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Effects of *Thymus schimperi* and *Moringa stenopetala* Leaf Extracts on Lipid Peroxidation and Total Antioxidant Status in Pre-eclampsia Rat Models

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Authors' contributions

This work was carried out in collaboration among all authors. Author KM designed the study, performed the experimental procedures, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YAM, TT, EM and SG wrote the 2nd draft of the manuscript and supervised lab experiments. Authors AA, KG, AT, AD and DA organized data, managed the literature searches, assisted plant material preparation. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hypertensive disorders of pregnancy (HDP) are common pregnancy complications, with a cumulative incidence of 7%. Pre-eclampsia (PE) is the most common clinical type of HDP and one of the five top leading causes of maternal mortality worldwide. There is imbalance

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between lipid peroxides and antioxidant system in PE. Established PE is associated with increased concentrations of oxidative stress markers including lipid peroxidation products, and a reduction in antioxidant concentrations.

Methods: A case control experimental method was employed on Wistar rats with induced pre-eclampsia using nitric oxide-nitro-L-arginine methyl ester (L-NAME). Lipid peroxide content was estimated according to the method of Ohkawa et al. 1979. Total antioxidant capacity was assayed using colorimetric azinobis 2, 2′3-ethyl-benzothiazoline-6-sulfonate (ABTS) radical cathion decolorization assay.

Results: Lipid peroxides of untreated PE rat models were significantly ($p<0.01$) higher (0.57±0.08 nmol of malondyaldehide (MDA) per gram tissue weight) compared to normal pregnant controls (0.11±0.03 nmol). PE rat models that received aqueous leaf extracts of *Thymus schimperi* (ALETS) had (0.09±0.01, 0.07±0.002 and 0.02±0.002 nmol) ($p<0.05$) while, those PE rat models that received aqueous leaf extracts of *Moringa stenopetala* (ALEMS) had (0.36±0.08, 0.20±0.003 and 0.13±0.02 nmol) ($p<0.05$) with daily doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. On the other hand, untreated PE rat models had significantly ($p<0.01$) lower levels of serum total anti-oxidants (24.5±0.9 µg/ml of ascorbic acid equivalent) compared to normal pregnant controls (28.1±0.4 µg/ml). ALETS or ALEMS treated PE rat models had significantly ($p<0.01$) higher levels of serum total anti-oxidants in a dose dependent manner compared to untreated PE controls; (27.6±0.3, 29.5±0.3, 31.2±0.4 µg/ml and 29.2±0.3, 29.7±0.3, 30.6±0.4 µg/ml) with daily doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. ALETS treated PE rat models had significantly ($p<0.05$) reduced total lipid peroxides compared to ALEMS treated counterparts.

Conclusion: ALETS and ALEMS might have significant therapeutic effects against PE syndrome through reducing lipid peroxides and increasing total anti-oxidants.

Keywords: PE; lipid peroxidation; anti-oxidants; treatment; ALETS; ALEMS.

ABBREVIATIONS

ABTS :Azinobis-2,2'3-ethyl Benzo-Thiazoline-6-Sulfonate
ALEMS :Aqueous Leaf Extracts o Moringa stenopetala
ALETS :Aqueous Leaf Extracts of Thymus schimperi
ANOVA :Analysis of the Variance
GD :Gestation Day
L-NAME :Nitro-L-Arginine Methyl Ester
MDA :Malondi-Aldehyde
OD :Optical Density
PE :Pre-Eclampsia
S.E.M :Standard Error of the Mean
SPSS :Statistical Package for Social Sciences
TBA :Thio-Barbituric Acid

1. INTRODUCTION

Pre-eclampsia (PE) is a hypertensive disorder of pregnancy characterized by generalized vasoconstriction and elevated peripheral vascular resistance [1]. The hallmark in the pathophysiology of PE is widespread systemic vasoconstriction which results in marked increase in peripheral resistance which starts 3-4 months prior to the development of hypertension [2]. The subsequent effects of this change include development of arterial hypertension, decrement of venous return and blood volume from reduced vascular space [3]. PE also causes capillary endothelial damage which is responsible for the formation of micro thrombi in different organs mainly liver, kidneys and brain [4].

PE is one of the major causes of maternal death both in the developed and developing countries [5]. It occurs in 5% of pregnancies accounting for 70% of hypertensive disorders of pregnancy worldwide [6]. The incidence of PE ranges between 2% and 10% of pregnancies. The incidence of eclampsia which is the malignant for of PE, is 0.1-0.5% [7].

In spite of tremendous advances in modern medicine, no effective drugs are available to

prevent and treat PE [8]. Results from ongoing basic and clinical studies, therefore, may provide, new and important information regarding the physiological mechanisms responsible for the elevation of arterial pressure and oxidative stress in women with PE [9].

Oxidative stress contributes to atherogenicity in PE [10-12]. Placental level of lipid peroxides increases in PE [13]. On the other hand, serum antioxidants are excessively utilized to counteract the cellular damage mediated by free radicals production [14]; while, deficient antioxidants as well as increased lipid peroxidations are important factors in the pathogenesis of PE [15,16].

Reduced perfusion as a result of abnormal placentation leads to ischemia reperfusion injury to the placenta. Placental oxidative stress, which results from the ischemia reperfusion injury, is being increasingly reported to be involved in the etio-pathogenesis of PE [17]. It has been proposed as a promoter of lipid peroxidation and the endothelial cell dysfunction that is commonly seen in this condition. PE is characterized by increased lipid peroxidation and diminished antioxidant capacity [18]. Lipid peroxides and blood oxidative imbalance could be part of the cytotoxic mechanisms leading to endothelial cell injury in PE [19]. Since antioxidant vitamins are significantly decreased in both severe and mild PE, early supplementation of antioxidants may be beneficial [20]. *Thymus schimperi* and *Moringa stenopetala* plant leaves are rich in anti-oxidant phytochemicals such as Tannins, Anthraquinones, Polyphenols and Flavonoids (identified by phytochemical screening tests). Both plant extracts are also potentially non-toxic as evidenced by their median lethal doses (LD_{50}) are >10,000mg/kg in acute toxicity tests [21,22].

2. METHODS

2.1 PE Animal Models

A case control experimental method was employed on Wistar rats with induced PE using nitric oxide synthase inhibitor, nitric oxide-nitro-L-arginine methyl ester (L-NAME) at oral dose of 50mg/kg/d from gestation day (GD) 11 to 20. The method was adopted from [23] with some modifications.

2.2 Measuring Lipid Peroxide Levels

Lipid peroxide content was estimated from tissue homogenate of extract-treated PE rat models and control group according to the method of [24]. In this method, acetic acid detaches the lipid and protein of the tissue. The protein in the reaction mixture was dissolved by the addition of sodium dodecyl sulphate, 2-thiobarbituric acid (TBA) which reacts with lipid peroxide, hydroperoxide and oxygen labile double bond to form the color products with absorption maxima at 532 nm. The extinction coefficient of malondi-aldehyde (1.56×10^5) was used to calculate the amount of lipid peroxide in the samples and results were expressed as nmol of MDA/gram tissue weight.

X= O.D x DF/E x wet Weight tissue (g)

Where: O.D-optical density

X= nmol of MDA/g tissue
E= Extinction coefficient of malondi-aldehyde ($1.56 \times 10^5 M^{-1}cm^{-1}$)
DF=Dilution Factor=10/3

2.3 Measuring Total Anti-oxidant Capacity

Total antioxidant capacity was assayed using the most widely used colorimetric method-azinobis 2, 2′ 3- ethylbenzothiazoline -6 sulfonate (ABTS) radical cathion decolorization assay which is a novel automated direct measurement method for determining a total anti-oxidant capacity using antioxidant assay Kit (Sigma-Aldrich, USA). In this study, metmyoglobin and hydrogen peroxide produce feryl myoglobin radical which oxidizes the ABTS to produce a radical cation, ABTS·+, a soluble chromogen that is green in color. Antioxidants suppress the production of the radical cation and the color intensity decreases proportionally.

2.4 Data Analyses

All experimental data were expressed as mean values ± S.E.M and were subjected to bio-statistical interpretation by SPSS windows version 21 statistical packages all the way through a one-way ANOVA followed by post-hoc test (Tukey Test) for multiple comparisons of the mean differences and responses of drugs and extracts. Statistical significance of $P < 0.05$ was considered as level of significance.

3. RESULTS

3.1 Comparative Effects of ALETS and ALEMS on Lipid Peroxidation

Both extracts were evaluated for their potential therapeutic effects against PE by administering to the rat models at different dose levels. The results indicated that the lipid peroxide levels were lower among ALETS or ALEMS treated PE rat models in a dose-dependent pattern compared to untreated PE controls. ALETS treated PE group had significantly lower levels ($p<0.001$) of lipid peroxides compared to ALEMS received counterparts in all (Table 1).

3.2 Comparative Effects of ALETS and ALEMS on Serum Total Anti-oxidant Capacity

PE rat models that were treated with either of the aforementioned extracts had significantly higher total serum anti-oxidant capacity in dose dependent manner compared to untreated PE group (G5). However, there was no significant difference in total anti-oxidant levels on those PE group that were treated with ALETS compared to those treated with ALEMS (Table 2).

4. DISCUSSION

Lipid oxidation is an important factor in the pathogenesis of PE. Serum levels of lipid peroxidation measured among untreated PE (G5) rat models was significantly higher ($p<0.01$) compared to normal pregnant controls (G11). The results were consistent with previous reports [25-27] that indicated elevated lipid peroxidation in women with PE due to increased oxidative stress. PE rat models that received either ALETS or ALEMS had significantly lower levels of lipid peroxides in a dose-dependent manner. The results implied that both extracts might have potential therapeutic effects in combating PE induced excess release of lipid peroxides. Untreated PE rat models had significantly lower levels of serum total anti-oxidants compared to normal pregnant controls. These results were in agreement with previous studies [28]. While, PE rat models that received either of the extracts have higher anti-oxidant levels which might indicate that both extracts could have potential protective effects against severe PE by enhancing the total serum anti-oxidants where their deficiencies are involved in the etio-pathogenesis of PE [29].

PE leads to over production of oxygen free radicals which the inbuilt anti-oxidant system cannot cope that results in imbalance between anti-oxidant capacity and production of oxygen free radicals thus, oxidative stress occurs [30]. Both plant extracts have phytochemicals such as Tannins, Anthraquinones, Polyphenols and Flavonoids that have anti-oxidant properties to fight free radicals which are produced during PE condition; as the presence of adequate amount of such anti-oxidants prevent lipid peroxidation in plasma [31]. Hence, the study extracts might prevent the progression of PE through their anti-oxidant constituents by scavenging and/or neutralizing the common oxidants [32]. The active components of the extracts also might initiate activation of endogenous anti-oxidants that counteract PE- induced free radicals such as super oxides.

Table 1. Comparative effects of ALETS and ALEMS on total lipid peroxides of PE rat models compared to normal (G11), negative (G5) and positive (G6) controls

Groups	Concentration of total lipid peroxides in MDA/gram of tissue (nmol)
G5 (Untreated PE rat models or negative control)	$0.57\pm0.080^{a^{**}c^{***}}$
G6 (PE rat models treated with nifedipine, 20mg/kg/d)	$0.09\pm0.002^{b^{***}}$
G7 (PE rat models treated with ALETS, 250mg/kg/d)	$0.09\pm0.010^{b^{***}d^{**}}$
G8A (PE rat models treated with ALETS, 500mg/kg/d)	$0.07\pm0.002^{b^{***}d^{*}}$
G8B (PE rat models treated with ALETS, 1000mg/kg/d)	$0.02\pm0.002^{a^{*}b^{***}d^{**}}$
G9 (PE rat models treated with ALEMS, 250mg/kg/d)	$0.36\pm0.080^{a^{**}d^{**}}$
G10A (PE rat models treated with ALEMS, 500mg/kg/d)	$0.20\pm0.003^{b^{**}d^{*}}$
G10B (PE rat models treated with ALEMS, 1000mg/kg/d)	$0.13\pm0.020^{b^{**}d^{**}}$
G11 (Normal pregnant rat control)	0.11±0.030

Mean ± SEM; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, ^{a}p-compared to normal pregnant controls (G11), ^{b}p-compared to negative controls, ^{c}P-compared with positive controls (G6), ^{d}p-compared to extract received counterparts, PE-pre-eclampsia, MDA- malondialdehyde, n=6

Table 2. Comparative effects of ALETS and ALEMS on serum Total Anti-oxidant Capacity (TAC) of PE rat models

Groups	Total anti-oxidant capacity (TAC) (µg/ml of ascorbic acid equivalent)
G5 (Untreated PE rat models or negative control)	$24.5\pm0.9^{a**c**}$
G6 (PE rat models treated with nifedipine 20mg/kg/d)	$28.4\pm0.2^{b**}$
G7 (PE rat models treated with ALETS, 250mg/kg/d)	$27.6\pm0.3^{b*}$
G8A (PE rat models treated with ALETS, 500mg/kg/d)	$29.5\pm0.3^{b***}$
G8B (PE rat models treated with ALETS, 1000mg/kg/d)	$31.2\pm0.4^{a*b***c*}$
G9 (PE rat models treated with ALEMS, 250mg/kg/d)	$29.2\pm0.3^{b**}$
G10A (PE rat models treated with ALEMS, 500mg/kg/d)	$29.7\pm0.3^{b**}$
G10B (PE rat models treated with ALEMS, 1000mg/kg/d)	$30.6\pm0.4^{a*b***}$
G11 (Normal pregnant controls)	28.1±0.4

Mean ± SEM; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, ^{a}p-compared to normal pregnant controls (G11), ^{b}p-compared to negative controls (G5), ^{c}P-compared with positive controls (G6), ^{d}p-as compared to extract received counterparts, n=6

5. CONCLUSION

Both ALETS and ALEMS could reduce lipid peroxides and increase total serum anti-oxidants of PE rat models, suggesting their potential therapeutic actions against PE through reducing levels of free radicals which are excessively released in serum of PE cases.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from Institutional Review Board of College of Health Sciences of Addis Ababa University with a protocol number of 029/16/032/16/ Physio, before commencement of the animal experimentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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