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# Fresh Raffia Palm Wine, Fermented Soya Bean Milk Supplementation, Enzymatic Antioxidant and Lipid Peroxidation Biomarkers in Lactating Female Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** Lactation is the most crucial period characterized by intensive caloric requirements to boast up female's nutritive system and breastfeeding life. Current study evaluated the effects of fermented soya bean milk (FSBM) and fresh palm wine (FPW) supplements on endogenous antioxidant enzymes activities and lipid peroxidation biomarker in thirty five (35) lactating Wistars rats.
**Methods:** Animals were obtained from the Experimental Research Animal House of University of Calabar, Nigeria**.** At parturition, the animals were randomly divided into five groups containing five

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(5) rats (n=5). Oral treatment done as follow: **Group I**: Control group, was given normal feed of protein content 20mg/kg and distilled water (1 ml/kg), **Group II**: Metoclopramide (5 mg/kg), **Group III**: 10ml /kg of FPW. **Group IV**: Three (3) sub-groups of group four (4) received 10%, 20% and 40% of FSBM respectively, **Group V**: Co-administration of 40% FSBM plus FPW (10ml /kg) supplements .Treatment was done every six hour daily for a duration of ten (10) days.

**Results:** Serum malondialdehyde concentration, a biomarker of Lipid peroxidation was significantly increased ($P < 0.05$) in metoclopramide treated group than control group, FSBM and FPW supplemented groups. There was a significant decrease ($P < 0.05$) in serum activities of four endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in metoclopramide treated group when compared to control group, FSBM and FPW supplemented groups.

**Conclusion:** while fermented soya bean milk and fresh palm wine supplementation increased lactation and endogenous enzymatic antioxidant activities without lipid peroxidation, Metoclopramide at 5 mg/kg concentration increased lactation with lipid peroxidation.

*Keywords: Soya bean; palm wine; lactation; lipid peroxidation; endogenous antioxidant enzymes.*

## 1. INTRODUCTION

According to the International Society of Antioxidant in Nutrition and Health (ISANH) and Medical Dictionaries, the term antioxidant was initially utilized to refer specifically to chemical products that prevent the consumption of oxygen [1- 3]. Endogenous antioxidants are agents which scavenge free radicals and prevent oxidative stress damage caused by excess free radicals in the human body. They are also known as antiradicals [4]. Endogenous enzymatic antioxidants can be defined as enzymes or molecules existing within the cellular fluid compartment of the body that are capable of preventing, inhibiting or delaying harmful oxidative reactions caused by high levels of free radicals or oxidants, thus playing an important role in forming antioxidant defensive mechanism and protective system of the body [5]. In recent times, antioxidants in the body in required, acceptable and appropriate levels have been reputed to help the body in repairing, equipping and preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species *in vivo* [6-8]. The fundamental classes of antioxidants that form the total antioxidant status of an individual include the primary or chain breaking antioxidant enzymatic systems, secondary and tertiary antioxidants. The primary or chain breaking antioxidant systems include superoxide dismutase, catalase, glutathione peroxidase and reductases. The secondary or preventive antioxidants include glutathione, vitamin C or ascorbate, vitamin E, vitamin A, uric acid, etc .The tertiary or repairing antioxidants include small molecules and plasma proteins such as albumin, transferrin, ceruloplasmin and antioxidant micronutrients such as iron, copper, selenium and manganese [9]. A number of these compounds are of exogenous nature and are obtained from certain food substances namely: antioxidants like α-tocopherol, β-carotene, and ascorbic acid, and some micro-nutrient elements such as zinc and selenium [10, 11]. Lactogenesis and lactation are essential for optimal feeding of infants during their first one year of dependable life and this has a direct impact on growth, development, immunity and health aspect in the neonatal period [12]. Mammalian lactating cells are therefore equipped with some sort of complex defiance mechanisms for radical detoxification [13].Oxidative stress (OS) is considered a metabolic disturbance that affects organ systems and its presence will affect not only the health status of the animals but also the quality and quantity of the final products, such as milk [14]. There is evidence that oxidative damage increases during lactation in some domesticated and laboratory animals [15, 16]. The soya bean is native to the Korean Peninsula and the Manchurian Area and has been one of the major sources of protein in Korean food [17]. Numerous foods are made by from soybeans, including doenjang (soya bean paste), ganjang (soya bean source), cheonggukjang (fast-fermented bean paste), bean curd, soybean milk, and bean-curd dregs [18]. Soybean consumption is effective for the prevention of osteoporosis, arteriosclerosis, strokes and dementia, and can reduce the risk of cancer and obesity [19, 20]. Palm wine is a whitish sap collected in vessels attached to the base of the palm tree from where some leaves have been removed. Fresh raffia palm wine (Raphia hooker) from these sources is sweet and contains little alcohol but, with fermentation, the alcohol content increases over time. Unbottled

palm wine has a slightly lower alcohol content (around 3%) than bottled palm wine (around 4%) [21,22]. Biochemical constituents of palm wine include carbohydrates (sugars), protein, amino acid, lipid, lactic acid, alcohol, vitamins such as vitamin B 6 , B1, B2, vitamin C and some antioxidants, mineral and trace elements like iron and selenium. The mean sugar content of fresh palm wine ranges between 0.10 in maltose and 8.74 mg/100ml in sucrose [23- 27]. The present study was aimed at evaluating the effects of fermented soya bean and fresh palm wine supplement on lipid peroxidation biomarker and activities of four endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in lactating Wistar rats in vivo.

## 2. METHODS

This experimental study was carried out from January 2018 to December 2018 at the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Science, University of Calabar, and the laboratory analysis of samples was carried out at the Chemical Pathology, and Haemalogy and Blood Transfusion Units, Department of Medical Laboratory Science, University of Calabar Teaching Hospital (UCTH) Calabar, Cross River State, Nigeria.

### 2.1 Animal Grouping and Drug Administration

Adult female apparently healthy Wistar rats weighing 180-200g were obtained from Biochemistry Department Experimental Research Animal House of University of Calabar, Calabar,Nigeria .Oral treatment was administered as follow**:-** **Group I**: (Control group) was given normal vital feed pellets and distilled water, orally (1 ml/kg). **Group II**: Metoclopramide (5 mg/kg), **Group III**: An oral treatment (through orogastric tube) of 10 ml/kg body weight per day of fresh raffia palm wine for 10 days. The dosage was calculated as 10 (ml)/1000 (g) × the body weight of the animal. Fresh undiluted palm wine from the sap of oil palm trees was collected at every 3 days interval from a local palm wine tapper. The palm wine on collection was administered to the animals and then conserved in a refrigerator for 3 days following which it was often replaced with a fresh collection.

**Group IV:** The three (3) sub groups of group four (4) received 10%, 20% and 40% soya bean, respectively. **Group V**: was co-administered 20% soya bean supplement and fresh palm wine (10ml/kg). Administration was carried out orally for a period of ten (10) days.

### 2.2 Sample Collection

After ten days, the rats were anaesthetized by chloroform inhalation in a closed chamber and blood samples were obtained via cardiac puncture into specimen bottles and allowed to clot, and separated by centrifugation at 4000 rev/per minutes for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany) at an average room temperature of 23˚C. The supernatant obtained was used for biochemical assays. Sample haemolysis was avoided.

### 2.3 Biochemical Assay of Malondialdehyde in Serum

Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid hydroperoxide decomposition and is most often measured as a biomarker and index of lipid peroxidation. MDA activity was estimated using the NWLSS™ Malondialdehyde commercial assay kits purchased from Northwest Life Sciences Specialized Product NWK-MDA01, Vancouver WA, Specificity: Malondialdehyde, Sensitivity: 0.08 µ) based on the method adapted by Janero, [28]. The test principle is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.

### 2.4 Biochemical Assay of Serum Superoxide Dismutase Activities in the Serum

Superoxidase Dismutase (SOD) activity was assayed using the North West Life Science Specialties (NWLSS™) SOD assays kit (product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe superoxide dismutase, sensitivity: 5 U/mL). The method is based on the principle of superoxide inhibition of auto-oxidation rate of hematoxylin as originally described by, Martin, [29], with modifications to increase robustness and reliability. About 920 µL of assay buffer was added to each cuvette. This was followed by addition of 40 µL of assay buffer (for blank) and 40 µL of sample. The mixture was incubated for two (2) minutes. After which 40 µL hematoxylin

reagent was added and mixed quickly to start the auto-oxidation reaction. An absorbance was measured at 560 nm for every 10 seconds for 5 minutes.

### 2.5 Biochemical Assay of Catalase Activities in the Serum

Catalase (CAT) activity was estimated using the North West Life Science Specialties (NWLSS™) NWLSS$^{TM}$ Catalase assay kits (Product NWK-CATO1, Specificity: 6.0 U Catalase/mL) based on the method of Beers, (1952) [30], with modifications to increase robustness and convenience. The test is based on monitoring the consumption of $H_2O_2$ substrate. The ultraviolet absorption by hydrogen peroxide which is considered as a function of time and was used to follow the catalase-peroxide reaction. The absorption spectrum of hydrogen peroxide was measured spectrophotometrically at the wavelength of 240nm. The optical density as a measure of peroxide concentration, increases linearly with peroxide concentration and directly proportional to catalase in the sample in accordance with the Beer-Lambert law. The reaction products, oxygen and water, do not absorb light in this spectral region nor does catalase.

### 2.6 Biochemical Assay of Glutathione Peroxidase Activity (GPx) in Serum

Glutathione peroxidase (GPx) activity was assessed using the North West Life Science Specialties (NWLSS™) cGPx (GPX1) ELISA kit (Product NWK-GPX02, Specificity: Glutathione peroxidase, Sensitivity: 12.5 pg/ml) based on the method described by Avissar, [31]. The assay is based on a sandwich Enzyme-Linked Immunosorbent Assay, where sample GPx concentration was determined by comparing the 450 nm absorbance sample wells to the absorbance of known standards.

### 2.7 Statistical Analysis

The analysis of data was computed using SPSS software version 20 (SPSS Inc., Chicago, USA) and Microsoft Excel (2007). The results of our study were analyzed with the aid of cross tabulations to explore proportional associations between variables and Chi square test was used to explore proportional association between groups. All data were expressed as mean ± standard error of the mean (mean ± SEM). Statistical significance was carried out using one way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Values of $P < 0.05$ were considered significant.

## 3. RESULTS

The results of the current study are shown in the following tables below.

Table 1 shows the effects of ten (10) days administration of fermented soya bean milk and fresh palm wine supplementation on lipid peroxidation biomarker (serum malondialdehyde (MDA) concentration in female lactating wistar rats. These results show that the mean values of MDA ( in µmol/L) after 10 days from **Group I** to **Group V** .The MTCL treated group had a higher level of MDA ($P < 0.05$) when compared to the control group, while **Group V** had the lowest level of MDA indicating the different degree of lipid peroxidation and lactation.

As shown in Table 1, the MTCL treated group had a highest level of MDA ($P < 0.05$) compared to the control indicating a high degree of lipid peroxidation and lactation, while group V had the lowest level of MDA among the test groups, indicating a lowest degree of lipid peroxidation and lactation. Group **III** and **IV** had moderate levels of MDA.

Table 2 shows the effects of ten (10) days administration of fermented soya bean milk and fresh palm wine supplements on the serum activities of four endogenous antioxidant enzymes in female lactating wistar rats namely:-

### 3.1 Superoxide Dismutase (SOD)

These results show that the mean values of SOD (IU/L) after 10 days from **Group I** to **Group V.** The MTCL treated group had the lowest level of SOD while group V had the highest level of SOD indicating the degree of elevation of endogenous antioxidants enzymes and less oxidative stress.

**Catalase (CAT):** These results show that the mean values of catalase -CAT (IU/L) after 10 days from **Group I** to **Group V** . The MTCL treated group had the lowest level of catalase while Group V had the highest level of catalase indicating the degree of elevation of endogenous antioxidant enzymes and less oxidative stress.

**Table 1. Lipid peroxidation biomarker (Serum MDA levels ) in female lactating Wistar rats treated with fermented Soya bean milk and fresh palm wine supplements, metoclopramide, distilled water and normal feed**

| Groups | Animal handling and treatment of groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | | IV | | V | |
| | Control | Treated with | Treated with | Treated with | Treated with | Treated with | Treated with | P-value |
| Substances Administered | Distilled water | METCL | FPW | FSBM | FSBM | FSBM | FSBM | |
| Quantity | 1ml/kg | 5mg/kg | 10ml/kg | 10% | 20% | 40% | 40%+FPW | |
| Mean±SEM MDA after 10 days (µmol/L) | 1.33± 0.11 | 1.79± 0.15 | 1.53 ± 0.09 | 1.43 ± 0.11 | 1.61 ± 0.07 | 1.44 ± 0.05 | 1.42 ± 0.07 | P< 0.05* |

***METCL** = Metoclopramide, **MDA** = malondialdehyde, **FPW** =fresh palm wine, **FSBM**= fermented soya bean milk*
*mean ± SEM= mean plus or minus standard error of the mean.*
** There was a significant difference in MDA levels in FSBM, and FPW treated groups (P < 0.05)-compared to the control group, FSBM and FPW supplemented groups*

**Table 2. Serum activities of four endogenous antioxidant enzymes (SOD , CAT and GPX ) in female lactating wistar rats treated with soya bean milk and fresh palm wine supplements, metoclopramide, distilled water and normal feed**

| Group | Animal handling and treatment of groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | | IV | | V | P-value |
| | Control | Treated with | Treated with | Treated with | Treated with | Treated with | Treated with | |
| **Substances Administered** | Distilled water | METCL | FPW | FSBM | FSBM | FSBM | FSBM | |
| **Quantity** | 1mg/kg | 5mg/kg | 10ml/kg | 10% | 20% | 40% | 40% +FPW | |
| **Mean±SEM activity (IU/L)** | 2.25± 0.09 | 1.69±0.07 | 2.18 ± 0.10 | 1.76± 0.10 | 1.85±0.05 | 2.34±0.13 | 2.98 ± 0.12 | $P<0.05^{\alpha}$ |
| **Mean ± SEM Catalase activity (IU/L)after 10 days** | 55.00 ± 0.71 | 48.20±1.55 | 66.80±1.59 | 65.00 ±1.14 | 67.20± 1.28 | 70.40± 1.17 | 78.20±1.02 | $P<0.05^{\beta}$ |
| **Mean ± SEM activity GPX(IU/L) after 10 days** | 49.40±0.93), | 45.20± 1.56 | 43.60±0.93 | 42.60 ±1.50 | 43.60± 1.50 | 49.00± 0.94 | 50.80 ± 0.86 | $P<0.05^{\gamma}$ |

***SOD**= superoxide dismutase, **CAT=** catalase, **GPX** =glutathione peroxidase, **METCL** = Metoclopramide, **FPW** =fresh palm wine, **FSBM**= Fermented soya bean milk , mean ± SEM= mean plus or minus standard error of the mean*

**Glutathione peroxidase (GPX):** These results show that the mean values of glutathione peroxidase (GPX) (IU/L) after 10 days from **Group I** to **Group V**. The MTCL treated group had the lowest level of Glutathione peroxidase (GPX) while group V had the highest level of glutathione peroxidase (GPX) indicating the degree of elevation antioxidants and less oxidative stress.

$^{\alpha}$There was a significant increase in SOD activities in FSBM and FPW treated group ($P < 0.05$) when compared to the control group and MTCL treated group. In Table 2 above it is seen that the MTCL treated group had a statistically significant decrease ($P < 0.05$) activity of SOD when compared to the control group and the treated group with FSBM 10%, 20%, 40% and FPW while Group V had the highest activity of SOD ($P < 0.05$) indicating the different degree of elevation in the activities of endogenous antioxidant enzymes and lactation with less lipid peroxidation.

$^{\beta}$ There was a significant increase in CAT activities in FSBM and FPW treated group ($P < 0.05$) when compared to the control group and MTCL treated groups. In Table 2 as shown above, the MTCL treated group had a significant decrease in ($P < 0.05$) the activity of catalase when compared to the control, FSBM 10% , FSBM 20% and FPW treated groups, while Group V had the highest activity of catalase indicating different degrees in the elevation of endogenous antioxidant enzymes activities and lactation with less lipid peroxidation.

$^{\gamma}$ There was a statistically significant increase in GPX levels in FSBM, and FPW treated group ($P < 0.05$) when compared to the control group and MTCL treated groups. In Table 2 as shown, the MTCL treated group had significant decrease ($P < 0.05$) in the activity of glutathione peroxidase (GPX) when compared to the control, FSBM 10%, 20%, 40% and FPW treated groups respectively, while group V had a significantly higher ($P < 0.05$) activity of glutathione peroxidase (GPX) indicating different degrees of elevation in endogenous antioxidant enzymes and lactation with less lipid peroxidation.

## 4. DISCUSSION

The mean ± SEM of serum MDA level after 10 days of the current study was displayed in Table 1.The results showed a statistically significant increase ($P < 0.05$) in serum MDA levels. In Group II subjects, which was treated with Metoclopramide as compared to Group I subjects, the control group which was treated only with distilled water and normal feed of protein content 20mg/kg Metoclopramide is a medication used to treat stomach disorders such as reflux, nausea, and vomiting (anti–emetic) [31], but in lactating subjects it can cause lactation induction because of its galactagogue properties and lactating augmentative action. These properties could be attributed to the mechanism of action of metoclopramide on its receptors located in the gastro-intestinal, gastro-esophageal mucosal, membranes of hepatocytes, breasts and central nervous system including others target sites, where poly unsaturated fatty acids are broken down with the resultant increase of lipid peroxidation which can subsequently assessed by the biomarker called malondialdehyde (MDA) formed and released as a by-product [32]. The common side effects of metoclopramide are sleepiness, headache, or restlessness, depression and tardive dyskinesia which are signs of increase oxidative stress and increased lipid peroxidation [33]. The Subgroup of Group IV which was treated with 20% soya bean showed an increased in the level of serum MDA compared to the other treated Groups. This could also be due to a possible pro-activity of the supplements on oxidants resulting in increased lipid peroxidation of cells. It could have also been due to the inability of the supplement at this concentration that effectively break down the lipid peroxidation chain reaction since at higher concentrations it helps to reduce oxidative stress because of its high antioxidant content, daidzein , genistein and aglycone composition.These are inline with [34,35]. However, the increase was not statistically significant compared to metoclopramide treated and the normal control groups ($P > 0.05$). Superoxide dismutase (SOD) activities as shown in Table 2 was decreases in the metoclopramide treated group compared to the control group although the results were not statistically significant. ($P > 0.05$). This result could be due to the depletion of the endogenous antioxidant as a result of its utilization in combating the existing oxidative stress as suggested by the level of increased MDA in metoclopramide treated group. The result also could have been due to the inability of metoclopramide to stimulate the release of endogenous antioxidants as well as its tendency to cause increase in reactive oxygen species (ROS) within the system. There was however, a remarkable increase in SOD activities , in the fresh palm wine treated group as shown in Table

2 which could have been be due to its antioxidant activity in fresh palm wine .These results agreed with [36]. The increase was however, rivaled by that of the soya bean 40% Group, which showed the highest increase in serum SOD activities. This result is suggestive of SB 40% Group as having an antioxidant activity more than the standard anti-oxidant or possibly a more lasting effect than that of fresh palm wine and agreed with [37]. This anti-oxidant activity of the supplement on serum SOD activities could be attributed to the presence of isoflavones [38]. Co-administration of SB and fresh palm wine increased the activities of SOD more than when the supplement was given singlely in the 10% and 20% treated groups as shown in Table 2. This suggests that the co-administration of the supplement and fresh palm wine could be more potent than the supplement alone at lower doses of 10% and 20%. In Table 2 there was a statistically significant decrease in the level of catalase observed in the metoclopramide, and the supplement treated groups at 10% and 20%. This result suggests the inability of metoclopramide and the supplement at 10% and 20% to specifically augment the release of endogenous catalase enzyme when compared to the activity of the supplement at 40%.This is expected because catalase and selenium-containing glutathione peroxidase have been demonstrated in human milk but their levels in colostrum is higher than that in corresponding mature milk and this agreed with [39]. However, this highest levels in colostrum usually decline during early lactation, despite the fact that total lipids increase as reported by Yuksel (2015) [40]. As shown on Table 2, there was a statistically significant decrease in the activities of GPx in the supplement treated groups at 10% and 20% and also that of fresh palm wine. This result suggests that the supplement at these concentrations also do not seem to have antioxidant activity, specifically on GPx release or synthesis [41].

## 5. CONCLUSION

Metoclopramide at 5 mg/kg concentration is capable of increasing lipid peroxidation according to this study and the FSBM supplement at 40% and fresh palm wine mixture showed an increase in endogenous enzymatic antioxidant activities with more lactation and less lipid peroxidation when compared to the control groups.

## WHAT IS KNOWN ABOUT THIS TOPIC

In many African cultures and tradition there is long practice that fermented soya beam milk and fresh raffia palm wine and is capable of augmenting lactation and clinically, metoclopramide is known to be a good galactogogue and side effects .What is not known till the time of this is the effect of the fermented soya bean milk and fresh raffia palm wine on the oxidative stress and the endogenous antioxidant enzymes and lipid peroxidation.

## WHAT THIS STUDY ADDS

Findings from this study have help highlighted the role of the combine effects of fresh raffia palm wine and fermented soya bean milk supplementation during lactation in viviparous mammals. In addition the long standing traditional dilemma and the superstitious belief about the usage of fresh palm wine fermented soya bean milk in humans have been elucidated by the findings of the current study .

## AVAILABILITY OF DATA AND MATERIALS

Data sets generated and analyzed in this study are available from the corresponding author on request.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee of University of Calabar on Animal Handling, consistent with standard animal welfare guidelines.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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