Soy Oil Reduces Antioxidants in Litters of Treated Pregnant Rats

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ABSTRACT

Background: Diet and lifestyle are important during pregnancy and breastfeeding, for health of mothers and their offspring.

Objectives: The aim of this study was to determine the effects of soy oil administration on pregnant Wistar rats, on the lipid peroxidation and antioxidant levels in the heart and liver of their litters.

Materials and Methods: Pregnant rats were divided into two groups. Group 1 served as the control while group 2 served as the test group and were given 2ml of soy oil through oral gavage. The administration continued until 2 weeks after the rats had delivered. At the end of the experimental period, the litters' livers and hearts were excised and stored in phosphate buffer for biochemical analysis.

Results: The results showed that soy oil had no significant effect on malondialdehyde (MDA) in the litters' heart and liver when compared with control (P=0.06 and 0.19 respectively). There was no significant effect on catalase (CAT) and superoxide dismutase (SOD) in the litters' heart and liver respectively when compared with control (P=0.17 and 0.07 respectively). However, SOD and CAT were significantly reduced in the litters' heart and liver respectively when compared with control (P=0.02 and 0.001 respectively).

Conclusion: Soy oil administration on pregnant Wistar rats significantly reduced liver CAT and cardiac SOD levels in litters. Thus, our findings suggest caution when consuming soy oil during pregnancy.

Keywords: antioxidants, free radicals, lipid peroxidation, reactive oxygen species, soy oil.

INTRODUCTION

Lipid peroxidation is a biochemical autoxidation phenomenon initiated by the interaction of free radicals with phospholipids or polyunsaturated fatty acids found in cell membranes. This process produces byproducts including aldehydes, ketones, alkanes, carboxylic acids, and polymerization products, which serve as indicators for assessing oxidative stress.[1] The lipid-rich composition of cell membranes, particularly the presence of polyunsaturated fatty acids, is essential in modulating the advancement of lipid peroxidation reactions, potentially resulting in the generation of free radicals and lipid peroxides. Antioxidants play a crucial role in maintaining a physiological environment by preventing the accumulation of reactive oxygen species to levels that could harm cellular integrity.[2] Among various biomolecules, lipids—particularly the polyunsaturated fatty acid components of phospholipids—exhibit the highest sensitivity to free radicals. The body employs antioxidant defense mechanisms to shield itself from the detrimental effects of these free radicals.[3] Free radicals are important in the oxidative stress balance between prooxidant and antioxidant activities. If the balance tilts towards prooxidants, the functional health of an organism is at risk.[4] Lipid peroxidation is the core reaction of ferroptosis, which is caused by the attack of oxidants on lipids. Uncontrolled lipid peroxidation leads to membrane rupture and cell death.[5] Therefore, disturbance of the balance between the prooxidation and the antioxidant defense against it can lead to oxidative stress in tissues,

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which has been implicated in the pathogenesis of many health challenges. Oxidative stress has been associated with several metabolic disorders due to unhealthy dietary pattern and may be alleviated by increasing the intake of antioxidants.[6] The effects of oxidative stress are related to the type of macronutrients consumed and the absolute amount taken.[7] Oxidative stress is a central player of metabolic ailments associated with excessive fat consumption and research has shown that intakes of macronutrients such as fats and oil can promote oxidative stress.[8]

A large amount of studies have demonstrated that free radicals contribute to initiation and progression of several pathologies including cardiovascular diseases and liver diseases. An Increased formation of lipid peroxides has been implicated in disease states such as atherosclerosis, ischemia-reperfusion, heart failure, Alzheimer's disease, rheumatic arthritis, cancer, male infertility and other immunological disorders.[9,10] Reducing the formation of lipid peroxidation products will help to limit the harmful effects of reactive oxygen species (ROS) in various pathological conditions.[10]

Soy oil is a common cooking oil derived from soybeans and it contains high polyunsaturated fatty acid. According to Szpunar-krok & Wondolowskar-Grabowska,[11] soy oil is the second highest produced and consumed vegetable oil worldwide. Various studies have shown that soy oil has varying effects on both animal and human subjects. Sometimes, these reports are conflicting. Soy oil has been shown to influence glucose metabolism in rats,[12] decrease inflammatory markers,[13] and improve lipid profile.[14] Conversely, a study by Patel *et al.,*[15] observed no significant effect on some inflammatory markers. Our previous studies showed that Soy oil has no effect on some liver enzymes and lipid profile.[16] A study carried out by Henkel *et al.,*[17] showed that a high fat diet rich in soy oil-derived omega-6-poly-unsaturated fatty acids caused liver damage in mice. The oxidation of vegetable oils such as soy oil yield both primary and secondary oxidation products such as hydroperoxides, malondialdehyde and hydrocarbons which produce undesirable sensory and biological effects.[18].

Diet and lifestyle are important during pregnancy and breastfeeding, for health of mothers and their offspring. Fetal programming is critical to lifelong health of offspring.[19] The consumption of balanced diet from the preconception period is important to ensure both maternal well-being and pregnancy outcomes.

Disruption in the overall health of the offspring may occur when there is maternal exposure to stress, under or over nutrition and any other types of biochemical insult.[20,21]

Soy oil contains polyunsaturated fatty acids and studies have shown increasing intake of polyunsaturated fatty acid in pregnant women.[22, 23,24] Despite the increase, there are limited information on the effects of consumption of soy oil during pregnancy on newborns. Thus, the aim of this study was to determine the effect of consumption of soy oil during pregnancy on oxidative and antioxidant status of newborn in Wistar rats

MATERIALS AND METHODOLOGY

Oil acquisition

Standard food grade Soy oil used in this study was purchased from Nkwo market in Nnewi, Anambra State, Nigeria.

Animals

Twenty-two (22) Wistar rats comprising of 16 female rats and 6 male rats which weighed between 140-180g were used for the experiment. The animals were kept in standard cages at a constant room temperature, humidity and light cycle (12 h: 12 h; light: dark) with free access to water and fed with chow *ad libitum.* The female rats were kept in different cages, separated from the male rats. The animals were acclimatized for 14 days after which the female rats were kept together with the male rats for mating. The female Wistar rats that became pregnant were separated and used for this study. Animals handling and all procedures were reviewed and approved by the Edo State University Animal Research Ethics Committee (EDSU-AREC) for laboratory animal care and use.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

Experimental designs

The pregnant Wistar rats were randomly grouped into two of six animals each. Group 1served as control and was administered with rat chow and water *ad libitum.* Group 2 served as treated group and was administered 2ml/kg/day soy oil. All animals were allowed access to food and water *ad libitum* and 12 hours light/dark cycle. Soy oil administration was done through oral gavage. The administration of soy oil continued throughout the gestation period until 2 weeks after parturition. The litters from each group were sacrificed and their liver and heart excised for biochemical analysis.

Biochemical analysis

Liver and cardiac tissues samples were immediately stored at 0ºC till analysis for the determination of lipid peroxidation, superoxide dismutase and catalase.

Preparation of homogenate

Each liver and cardiac tissue was homogenized in cold 0.1M phosphate buffer (1:4(w/v), pH 7.4) using a mortar and pestle and the homogenates were centrifuged at 12000rpm for ten (10) minutes at 40C.

Determination of Lipid peroxidation activity: Malondialdehyde (MDA) level

MDA level was determined by the colorimetric method of Gutteridge and Wilkins.[25]

Procedure in brief:

To each test tubes of 0.1 ml of test samples (liver and heart homogenates), 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (0.05 M sodium hydroxide) was added. The content was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. Calculation:

The level of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105 M-1cm-1).

MDA (nmol/ml) = (OD X 1000000)/ E532

Where:

E532 = Molar extinction coefficient for MDA (1.56x105 M-1cm-1)

1000000 = conversion of mMol to nMol

Determination of superoxide dismutase (SOD) activity

SOD was assayed by colorimetric method of Misra and Fredovich.[26].

Procedure in brief:

Eighty (80µl) of sample/blank was added into a clean test tube containing 1000 µl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly and allowed to equilibrate by incubating at 37 °C for 5 minutes. Thereafter, 600 µl of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80µl of distilled water was used instead of plasma. The changes in absorbance of both test and blank were determined. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the organ SOD activity was expressed as U/ml as shown below. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

Calculation:

Actual OD reading (R) = OD150 – OD30/2

% inhibition = (Rblank – Rtest/ Rblank) X 100

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor.

Determination of catalase(CAT) activity

Catalase activity was determined by colorimetric method of Sinha.[27].

Procedure in brief:

One millilitre (1 ml) of phosphate buffer (pH 7.0) was mixed with 0.4 ml of 0.2 M hydrogen peroxide and allowed for 3 minutes to equilibrate at room temperature. 0.1 ml of sample was added into the reaction mixture and was gently swirled at room temperature. 2 ml dichromate/acetic acid reagent was added after one minute and was thoroughly mixed. The solution was incubated in same way except that dichromate/acetic acid mixture was added before the addition of the sample. The colour developed was read at 570 nm and the activity of catalase in U/ml which is equivalent to the amount of hydrogen peroxide (µmoll) degraded per minute was calculated using 40 µmoll of hydrogen peroxide as standards.

Calculation

Actual OD of test = ODblank – ODtest

Activity of catalase (U/ml) = Actual ODtest/ODstd X Std concentration.

Statistical analysis

Results are expressed as Mean ±SEM. Data were analyzed using statistical package for social sciences (SPSS) version 23. Student's T-test was used to test for significance. P values less than 0.05 were considered significant.

RESULTS

Litters' liver tissue malondialdehyde (MDA)

Our result (figure 1) showed that administration of soy oil to pregnant Wistar rats had no significant effect on liver tissue MDA of their litters when compared with the litters of control (P=0.19)

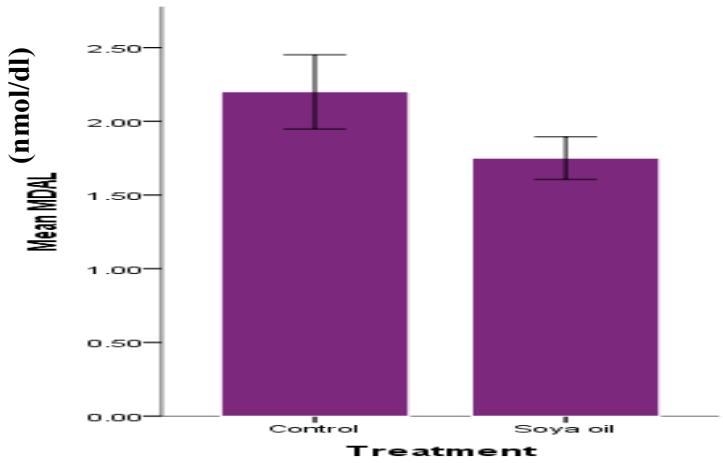

Figure1: Litters' MDA levels in liver tissue.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

Litters' liver tissue superoxide dismutase (SOD)

Our result (figure 2) showed that soy oil administration to pregnant Wistar rats had no significant effect on their litters' liver tissue SOD when compared with that of the litters of control rats (P=0.07)

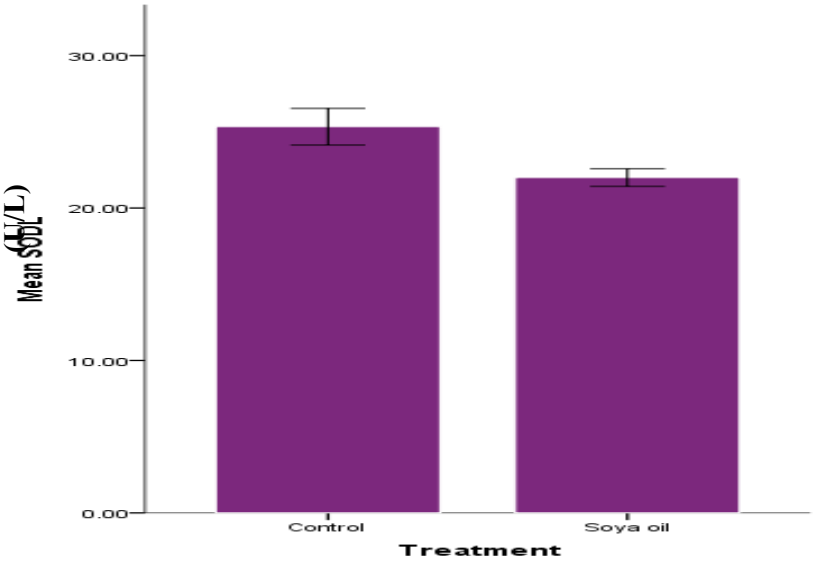

Figure 2: Litters' SOD levels in liver tissue.

Litters' liver tissue catalase (CAT)

Soy oil administration on pregnant Wistar rats significantly reduced (P=0.001) their litters' liver tissue catalase level when compared with that of the litters of control rats (figure 3)

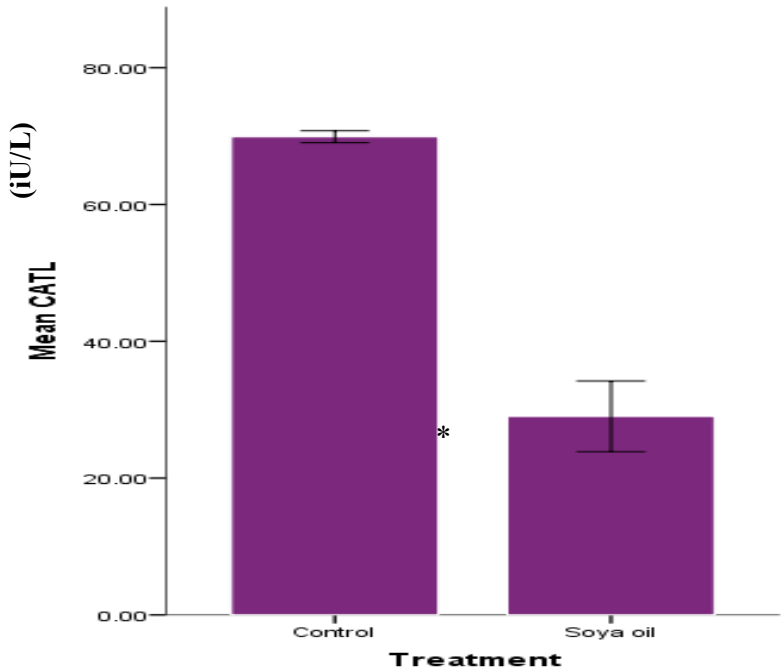

Figure 3: Litters' catalase levels in liver tissue.

Litters' cardiac tissue malondialdehyde (MDA)

Soy oil administration on pregnant Wistar rats had no significant effect (P=0.06) on their litters' cardiac tissue MDA level when compared with that of the litters of control rats (figure 4)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

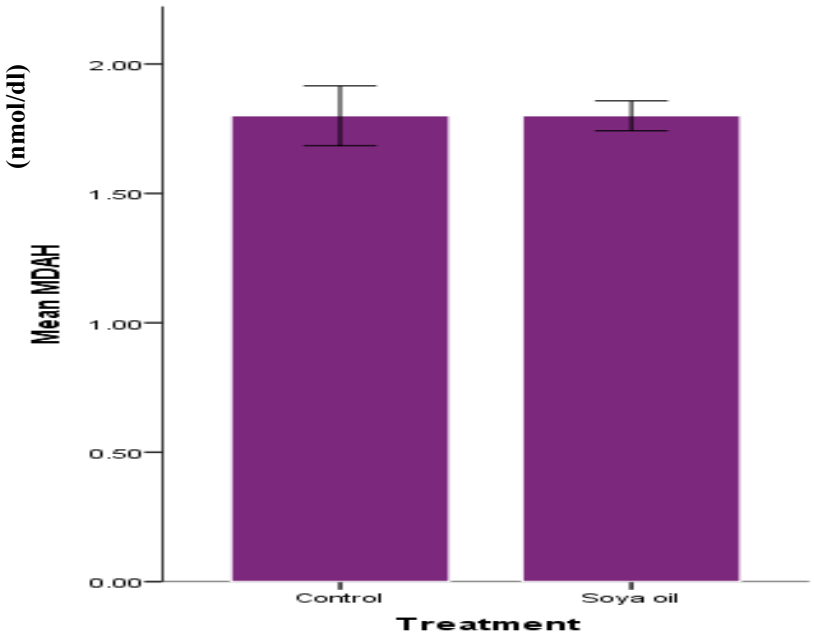

Figure 4: Litters' MDA levels in Cardiac tissue.

Litters' cardiac tissue superoxide dismutase (SOD)

Soy oil administration on pregnant Wistar rats significantly reduced (P=0.02) their litters' cardiac tissue SOD level when compared with that of the litters of control rats (figure 3)

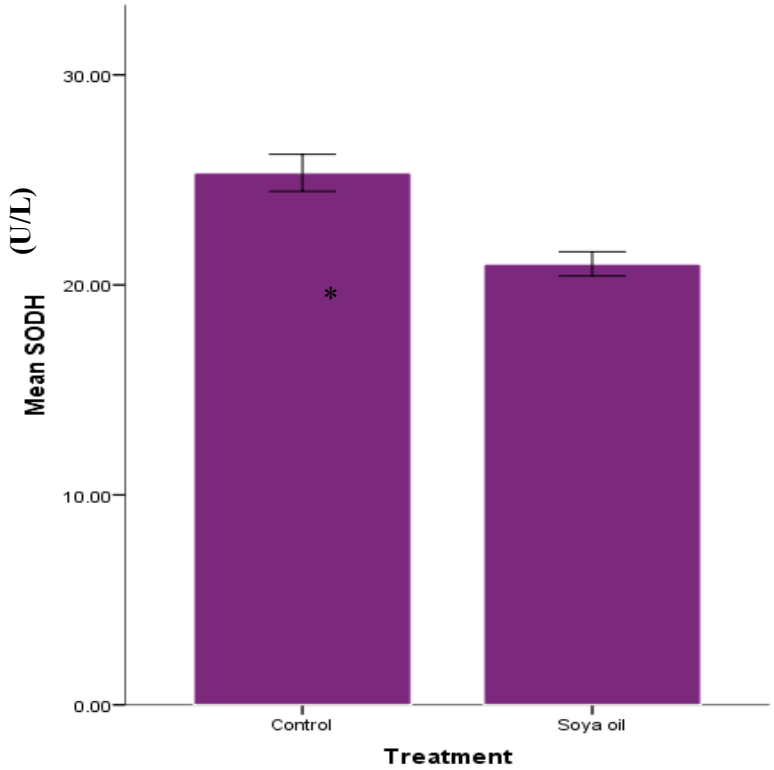

Figure 5: Litters' SOD levels in Cardiac tissue.

Litters' cardiac tissue catalase (CAT)

Soy oil administration on pregnant Wistar rats had no significant effect (P= 0.17) on their litters' cardiac tissue CAT level when compared with that of the litters of control rats (figure 5)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

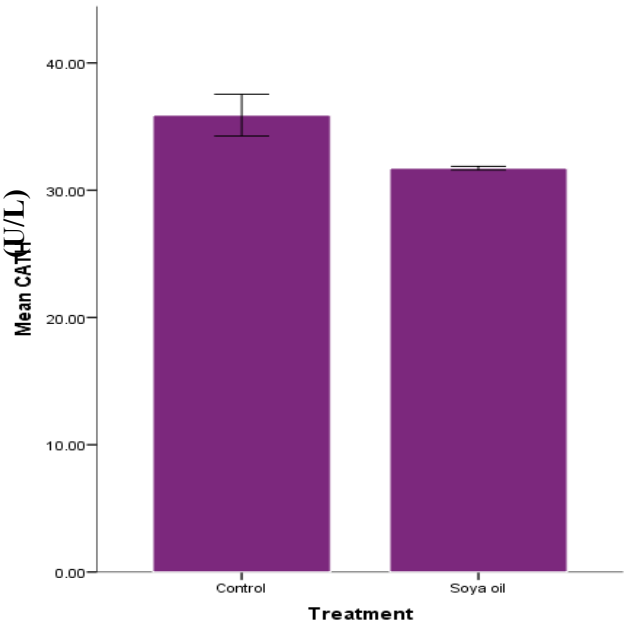

Figure 6: Litters' catalase levels in Cardiac tissue.

DISCUSSION

In this study, soy oil was administered to pregnant Wistar rats in order to determine its effects on malondialdehyde, superoxide dismutase and catalase in the cardiac and hepatic tissues of their litters after parturition. Our findings showed that consumption of soy oil by pregnant Wistar rats had no effect on MDA but hepatic CAT and cardiac SOD levels were significantly reduce in their litters.

Malondialdehyde, a product of lipid peroxidation is a known biomarker of oxidative stress. According to Gawel *et al.,*[28] MDA is one of the final products of polyunsaturated fatty acids in the cells. It is highly toxic.[29] It is a product of free radical injury on membrane lipid.[30]

Administration of soy oil had no significant effect both on the liver and heart MDA levels in the litters when compared to control ($P>0.05$). This finding suggest that consuming soy oil during pregnancy may not produce oxidative stress due to lipid peroxidation in the livers and hearts of newborns. This is in disagreement with the study carried out by Liang *et al*.,[31] where MDA level significantly increased in the plasma and jejunum of young birds. Although, this discrepancies may be as a result of differences in animal species or due to differences based on fetal programming in Wistar rats as used in this study.

Superoxide Dismutase catalyzes the dismutation of superoxide anion free radical into molecular oxygen and hydrogen peroxide thereby decreasing the level of superoxide anion which damages the cells at excessive concentration.[32]. When there is an increased level of superoxide anion and a reduced SOD, oxidative stress may develop, damaging essential biomolecules and altering their physiological functions.[33]. Although, optimum production of superoxide anion radical can be essential to life as it can act as a signalling molecule, regulating numerous processes including apoptosis.[33] Administration of soy oil had no significant effect both on the liver SOD levels in the litters when compared to control ($P>0.05$) in this study. Unlike in the liver tissue, SOD was significantly reduced in the heart tissue of the soy oil administered group. This finding is suggestive of the damaging potential of Soy oil to cardiac tissues and therefore, may alter its physiological function in newborns. This finding is in agreement with the study carried out Liang *et al.,*[31] where soy oil decreased SOD.

Catalase has the capacity to scavenge reactive oxygen specie particularly, hydrogen peroxide.

On the catalase level, soy oil administration had no significant effect on the cardiac tissues of the litters when compared to control ($P>0.05$). However, there was a significant decrease in the liver catalase level of the litters

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when compared to control ($P<0.05$). This reduction in catalase may reduce the ability of the antioxidant to neutralize hydrogen peroxide in the liver. This may suggest that the livers of the litters suffered oxidative stress due to the buildup of hydrogen peroxide in the livers. This is in agreement with the study carried out by Tan *et al.,*[34] where soy oil administration reduced the catalase level in young birds.

This study therefore shows a balance between malondialdehyde, and catalase in the heart of the litter as well as a balance between malondialdehyde and superoxide dismutase in the liver of the litter. However, the significant reduction in catalase and SOD in hepatic and cardiac tissues respectively may significantly affect the potential of newborn to combat oxidative stress.

Strength And Limitation Of This Study

The strength of this include the use of commercially produced soy oil generally used in cooking and focusses on fetal programming following consumption of soy oil throughout the gestation period. The limitation of this study include lack of funding needed to expand and analyze other parameter in the litters.

CONCLUSION

Soy oil administration on pregnant Wistar rats significantly reduced liver CAT and cardiac SOD levels in litters. Thus, soy oil consumption during pregnancy should be reduced

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Author contribution: POO conceptualized and designed the study. COO contributed to the implementation of the project and prepared the manuscript draft. All authors were involved in the revision of the manuscript. The authors read, approved the final manuscript and agree to be accountable for all aspects of the work

Data availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Funding source: No funding sources

Conflict of interest: We declare no conflict of interest

Ethical approval: This study was approved by the Institutional Ethics Committee of Edo State University

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