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Serum Antioxidant Status of Induced Preeclamptic Wistar Rats Exposed to Methanolic Leaf Extracts of Some Selected Herbal Plants

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Abstract

Preeclampsia is known to cause an accumulation of free radicals, putting the cells in a state of oxidative stress. Antioxidants are produced by cells as a defense mechanism against these stressors. Antioxidants are natural chemicals that can prevent or postpone various types of cell damage by acting as oxidative process inhibitors. Food and medicinal plants are the primary sources of antioxidants. The goal of this study was to see how methanolic leaf extracts of *Jatropha curcas*, Alchonnea cordifolia, and Secamone afzelii affected the antioxidant state in induced preeclamptic Wistar rats. Age-matched female Wistar rats weighing 220 to 256 g were used for the study. Preeclampsia was induced using the Adriamycin Model. When preeclampsia was confirmed in the induced Wistar rats by an increase in blood pressure from 124/98 mmHg to 177/121 mmHg, as well as significant proteinuria, the preeclamptic and control rats were administered methanolic extracts of *Jatropha curcas*, *Alchornea cordifolia*, and *Secamone afzelii.* In Wistar rats, preclampsia resulted in a substantial decrease in gluthathione peroxidase activity (461.2 U/l). However, *J. curcas* administration considerably increased antioxidant activity (756.8 U/l), outperforming the reference medication (666.9 U/l). Malondialdehyde levels, which were elevated in preeclamptic rats (21.9 M), were considerably lowered when plant extracts (10.8 – 15.1 M, p0.05) were administered. Given that preeclampsia reduces antioxidant capacity in pregnant Wistar rats, treatment of plant extracts improved antioxidant status in preeclamptic Wistar rats, lowering oxidative stress in preeclamptic Wistar rats.

Keywords: Antioxidants; Preeclampsia; *Jatropha curcas; Alchonnea cordifolia; Secamone afzelii*

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Introduction

The biochemical reaction, which takes place within the cell organelles, is one of the main driving forces, which help in sustaining human life [1]. For the past, there is an increasing awareness among people in prevention of diseases, especially caused by the free radicals, which are continuously produced by the body's normal use of oxygen [2] as biological phenomena, its oxidative property plays a vital role, and oxygen being essential for life can also aggregate the damage within the cell by oxidative events [3]. However, the potential harmful effects of oxygen is due to the formation and activity of chemical compounds, known as reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), which have the tendency to donate oxygen to other substances. ROS and RNS play importance role, i.e, toxic and beneficial, in human body, thus maintaining balance between these two ant agonistics in the body is an important aspect of life [4].

Antioxidants are natural substances, which may prevent or delay some types of cell damage. Even at relatively small concentration, they act as an inhibitor of the oxidative process and thus have different physiological role in human body. For plant origin antioxidants act as radical scavengers and help in converting the radicals to less reactive species. Antioxidants prevent tissue and

cell damage. In excessive free radicals, cells have the defense and preventative mechanisms, repair mechanisms and antioxidant defenses (Jacob, 1995). Study showed that the increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation of oxidative chain reactions, acting as free radical scavengers, quenchers of oxygen and reducing agents [5].

Antioxidants are mainly derived from food and medicinal plants, such as fruit, vegetables, cereal, mushroom [6, 7]. Agricultural by-products are also potentially important sources of natural antioxidants [8]. Natural antioxidants from plant materials are mainly polyphenols (phenolic acids flavonoids, anthocyains). Generally, natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, such as antibacterial, antiviral, anti-aging anti-inflammatory and anticancer [9]. Generally, natural antioxidants are the chain breaking antioxidants, which react with lipid radicals and convert them into more stable products. They include vitamin A (retinoids), bioflavomoids (citrin), polyphenols (hydroxytyrosol), tocopherols (vitamin E) and ascorbic acid (vitamin C). They play a significant role in the prevention of heart disease, cancer, ageing and immune deficiency diseases. Antioxidants mainly have phenolic structures including antioxidants minerals, antioxidants vitamins and phytochemicals [10]. Generally, there are three main types of antioxidants found in nature; phytochemicals, vitamins and ezymes but antioxidants found in plants are most powerful since the plants are exposed to UV light throughout the day. However, antioxidants are synthesized within the human body but can be obtained from vegetables and fruitsincluding nuts, grains seeds, and meats and fish in the form of Vitamin E, C and beta-carotene [11]. Enzymatic antioxidants are produced by the body and are include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx) as reported by Bouayed and Bohn (Bouayed and Bohn, 2010). There are significantly in human body as they break down and remove the free radicals from the body. They can wash out harmful oxidative products by converting them into hydrogen peroxide and then into water.

Plants have been a source of dietary antioxidants [12] reported that two-thirds of the world's plants species have medicinal importance, and almost all of these have excellent antioxidant potential. The antioxidant potential of plants had received a great deal of attention because increased oxidative stress had been identified as a major causative factor in the development and progression of several life threating disease, including cardiovascular disease and neurodegeneration. Demonstrated that supplementation with dietary antioxidants or boosting of endogenous antioxidant defenses of the body had been found to be promising method of counting the undesirable effects of oxidative stress [13].

Preeclampsia had been described to be the most serious and life-threatening pregnancy complication for both mother and embryos. Smith and Baker, 2005 shown various risk factors associated with preeclampsia including age, obesity, chronic hypertension, renal disease and diabetes mellitus. Some evidence suggests that placental and systemic oxidative stress play a crucial role in the development of preeclampsia [14, 15]. Generally, the potential causes of oxidative stress may be ascribed to a series of physiological change, mineral deficiencies and increased oxygen consumption during pregnancy. Evidences showed that reduced perfusion and ischemic reperfusion in placenta result in placental hypoxia and, as a consequence, leading to raised synthesis of the free radical including superoxide anion in placenta. However, production of free radicals in endothelial cells is relatively low. Chemical scavengers or antioxidant molecules such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are neutralized by active defense systems. Changes in the level of various enzymatic and non-enzymatic antioxidants during pregnancy could affect pregnancy outcome through alteration in maternal and fetal metabolism. Study showed that oxidative stress occurs prior to the onset of preeclampsia and the placenta is referred as central organs responsible for free radical generation in women with preeclampsia [16]. Nevertheless, these studies were not comprehensive because of small sample size. Some authors suggest that antioxidant supplementation reduces the risk of preeclampsia, but others did not find such effect. The aim of this current study therefore was to investigate the antioxidant capacity of induced preeclamptic Wistar rats and also evaluate their responses after exposure to the methanolic extract of *Jatropha curcas; Alchonnea cordifolia; Secamone afzelii.*

Materials and Methods

Collection and Preparation of Plant Samples

Plant samples (leaves) were collected from First Generation Farms in Edo State, Nigeria, in Iguosula, Uhunmwonde Local Government Area. They were identified and verified at the Phytomedicine department of the University of Benin's Department of Plant Biology and Biotechnology in Benin City. For Jatrophacurcas, *Alchornea cordifolia* and Secamone afzelii, specimen numbers UBH-J404, UBH-A560, and UBHS566 were assigned. The plant samples were cleansed with distilled water several times, then air-dried for two weeks before being crushed into powder with a Panasonic® medium kitchen blender, model MX-GX1021WTZ. The extracts were filtered using Whatman Filter Paper No. 42 (125mm) after soaking 100g of each powder sample in 200ml of methanol for 12 hours.

Study design

Female Wistar rats weighing 220 to 256 g were employed in the study, and they were age-matched (three days) (mean, 237 g). During the month of May 2019, the animals were kept in a well-ventilated Animal House at the University of Benin's Department of Biochemistry in Benin City, with daily light and darkness fluctuations. The animals had free access to a standard meal (0.35 g NaCl, 20 g protein, and 1.17 g Arginine per 100 g chow) and ad libitum tap water (pH range 6.8–7.2). Before the study began, they were given a one-week acclimatization period and were cared for in accordance with international guidelines for the care and use of laboratory animals.

In this experiment, the Wistar rats were randomly separated into fifteen groups, each with ten rats. The first group acted as a positive control, while the second, third, and fourth groups

functioned as negative controls. The remaining groups are listed in (**Table 1**); the rats were given extracts as well as standard medicines as soon as preeclampsia was established. This was done once a day, orally, at the appropriate doses, until the rats were delivered of their pops.

Induction of preeclampsia

Preeclampsia was induced using the Adriamycin Model developed by [17]. Rats were given Adriamycin (Adriablastina, Abic) at a dose of 3.5 mg/kg i.v. into the femoral vein under mild ether anesthesia. For four days, the rats were paired with a reproductive male. On the first day of pregnancy, spermatozoa were discovered in the vaginal smear. Elevated blood pressure and substantial proteinuria confirmed preeclampsia (**Table 2**). The Wistar rat's blood pressure was measured using the CODA® High Throughput System with 2 Activated Channels (CODA-HT2) by Kent Scientific Corporation in the United States. The rats were carefully placed in the CODA System's restrainer, and the back hatch was replaced to keep the rat contained. After putting all of the Wistar rats in their restrainers to be tested on the same CODA system, the rats were given 5 minutes to adjust to the restrainer before starting the blood pressure measurement process. The Wistar rats relax and warm up at this time, allowing blood to flow to the tail. After a 5-minute acclimatization period, the blood pressure measurement procedure bg2an.

Table 1. Designation of experimental groups.

Group	Description
Group 1	Control
Group 2	Administered with Ext-JC (No induced Preeclampsia)
Group 3	Administered with Ext-AC (No induced Preeclampsia)
Group 4	Administered with Ext-SA (No induced Preeclampsia)
Group 5	Induced Preeclampsia, no treatment provided
Group 6	Induced Preeclampsia + 100 mg/kg Standard drug
Group 7	Induced Preeclampsia + 50 mg/kg Ext-JC
Group 8	Induced Preeclampsia + 100 mg/kg Ext-JC
Group 9	Induced Preeclampsia + 200 mg/kg Ext-JC
Group 10	Induced Preeclampsia + 50 mg/kg Ext-AC
Group 11	Induced Preeclampsia + 100 mg/kg Ext-AC
Group 12	Induced Preeclampsia + 200 mg/kg Ext-AC
Group 13	Induced Preeclampsia + 50 mg/kg Ext-SA
Group 14	Induced Preeclampsia + 100 mg/kg Ext-SA
Group 15	Induced Preeclampsia + 200 mg/kg Ext-SA

Ext-JC, Metholic leaf extract of *Jatropha curcas;* Ext-AC, Metholic leaf extract of *Alchonnea cordifolia;* Ext-SA, Metholic leaf extract of *Secamone afzelii.* Standard drug was methyl DOPA (Aldomet®)

Table 2. Confirmation of preeclampsia.

Treatments	Parameter	Control	Induced
Blood pressure			
Third trimester	Systolic (mmHg)	124	177
	Diastolic (mmHg)	98	121
Post-partum	Systolic (mmHg)	121	160
	Diastolic (mmHg)	96	125
Proteinuria			
Third trimester	Proteinuria	Negative	+++
Post-partum	Proteinuria	Negative	+

Determination of proteinuria

The dipstick (combi2) method was used to determine proteinuria.

Confirmation of preeclampsia

Preeclampsia was confirmed in the induced Wistar rats by an increase in blood pressure from 124/98 mmHg to 177/121 mmHg, as well as significant proteinuria.

Ethical issues

The Research and Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City, granted ethical permission with reference LS19017, dated March 7, 2019.

Processing blood for serum

Cardiac puncture was used to collect blood, which was then allowed to clot and retract in simple bottles. The sample was then centrifuged for 15 minutes at 3000 rpm to get the supernatant serum.

Sacrifice of experimental animal

The animals were anaesthetized with chloroform and humanely sacrificed 24 hours after the last dosage of the standard medicine and various treatment extracts were administered to the relevant groups.

Determination of serum antioxidant levels

The action of glutathione reductase (GR) was monitored by following the disappearance of the co-substrate NADPH at 340 nm [18]. This assay took advantage of glutathione disulfide (GSSG) formed by the enzymatic action of glutathione peroxidase (GPx) and is regenerated by excess GR in the assay. Catalase activity was determined by a spectrophotometric procedure by measuring peroxide removal, a direct assay with pseudo-first order kinetics [19].

Superoxide dismutase (SOD)

Activity was evaluated by measuring the inhibition of pyrogallol autoxidation that is catalyzed by the superoxide radical [20]. The colorimetric approach was used to assess malondialdehyde (MDA). The catabolite of lipid peroxide reacts with thiobarbituric acid (TBA) to form a crimson molecule with a maximum absorption peak of 532 nm in this experiment [21].

Data analysis

Data collected were analyzed using SPSS version 20. Results were presented in Tables and Quantitative variables were expressed as mean ± standard deviation

Results and Discussion

GPx activity in the control was 794.5U/l during the 3rd trimester as against 736.4 U/l at Postpartum (**Table 3**). When these Wistar rats were induced, there was reduction in the GPx levels (461.2 U/l). However, administration of the plant extracts, particularly 50 mg/kg of *J. curcas* significantly enhanced antioxidant activity (756.8 U/l) even better than the standard drug (666.9 U/l) respectively. Increased activity of MDA usually indicates cellular

Table 3. Antioxidants at 3rd trimester and post-partum.

Parameter	3rd trimester					post-partum				
	GPX	GR	MDA	CAT	SOD	GPX	GR	MDA	CAT	SOD
Control	794.5	0.87	13.1	23.9	1.05	736.4	1.00	11.9	18.4	0.5
Only Ext-A (No induced PreEc)	544.0	0.94	17.5	17.4	0.33	508.8	1.03	16.0	16.0	0.6
Only Ext-AC (No induced PreEc)	552.1	0.99	12.1	19.0	0.09	679.1	0.94	15.5	21.3	0.9
Only Ext-SA (No induced PreEc)	574.4	1.00	11.9	22.9	0.39	700.5	1.10	15.9	30.2	0.8
Induced PreEc, no treatment provided	461.2	0.84	21.9	8.1	0.18	979.8	0.98	16.1	37.1	1.4
Induced PreEc + 100 mg/kg StdD	666.9*	0.90	15.1*	13.8	0.57*	673.9*	0.97	13.2*	20.4	0.2*
Induced PreEc + 50 mg/kg Ext-JC	756.8*	1.03	13.1*	17.0*	0.57*	629.4*	1.16*	13.0*	19.6	0.5*
Induced PreEc + 100 mg/kg Ext-JC	463.6	1.10	12.3*	7.9	0.95*	722.9*	0.97	12.4*	28.5	1.3
Induced PreEc + 200 mg/kg Ext-JC	459.4	1.03	12.4*	7.2	0.57*	607.8*	0.97	12.2*	14.2	0.3*
Induced PreEc + 50 mg/kg Ext-AC	474.6	1.07	12.3*	10.7	0.62*	587.9*	1.06	15.1	21.3	0.8*
Induced PreEc + 100 mg/kg Ext-AC	584.4	0.97	10.8*	16.1*	0.24	478.4*	0.96	12.2*	19.1	0.7*
Induced PreEc + 200 mg/kg Ext-AC	564.0	0.99	11.0*	12.7	0.20	439.6*	0.91	13.5	11.1	0.3*
Induced PreEc + 50 mg/kg Ext-SA	588.9	0.99	11.1*	20.4*	0.34*	603.6*	0.93	11.5*	23.0	0.3*
Induced PreEc + 100 mg/kg Ext-SA	449.8	1.03	13.9*	16.2*	0.63*	574.2*	0.85	11.9*	18.3	0.2*
Induced PreEc + 200 mg/kg Ext-SA	491.4	0.98	12.2*	19.9*	0.67*	434.0*	0.95	15.9	16.6	0.5*
LSD (0.05)	185.3	0.73	5.4	6.4	0.09	277.3	0.15	2.8	11.2	0.5
F-test	4.037	0.931	0.738	1.766	3.237	3.590	1.270	1.356	1.684	2.212
p-value	0.001	0.539	0.721	0.094	0.003	0.002	0.281	0.235	0.113	0.033

*Preeclampsia means with asterisks superscript significantly from the negative control (Induced PreEc, no treatment provi0DED) ($p<0.05$)
Ext-JC, Metholic leaf extract of *Jatropha curcas*; Ext-AC, Metholic leaf extract of *Alchonnea cordifolia*; Ext-SA, Metholic leaf extract of *Secamone afzelii*. Standard drug was methyl DOPA (Aldomet®)

stress especially due to membrane breakdown. MDA levels were heightened in the preeclamptic rats (21.9 µM). The administration of the plant extracts significantly reduced the MDA levels in preeclampsia (10.8 – 15.1 µM, $p<0.05$). Elevated MDA levels during postpartum was also reduced upon administration of plant extracts.

When compared to the control, SOD activity during preeclampsia was lower in the third trimester (0.18 U/ml), but higher in the postpartum period (1.4 U/ml). The administration of plant extracts considerably rectified these abnormalities ($p<0.05$).

Several studies have found significantly higher total antioxidant capacity inpreeclampticwomem, whereas other studies have found significantly lower total antioxidant capacities [22]. The researchers discovered that when preeclamptic wistar rats were exposed to various leaf extracts and conventional medications throughout the third trimester, their antioxidant capacity rose, whereas the antioxidant capacity of preeclamptic subjects was higher during the postpartum period.

By deforming or neutralizing free radicals, certain antioxidants can directly protect maternal and fetal tissues from oxidative harm. Superoxide dismutase (SOD) neutralizes free radicals by dismutating O_2-radicals, protecting cell membranes from lipid peroxidation caused by free radicals [23]. Several studies found significantly higher SOD activity in preeclamptic women [24, 25], whereas [22] found significantly lower SOD activity in preeclamptic women. The SOD activity of preeclamptic rats was shown to be lower in this investigation. The free radicals produced during preeclampsia likely overwhelmed the cell's ability to produce SOD, resulting in the decrease. Preeclamptic wistar rats had much lower GPx activity than normal rats, according to this study. However, a few studies have revealed that preeclamptic women have much decreased GPx activity. Evaluated GPx activity in blood samples and discovered that lower GPx activity was linked to increased preeclampsia risk and severity [26]. In this investigation, GPx levels were greater at post-partum in preeclampsia-affected wistar rats than in normal controls. GPx levels have been found to be greater in preeclampsia mothers in various investigations [27]. GPx plays a direct role in the removal of reactive oxygen species, which helps to prevent lipid peroxidation, particularly in membrane phospholipids.

In the current study, Wistar rats had higher maternal plasma MDA levels in the third trimester and postpartum than normal controls. Endothelial dysfunction direct damage to cell membranes and endothelium-dependent vasorelaxation have all been linked to MDA, a lipid peroxidation result [28]. According to study, higher MDA levels are associated with the severity of preeclampsia [29]. As one of the principal defense mechanisms and free radical scavengers, glutathione and glutathione-related enzymes play an important role in oxidative stress management. Free radicals are detoxified by SOD, GPx, and catalase, whereas GSH is a broad scavenger.

Conclusion

According to the findings of this study, third trimester preeclampsia has a reduced antioxidant enzyme status, meaning that there is indirect evidence of oxidative stress in preeclampsia. Antioxidants may be useful as an adjuvant treatment for preeclampsia because they help to lessen the oxidative stress induced by the illness. Methanolic plant extracts of *Jatropha curcas, Alchonnea cordifolia,* and *Secamone afzelii* were used to corroborate this, and they significantly lowered MDA levels in induced preeclamptic Wistar rats.

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