspective, given the well-known ill effects on reproductive and sexual function as well as general health. Almost 15% of all couples trying to conceive are affected by infertility, and in almost half of these cases, male infertility is the sole or contributing factor (7). The decline of male fertility is not an empty threat, evidence points to a steadily progressive decline in sperm concentration over the past 35 years (6). The greatest smoker prevalence (46% of smokers aged 20 to 39 years) is reported in young adult males during their reproductive lifetime (8).

Tobacco smoke is an endocrine disruptor that has been found to produce increased sperm DNA fragmentation, sperm mutagenesis, and sperm polyploidy, as well as many genetic alterations, all of which decrease male fertility (9). Despite global anti-smoking programs, cigarette smoking remains widespread and the overall number of couples attending an infertility clinic is on the increase (10). Lower testosterone levels may contribute to the risk of infertility linked with smoking. Men who smoke may have a decreased sperm count than non-smokers. According to the American Society for Reproductive Medicine, smokers have reduced sex desires and less frequent sex (11). Smoking among the young population is getting increasingly prevalent (12). *Terminalia catappa* is a large tropical tree in the leadwood tree in the family of Combretaceae, which grows mainly in the tropical regions of Asia, Africa, and Australia (13). Common names in English include Country almond, Indian almond, Malabar almond, Sea almond, Tropical almond, Beach almond, and False Kamani (14). Jahurul et al. (15) reported that *Terminalia catappa* kernel oil has an interesting fatty acid composition, displaying the lowest atherogenicity and thrombogenicity indices, highest polyunsaturated/saturated fatty acids, high total phenolic content (TPC) and hypocholesterolemic/hypocholesterolemic ratios, respectively. The application of essential oil has spread evenly throughout the whole world as well as its analysis, which had led to a tremendous increase in the yield and quality of essential oil production (16).

Therefore, *Terminalia catappa* seed oil (TCSO) which is rich in essential oil is being explored for its therapeutic potential in the management of hormonal dysfunction and redox imbalance enlisted as the predisposing factor to male infertility

METHODS

Chemicals and Reagents

The chemicals and reagents used for this study were of analytical grade. The testosterone, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Estradiol (E2) used were Enzyme-Linked Immunoassay Test Kits. MDA, GHS, SOD, and CAT Kits used were of analytical quality and were bought from Sigma Aldrich (St. Louis Mo USA). The ascorbic acid that was used for this study was bought from CeeJay Global Resources Limited, Abakaliki, Ebonyi State, Nigeria. The cigarettes (King) used in the study were bought from the dealer at Abakaliki, Ebonyi State, Nigeria

Plant Materials

Fresh seeds of Terminalia catappa were collected from Ndinwali village in the Igbeagu community, Izzi Local Government Area of Ebonyi State, Nigeria. The plant sample was identified and authenticated by a taxonomist Mr. Nwankwo Onyebuchi Ephraim of Applied Biology Department, Faculty of Science, Ebonyi State University, Nigeria. A total of 25 kg of the fruits of Terminalia Catappa were collected from the same species. The fruits of *Terminalia catappa* were sun-dried after which the fruits were broken to remove the seeds from the seed coats. The dry seeds were carefully ground to powder meal using a grinding machine. The seed oil of the Terminalia catappa was extracted from the powdered seed meal using a soxhlet extractor according to the method of Abarikwu et al. (17).

Animal handling

The animals were used according to the Departmental Ethical Review Committee guidelines (Approval Number: EBSU/BCH/ET/18/005) and per the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised in 1996) (18).

Acute toxicity of TCSO

According to OECD/OCDE Guidelines no. 425, the acute toxicity study was carried out using the limit dose up and down method. Male Wistar albino rats (aged 6 weeks) were used in the experiment, and they were acclimatized to the laboratory condition for seven days before starting. A male rat was given 50 ml/kg of TCSO orally after an overnight fast. Following TCSO administration, the animal was closely monitored for the first 30 min for physical or behavioral changes, then for the next 24 h, and then every day for the next 14 days. Food was given after 3 h of TCSO administration. Since the first rat survived, four more male rats were recruited and fasted for 4 h. They were then given the same dosage of TCSO and subjected to the same stringent monitoring for the next 14 days for any signs

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of toxicity (19, 20). Within the 24 h and 14-day testing periods, the rats did not exhibit any signs of gross physical or behavioral modifications such as hair erection, decrease in eating, or motor movements at the limited test dose of 50 ml/kg. For this reason, based on OECD guideline No 425, 10 % of the limit dose (5 ml/kg) was chosen as the middle/intermediate dose, half of it (2.5 ml/kg) as the lower dose, and 1.5 times the middle dose (7.5 ml/kg) as the higher dose (21).

Experimental design

A total of 36 Wistar male albino rats were randomly divided into six groups of A-F (N = 6); Group A (normal ontrol) rats were fed on normal rat pellet and allowed free access to water without restriction; group B (Negative control) was exposed to cigarette smoke for a period of 90 minutes; group C (Cigarette and 5 ml/Kg bw of TCSO) was exposed to cigarette smoke for a period of 90 minutes and administered 5 ml/Kg bw of TCSO; group D (Cigarette and 2.5 ml/Kg bw of TCSO) was exposed to cigarette smoke for a period of 90 minutes and administered 2.5 ml/Kg bw of TCSO; group E (Cigarette and 7.5 ml/Kg bw of TCSO) was exposed to cigarette smoke for a period of 90 minutes and administered 7.5 ml/Kg bw of TCSO and group F (Standard group) was exposed to cigarette smoke for a period of 90 minutes and administered 100 mg/ Kg bw of Ascorbic acid. Exposure to cigarette smoke was done by inhalation using box method once daily while administrations of the seed oil and ascorbic acid were done by oral intubation twice daily for 28 weeks.

Blood sample collection

The blood samples were collected via cardiac puncture under mild anesthesia using diethyl ether following overnight fast of the animals using 5 ml syringes into plain tubes for the determination of the levels of testosterone, follicle-stimulating hormone, luteinizing hormone, and estradiol using enzyme linked immunosorbent assay kits. While the activities of catalase, SOD, and the levels of GSH and MDA were determined with their respective rat kits.

Hormonal Determination

The serum testosterone, follicle-stimulating hormone, luteinizing hormone and estradiol levels were determined based on method described by Turkes et al. (22)

Oxidative Stress Markers Determination

Superoxide dismutase (SOD) activity was determined by the method of Sun et al. (23). Catalase (CAT) activity was assayed by monitoring the decomposition of H2O2 at 240 nm as described by Aebi (24). Reduced glutathione (GSH) and malondialdehyde (MDA) levels were determined by the method described by Ozdemir et al. (25).

Statistical Analysis

Data were expressed as means ± SD. The differences in mean were compared for statistical significance by Bartletts ANOVA tests. Difference was considered significant at $p<0.05$. The statistical analysis was

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performed using graph pad prism version 8.

RESULTS

Effects of Terminalia catappa seed oil (TCSO) on fertility hormones of rats exposed to cigarette smoke

Exposure of cigarette smoke to rats significantly ($p<0.05$) elevated the level FSH and significantly ($p<0.05$) reduced the levels of LH, testosterone and estradiol as shown in Figures 1-4. Treatment of rats exposed to cigarette smoke with TCSO significantly ($p<0.05$) reduced the FSH level and elevated LH, testosterone and estradiol levels (Figures 1-4).

Effects of Terminalia catappa seed oil (TCSO) on Oxidative stress indices of rats exposed to Cigarette smoke

Exposure of cigarette smoke to rats significantly ($p<0.05$) elevated the level of MDA and significantly ($p<0.05$) reduced the activities of catalase, SOD and level of GSH (Figures 5-8). Treatment of rats exposed to cigarette with TCSO significantly ($p<0.05$) reduced the MDA level and elevated the activities of catalase, SOD and level of GSH as shown in Figures 5-8.

DISCUSSION

Cigarette smoke contains compounds that are suspected to cause reproductive damage and possibly affect hormone activity by generating free radicals. Carrao et al. (26) reported that nicotine and carbon monoxide are part of the compositions of cigarette smoke, which are implicated in most biochemical pathways as competitive inhibitors and producers of reactive oxygen species (ROS) which are two toxic and addictive chemical constituents that may be responsible for oxidative stress. These patterns suggest that chemicals in tobacco smoke alter endocrine function, perhaps at the level of the testes, which in turn effects release of the pituitary hormones. This endocrine disruption likely contributes to the reported associations of smoking with adverse reproductive outcomes, including infertility and earlier menopause (27).

Our present study revealed that exposure to cigarette smoke in rats significantly ($p<0.05$) elevated the serum level of follicle-stimulating hormone (FSH), and significantly ($p<0.05$) reduced the levels of luteinizing hormones (LH), testosterone, and estradiol (Figures 1-4). However, co-administration of TSCO or ascorbic acid to rats exposed to cigarette smoke significantly ($p<0.05$) reduced the FSH level and significantly ($p<0.05$) elevated LH, testosterone and estradiol levels as shown in Figures 1-4. In animal studies, exposure to cigarette smoke in rodents have been revealed to have degenerated and lower number of Leydig cells (27), as well as a marked decrease in germ cell count and seminiferous tubule diameter in the testis (28, 29). Tobacco smoke is an endocrine disruptor that has been found to produce increased sperm DNA fragmentation, sperm mutagenesis, and sperm polyploidy, as well as many genetic alterations, all of which decrease male fertility (9). Decreasing LH will cause the number of leydig cells to decrease in its activities so that the testosterone produced will also decrease. This findings

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correlate with the results from the similar investigation according to Bakheet and Al-Refaecy (30) and Eze et al. (31) which investigated the relationship between tobacco smoking and serum concentrations of male reproductive hormones (luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and prolactin). The risk of infertility associated with smoking may be attributed to lower levels of testosterone. This decrease in testosterone levels may translate to a decrease in fertility as testosterone is the principal reproductive hormone in males.

Our study also revealed a marked elevation in MDA level with a reduction in the level of GSH and activities of SOD and catalase (Figures 5-8). But co-administration of TCSO or ascorbic to rats exposed to cigarette smoke restored the elevated serum MDA level to a level comparable to a level observed in the normal control group (Figure 6). Similarly, serum GSH level and activities of SOD and catalase were restored to a comparable status with the control group (Figures 5, 7 and 8). These results showed that Cigarette smoke exposure was able to alter antioxidant capacity negatively in rats. Our present result is in accordance with the study of Ge et al. (32) which reported that the levels of serum NF-κB were significantly increased in cigarette smoke-exposed rats ($P < 0.05$), while the SOD level was decreased ($P < 0.05$). However, our present result is also in line with the study of Mohamed et al. (27) which reported that supplementation of honey in rats exposed to cigarette smoke significantly ($p<0.05$) reduced histological changes and thiobarbituric acid reactive substances (TBARS) level, increased total antioxidant status (TAS) level, as well as significantly ($p<0.05$) restored activities of GPx, SOD and CAT in rat testis exposed to cigarette smoke.

Smoke-related free radicals can increase the levels of reactive oxygen species (ROS) produced by phagocytes, causing them to enter the bloodstream, alter antioxidant capacity in the blood, and hence various complications (33). Lipid peroxidation is a chain reaction starting with oxidation of polyunsaturated fatty acids in the cell membrane by ROS, continuing with the formation of lipid hydroperoxides, and ending with many by-products (34). Malondialdehyde is one of the final products of lipid peroxidation and generally accepted indicator of oxidative stress (35). The GSH levels reduced due to the increased lipid peroxidation by cigarette smoking.

In animal studies, exposure to cigarette smoke in rodents has been revealed to have degenerated and lower number of Leydig cells (27), as well as a marked decrease in germ cell count and seminiferous tubule diameter in the testis (28, 29). The exact mechanisms leading to the adverse effects on sperm indices, fertility hormones and testis are still speculative. But nicotine and carbon monoxide are twin products of cigarette smoke, which are implicated in most biochemical pathways as competitive inhibitors and producers of reactive oxygen species (ROS), and are toxic and addictive chemical constituents that may be responsible

for oxidative stress (12). However, it has been linked that cigarette smoke produces oxidative stress by the release of free radicals which could be responsible for the marked elevation in the level of malonaldehyde, a marker of lipid peroxidation, in rat testis after 45 days of exposure to cigarette smoke as compared to the control group (27). This is also associated with a significantly lower glutathione level and activity of glutathione peroxidase in rat testis (28).

CONCLUSION

The findings of this study shown that TCSO was able to restored male hormonal dysfunction and redox imbalance in rats exposed to cigarette smoke and hence could be beneficial in the management of male hormonal and oxidative imbalance associated with cigarette smoking linked to male infertility.

RESULTS

The descriptive characteristics of the neonatal parameters for males and females were presented in Table 1. The mean birthweight and placenta weights were 3.24 ± 0.51 and 0.59 ± 0.14, respectively. There were more deliveries through SVD compared to C/S.

Table 3 represents the mean and test of mean difference in the neonatal parameters and sex of the neonates. The difference in the weight of birth (p 0.148), weight of placenta (p 0.831) and occipitofrontal circumference (p 0.467) was not significant in males and females. The mean S.D of males were WOB (3.19±0.48), WOP (0.59±0.17) and OFC (34.72±1.36). The mean S.D of females were WOB (3.28±0.53), WOP (0.60±0.12) and OFC (34.86±1.52).

The mean and test difference between the neonatal parameters and mode of delivery was presented in Table 4. The difference in the weight of birth (p 0.780), weight of placenta (p 0.147) and occipitofrontal circumference (p 0.089) were not significant in males and females with mode of delivery. The mean S.D of SVD were WOB (3.23 ± 0.48), WOP (0.58± 0.14) and OFC (34.67±1.44). The mean S.D of C/S were WOB (3.25±0.56), WOP (0.61 ± 0.16), and OFC (35.00 ± 1.43).

Table 5 shows the mean (S.D) and the test of mean difference of the neonatal parameters and mode of delivery for male and female neonates. In SVD, the difference in the weight of birth(kg), weight of placenta(kg) and occipitofrontal circumference(cm) of males and females was not significant (p 0.474, 0.802 and 0.621 respectively). In C/S, the difference in the weight of placenta (kg) of males and females was not significant (p 0.876). However, weight of birth (kg) and occipitofrontal circumference (cm) of males and females showed significant difference (p 0.002 and 0.038 respectively). Females weighed more and had larger occipitofrontal circumference than males.

Table 6 shows the correlation between the neonatal parameters stratified by sex and the mode of delivery. For males delivered through SVD, it was found that

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there was a positive correlation between the weight of birth and occipitofrontal circumference (P<0.001), a positive correlation between the weight of birth and weight of placenta (P=0.009) and a positive correlation between the weight of placenta and occipitofrontal circumference (P<0.001). While in C/S, a positive correlation was found only between the weight of birth and occipitofrontal circumference (0.001). However, there's no significant correlation between the weight of birth and weight of placenta and the weight of placenta and occipitofrontal circumference. For female delivered through SVD, a positive correlation was found only between the weight of birth and occipitofrontal circumference (P<0.001). However, there was no significant correlation between the weight of birth and weight of placenta and the weight of placenta and occipitofrontal circumference. In C/S, a positive correlation was found between the weight of birth and occipitofrontal circumference (P<0.001), the weight of birth and weight of placenta (0.021), and the weight of placenta and occipitofrontal circumference (P<0.001).

DISCUSSION

Determination of sex is important in establishing the identity of an individual. The sex of embryo affects the size of both the fetus and the placenta, and the ability of the placenta to respond to adverse stimuli. Differences in how male and female placentas cope with stressful conditions helps us to understand how it contributes to sexual dimorphism later in life [10].

Past study found that the mean birth weight of male neonates is comparatively greater than that of female neonates [11] which is in contrast to this present study which shows that there's no significant difference between the mean birth weight of male neonates and female neonates. In previous studies conducted, they concluded that because placental weight has a relationship with birth weight, there should be a positive correlation between infant sex and placental weight.[12,13,14] However, another study found no positive correlation between infant sex and placental weight,[15] which is similar to this present study. This may be perhaps due to differences in ethnic and/or genetic factors [15]. Birth weight is dependent of head circumference; birth weight and head circumference are dependent on sex [3]. The study found that male neonates had a higher birth weight and occipitofrontal circumference compared to that female neonates [3]. However, in this present study, it was discovered that there was no significant difference between the occipitofrontal circumference of male neonates and female neonates.

In this study, it was found that there's a positive correlation between the placental weight and the birth weight in neonates delivered through SVD, that is, an increase in the placental weight will lead to a significant increase in the birth weight of neonates, whereas no positive correlation was found between the placental weight and birth weight in neonates delivered through

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C/S. This is similar to a study carried out which found a positive but weak correlation between the placental weight and birth weight.[9] Also, in another study carried out it was discovered that the placental weight increases are associated with rise in birth weight in normal pregnancy.[16]

Panti et al.[9] reported a high mean placental weight, and lower birth weight for newborns delivered through C/S compared to those delivered vaginally. The finding for placenta weight was same in this study, but differ for the birth weight. Differences in cord clamping time have been suggested to explain these differences since the umbilical cords are frequently clamped relatively late in vaginal delivery (so as to optimize blood transfer to the fetus) while in caesarean section early clamping of the cord is usually the rule. Furthermore, due to the absence of uterine contractions during caesarean section, the intervillous space in the placenta is more expansive and likely to contain more maternal blood than in vaginal deliveries where maternal blood is squeezed out of the placenta by contraction of the uterus[9].

CONCLUSIONS

From this present study, it was concluded that the neonatal parameters are not enough to determine the sex of neonates. Sex of neonates couldn't be determined from birth weight, placental weight and occipitofrontal circumference because there was no significant difference between the male and female neonates. There was a positive relationship between the sex of the neonates and occipitofrontal circumference which was negligible notwithstanding. However, some correlations were found between the neonatal parameters. An increase in the birth weight leads a significant increase in the placental weight. Also, the placental weight was significantly different in terms of the mode of delivery; it being higher in neonates born via C/S.

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Plate 1: *Terminalia catappa* Fruit

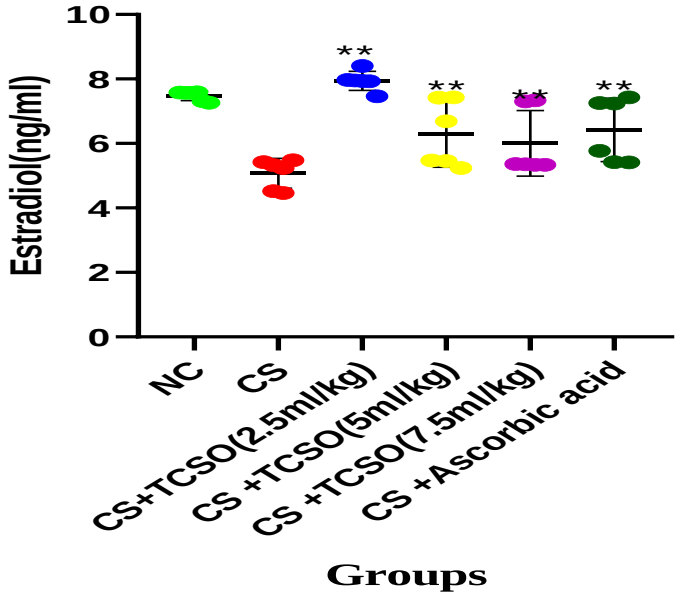

Figure 1: Effect of TCSO on serum Estradiol level in rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS)

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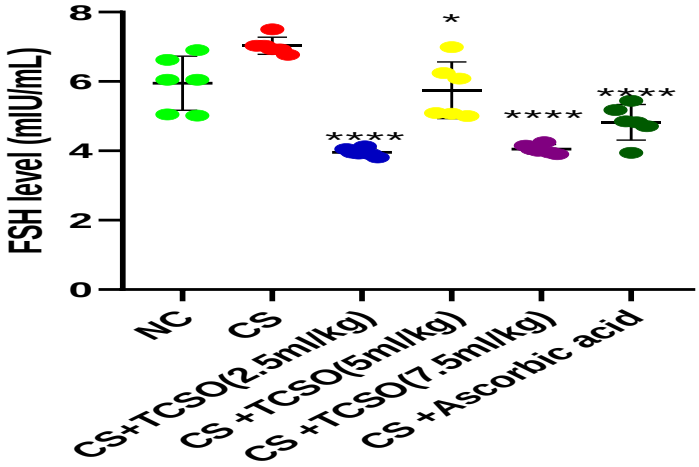

Figure 2: Effect of TCSO on serum Follicle stimulating hormone level in rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS)

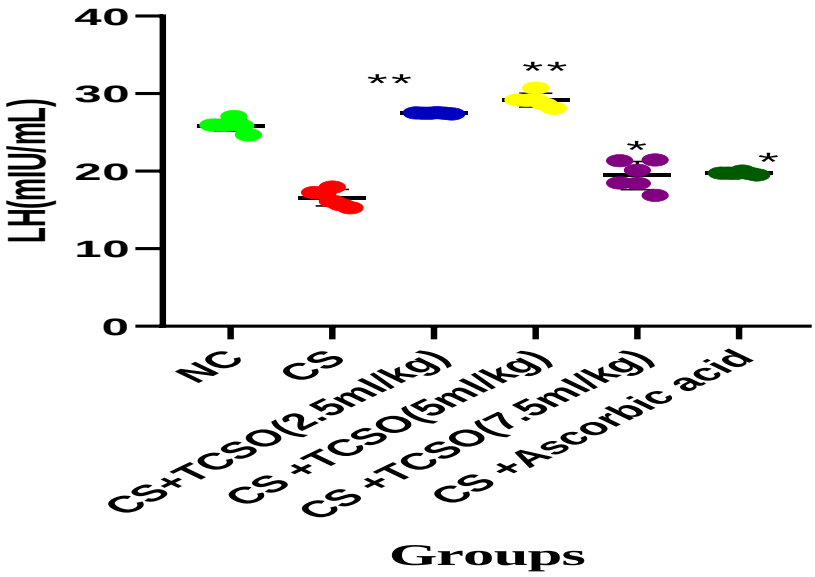

Figure 3: Effect of TCSO on serum Luteinizing hormone level in rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS)

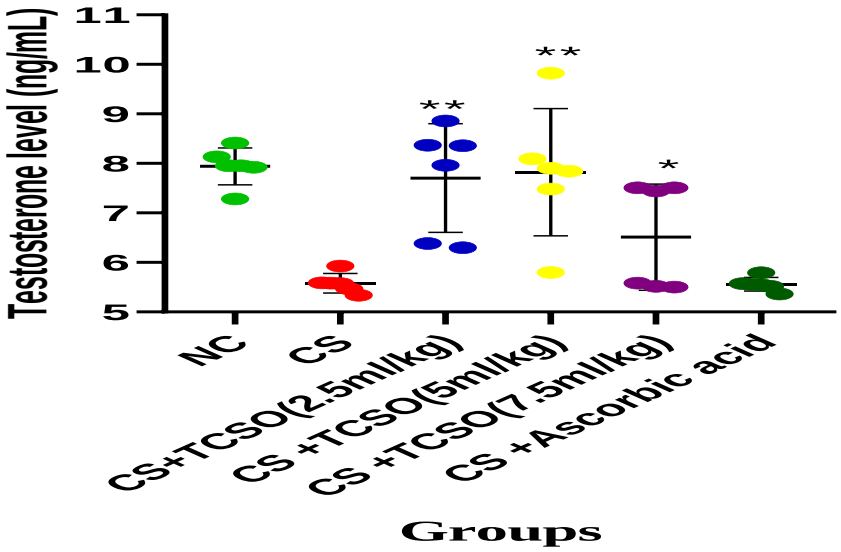

Figure 4: Effect of TCSO on serum Testosterone level in rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control (CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke (CS)

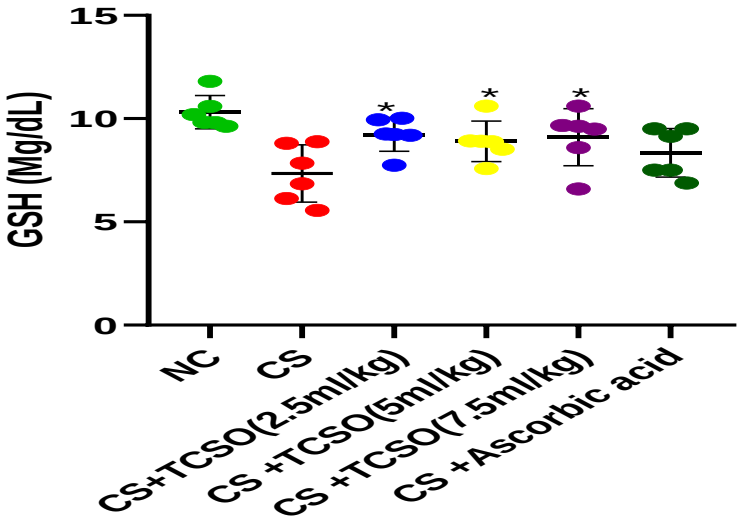

Figure 5: Effect of TCSO on serum Reduced glutathione level of rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS)

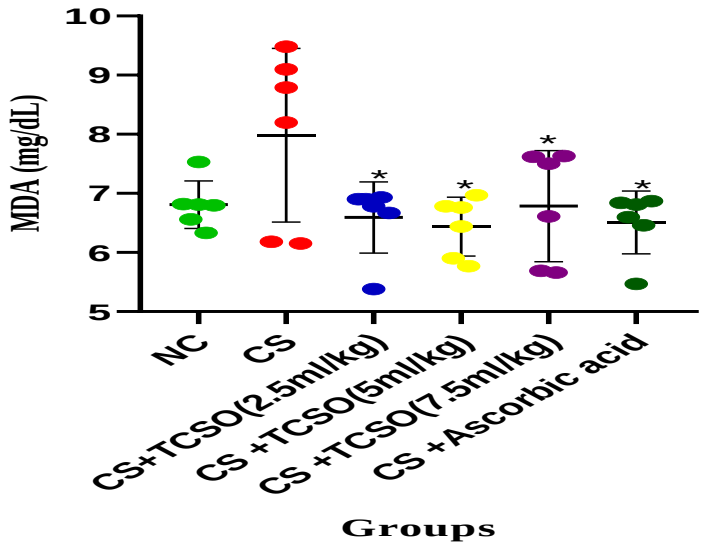

Figure 6: Effect of TCSO on serum Malondialdehyde level of rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS).

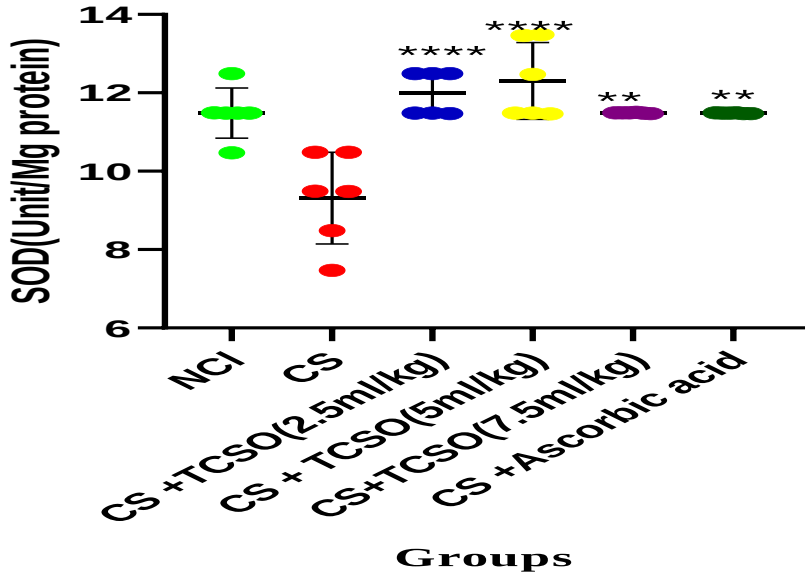

Figure 7: Effect of TCSO on serum Superoxide dismutase (SOD) Activity in rats exposed to cigarette smoke Data are shown as mean ± S.D (n=6). *Mean values of different groups were

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compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke(CS).

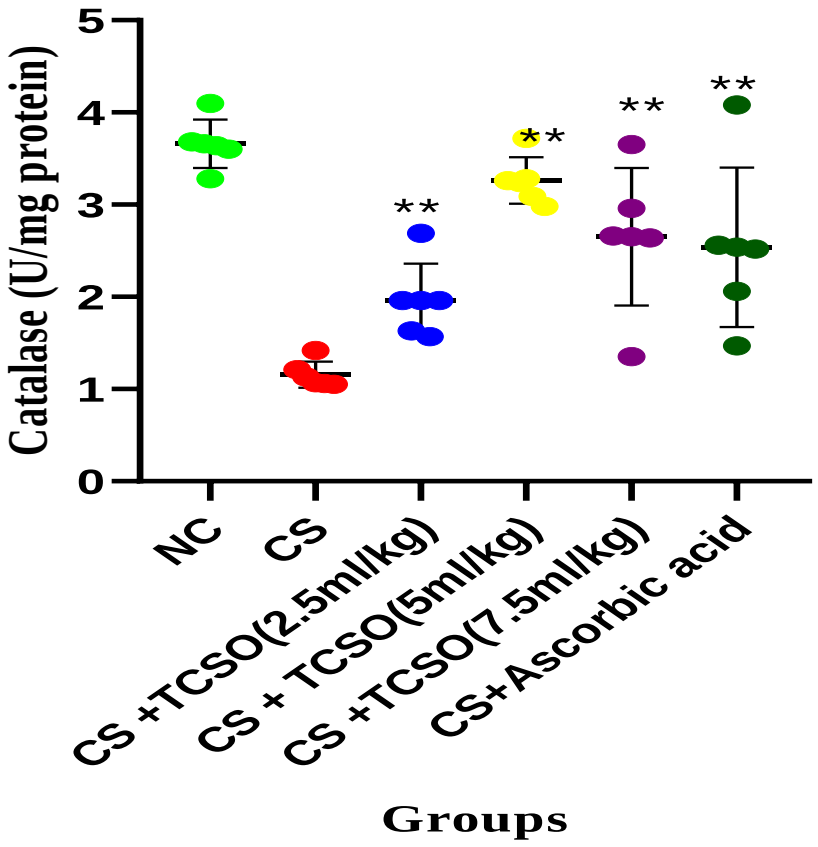

Figure 8: Effect of TCSO on serum Catalase Activity in rats exposed to cigarette smoke. Data are shown as mean ± S.D (n=6). *Mean values of different groups were compared with the control(CS) using Bartletts ANOVA with a significant difference at P<0.05. *Terminalia catappa* seed oil (TCSO), Normal control (NC) Cigarette smoke (CS)

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