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Lipid Ratios in Adriamycin-induced Pre-eclamptic Wistar Rats Exposed to Methanolic Plant Extracts

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ABSTRACT: The study accessed the lipid ratios in preeclamptic Wistar rats exposed to methanolic leaf extracts of *Jatropha cacus, Alchonnea cordifolia,* and *Secamone afzelii*. Plant samples (leaves) were washed severally with distilled water, air-dried, and crushed to powder and were filtered, then soaked in 200ml of methanol for 12 hours. The LD50 was determined to ascertain the safety of the plant extracts for use. Female Wistar rats, aged 3 days apart, used in the study, were acclimatized for one week. Peeclampsia was induced used the Adriamycin Model. Results showed that there was elevation of blood pressure (bp) due to preeclampsia. At 3rd trimester, systolic bp (177 mmHg) was higher than at postpartum (160 mmHg). The administration of plant extracts caused a significant reduction in systolic (127 – 150 mmHg) and diastolic (86 – 103 mmHg) bp during the 3rd trimester. Proteinuria was reduced to trace levels when *Alchonnea cordifolia* was used. Total cholesterol levels were higher in the third trimester (71.6 mg/dl) and postpartum (74.3 mg/dl), respectively. Preeclampsia was linked to similar increases in triglycerides, and low density lipoprotein cholesterol; with a reduction in high density lipoprotein cholesterol. The treatment with the various plant extracts lowered the incidence of arteriosclerotic cardiovascular events during preeclampsia. Although the extracts had a negative effect on systolic blood pressure and proteinuria during the third trimester, indicating that they were beneficial in reducing preeclampsia outcomes, there was no effect on blood pressure or proteinuria during the postpartum period.

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Pre-eclampsia is a pregnancy-related condition marked by high blood pressure and proteinuria that goes away after the baby is born. It's is a prevalent systemic pregnancy condition that is also one of the most common pregnancy disorders, as well as a major source of maternal and perinatal morbidity and mortality (Enquobahrie et al., 2004). Preeclampsia affects 7–10% of all pregnancies worldwide (Raijmakers et al., 2004). In Northern Nigeria, it accounts for 40% of maternal death, with a strong link to health-care workers' lack of understanding and a poor therapeutic interaction, however in Southern Nigeria, the prevalence rate of pre-eclampsia ranges between 5.6 and 7.6%. (Adeosun et al., 2015). According to several studies, women with greater levels of oxidized low-density lipoprotein (LDL) and triglycerides (TG) are more likely to develop pre-eclampsia than normotensive pregnant women (Qiu *et al*., 2006; Timalsina *et al*., 2016). The oxidative conversion of LDL-cholesterol to oxidized LDL is a critical event in the onset and progression of atherosclerosis and hypertension (Qiu *et al*., 2006). Dyslipidemia in early pregnancy is associated with increased risk of preeclampsia (Enquobahrie *et al*., 2004). During the first trimester of pregnancy, the mother is anabolic, and the lipid acts as a source of calories for both the developing fetus and the mother. During the third trimester, the mother is catabolic, and the lipid serves as a source of calories for both the growing fetus and the mother (Lain and Catalano, 2017). A number of studies have found that abnormalities in lipid profiles may be related to the risk of preeclampsia. Triglycerides, LDL and total cholesterol in the preeclamptic women are greater than regular pregnancies, however in preeclamptic women high density level of lipoprotein (HDL) is lower compared to normal pregnancy (De *et al*., 2006; Baker *et al*., 2009; Thathagari and Kumar, 2018). Although

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there is emerging evidence that excessive levels of oxidized low density lipoproteins and triglycerides enhance the risk of preeclampsia (Qiu *et al*., 2006), the role of lipid peroxidation in the pathophysiology of preeclampsia remains unclear. The aim of the study is to assess the changes in lipid profile and lipid ratio that occurs during preeclampsia, as well as the impact of plant extracts on the management.

MATERIALS AND METHODS

Collection and preparation of plant samples: Plant samples (leaves) were collected from first-generation farms located at Iguosula, Uhunmwonde Local Government area, Edo State. They were identified and verified at the Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, Benin City. 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 after soaking 100g of each powder sample in 200ml of methanol for 12 hours (125mm).

Study design: The study employed age-matched (three days) female Wistar rats weighing 220 to 256 g (mean, 237 g). The animals were provided unrestricted access to a regular diet with the following composition; 0.35 g NaCl, 20 g protein, and 1.17 g arginine per 100 g food. They were also provided tap water (pH range 6.8–7.2) *ad libitum*.

In this experiment, the wister rats were randomly divided into fifteen (15) groups, each with ten (10) rats. The positive control was Group 1, whereas the negative controls were Groups 2, 3, and 4. The rest of the groups are listed below (Table 1).

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

Preeclampsia induction: The Adriamycin Model adopted by Podjarny *et al* (1992) was used. Under light ether anesthesia, rats were given Adriamycin (Adriablastina, Abic) at a dose of 3.5 mg/kg IV into a superficial femoral vein. The rats were mated with a fertile male for four days after two weeks.

Experimental animal care and management: The animals were cared for and used in compliance with international guidelines for laboratory animal care and use (NRC, 2011).

Experimental animal sacrifice: Twenty four (24) hours after administration of the last dose of the standard drug and various treatment extracts to the respective groups, the animals were anaesthetized with chloroform and humanely sacrificed (Rowell, 1977).

Processing blood for serum: The approach described by Dave and Lewis (1986) for processing blood for serum was employed. Blood samples were taken through heart puncture and placed in a simple vial. After allowing the sample to clot, it was centrifuged for 15 minutes at 3000rpm to get clear slightly yellow supernatant serum.

Determination of Lipid profile levels: The lipid analysis was carried out using a spectrophotometer, and the kits were provided by Randox Laboratory Limited. The procedure employed was the standard operational assay. The blood lipid indices were calculated using the equations below;

Friedewald equation for LDL Chol. = TC – TG/5 – HDL Chol. (mg/dl)

Atherogenic index plasma, AIP = Log (TG/HDLChol.)

Atherogenic cooefficient, AC = (Non HDL Chol/ HDL Chol.)

Non-HDL cholesterol, NHDL = VLDL + LDL Chol.
Very low density lipotrotein, VLDL = Triglycerides/5
Castelli Risk Index I, CRI I = TC/ HDL Chol.
Castelli Risk Index II, CRI II = LDLC/HDLC

Statistical analysis: Data collected were analyzed using SPSS version 20. Results were presented in Tables and Quantitative variables were expressed as mean ±SD.

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.

RESULTS AND DISCUSSION

Figure 1 shows the LD_{50} of the extract administered to the Wistar rats. There was no mortality recorded upon administration of the various plant extracts, thus indicating safety of use. Preeclampsia caused an increase in blood pressure (BP) (Table 2). The systolic blood pressure (177 mmHg) was greater in the third trimester than it was postpartum (160 mmHg). During the third trimester, plant extracts generated a significant drop in systolic (127–150 mmHg) and diastolic (86–103 mmHg) blood pressure. The lowest bp (118/86 mmHg) was obtained when preeclamptic animals were administered 100mg/kg of *Jatropha cacus.* Generally, systolic bp during postpartum were lower than at 3rd trimester. Table 3 shows the effects of plant extract treatment on the incidence of proteinuria in rat models.

As presented, the control animals as well as those administered the plant extracts did not show signs of proteinuria. However, incidence of preeclampsia presented positive proteinuria cases for the test animals. At 3rd trimester proteinuria level was 3 times that at postpartum. Administration of plant extracts reduced proteinuria during 3rd trimester generally by one-thirds. At post-partum however, treatment of test animals with plant extracts reduced proteinuria by two-thirds with the use of 100 mg/kg *Jatropha cacus* and *Secamone afzelii* respectively. The use of *Alchonnea cordifolia* resulted in reduction of proteinuria to trace levels.

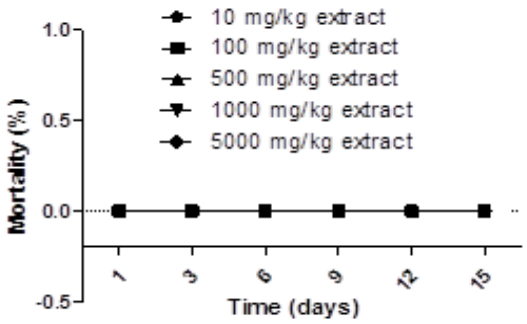

Fig 1: Presentation of LD_{50}

Table 2: Presentation of blood pressure results after administration of selected plant extracts to preeclamptic Wistar rats

Treatments	(n)	Systolic	Diastolic	(n)	Systolic	Diastolic
	3rd trimester			**Post-partum**		
Control	14	124	98	8	121	96
Only Ext-A (No induced PreEc)	15	119	85	12	110	85*
Only Ext-B (No induced PreEc)	19	132	101	13	122	94
Only Ext-C (No induced PreEc)	19	131	95	12	123	91
Induced PreEc, no treatment	26	177*	121*	12	160*	125*
Induced PreEc + 100 mg/kg StdD	36	141	103	10	135	104
Induced PreEc + 50 mg/kg Ext-JC	16	143	99	7	117	93
Induced PreEc + 100 mg/kg Ext-JC	12	118	86	4	129	101
Induced PreEc + 200 mg/kg Ext-JC	12	145	100	3	119	99
Induced PreEc + 50 mg/kg Ext-AC	14	143	97	4	113	90
Induced PreEc + 100 mg/kg Ext-AC	10	136	96	5	113	87
Induced PreEc + 200 mg/kg Ext-AC	19	127	87	10	127	94
Induced PreEc + 50 mg/kg Ext-SA	11	140	98	6	136	102
Induced PreEc + 100 mg/kg Ext-SA	16	141	101	6	116	86
Induced PreEc + 200 mg/kg Ext-SA	13	150*	92	8	121	106
LSD(0.05)		22	14		16	11
F-test		5.913	7.795		4.332	4.241
p-value		<0.001	<0.001		<0.001	<0.001

* Means are significantly different from the Control ($p<0.05$). Values presented are to the nearest integer. Ext-JC, Metholic leaf extract of *Jatropha cacus;* Ext-AC, Metholic leaf extract of *Alchonnea cordifolia;* Ext-SA, Metholic leaf extract of *Secamone afzelii.* Standard drug was methyl DOPA (Aldomet®)

Table 3: Effects of administration of plant extracts on incidence of proteinuria in the preeclamptic Wistar rats

Group	Baseline	At Third trimester	At post-partum
Control	Negative	Negative	Negative
Only Ext-A (No induced PreEc)	Negative	Negative	Negative
Only Ext-B (No induced PreEc)	Negative	Negative	Negative
Only Ext-C (No induced PreEc)	Negative	Negative	Negative
Induced PreEc, no treatment provided	+++	+++	+
Induced PreEc + 100 mg/kg StdD	NA	+	Trace
Induced PreEc + 50 mg/kg Ext-JC	NA	+	Trace
Induced PreEc + 100 mg/kg Ext-JC	NA	++	+
Induced PreEc + 200 mg/kg Ext-JC	NA	++	Trace
Induced PreEc + 50 mg/kg Ext-AC	NA	++	Trace
Induced PreEc + 100 mg/kg Ext-AC	NA	++	Trace
Induced PreEc + 200 mg/kg Ext-AC	NA	++	Trace
Induced PreEc + 50 mg/kg Ext-SA	NA	++	Trace
Induced PreEc + 100 mg/kg Ext-SA	NA	++	+
Induced PreEc + 200 mg/kg Ext-SA	NA	++	Trace

Present + (The number of "+" indicates level of severity); NA not applicable

Table 4 shows the dyslipidemia observed in induced test animals after exposure to plant extracts. There was elevated total cholesterol (TC) at both 3rd trimester (71.6 mg/dl) and postpartum (74.3 mg/dl) respectively. Similar increases in Triglycerides (TG), High density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) were reported due to preeclampsia. It was observed that there were significant differences between the TC, TG, HDL and LDL at the 3rd trimester. At post-partum, there were no significant differences between the TC and LDL among the groups treated with the plant extracts. While, there were significant differences between TG and HDL. There was significant decrease in lipids parameters in the Induced PreEc + 100 mg/kg Ext-C, Induced PreEc + 50 mg/kg Ext-C and Induced PreEc + 50 mg/kg Ext-B group compared to the control group in the 3rd trimester, but significant increase across the other groups.

Table 4: Lipid profiles in induced test animals after exposure to plant extracts

Treatments	TC	TG	HDL	LDL	TC	TG	HDL	LDL
	3rd Trimester				**Post-partum**			
Control	43.2	51.4	17.4	32.9	39.9	47.1	13.5	31.2
Only Ext-A (No induced PreEc)	42.5	46.6	18.3	33.2	43.2	38.2	15.6	33.7
Only Ext-B (No induced PreEc)	51.3	41.5	27.7	41.2	46.3	40.3	15.2	36.4
Only Ext-C (No induced PreEc)	46.8	61.2	32.4	33.8	40.5	38.4	37.3	33.2
Induced PreEc, no treatment provided	71.6	66.2	16.4	57.2	74.3	59.7	11.3	63.9
Induced PreEc + 100 mg/kg StdD	59.2	52.1	15.7	46.8	47.3	43.4	14.7	38.1
Induced PreEc + 50 mg/kg Ext-JC	69.7	59.6	19.3	56.4	36.7	33.1	13.8	39.2
Induced PreEc + 100 mg/kg Ext-JC	50.4	36.7	13.3	44.3	55.3	42.4	16.1	46.8
Induced PreEc + 200 mg/kg Ext-JC	48.2	39.2	18.0	41.3	44.5	40.3	37.6	36.4
Induced PreEc + 50 mg/kg Ext-AC	33.7	31.2	12.4	27.5	49.7	44.3	16.2	39.4
Induced PreEc + 100 mg/kg Ext-AC	46.1	41.3	34.8	37.2	34.4	30.3	15.4	29.1
Induced PreEc + 200 mg/kg Ext-AC	43.3	38.2	34.9	36.5	57.3	47.2	14.3	47.9
Induced PreEc + 50 mg/kg Ext-SA	38.2	30.1	12.3	33.2	56.8	38.7	15.3	45.2
Induced PreEc + 100 mg/kg Ext-SA	39.4	33.7	9.7	32.6	53.4	47.7	15.6	42.5
Induced PreEc + 200 mg/kg Ext-SA	55.6	44.0	18.7	46.8	40.4	39.4	32.6	33.2
LSD(0.05)	12.1	7.2	11.2	18.4	14.13	4.03	3.91	6.31
p-value	0.121	0.138	0.329	0.422	<0.001	0.005	0.568	<0.001

***Key: TC** Total cholesterol, TG Triglycerides, HDL High density lipoprotein cholesterol, LDL low density lipoprotein cholesterol*

Castelli risk ratio (CRI, 1) was 2.48 in the control, but was elevated (4.37) in the preeclampstic rats (Table 5). However, CRI (I) in the 3rd trimester was during postpartum (6.58) in the preeclamptic rats. Reductions in CRI as well as other lipid ratios was reported in both 3rd trimester and postpartum when rats were administered different doses of the plant extracts. The risk of arteriosclerotic cardiovascular events during preeclampsia was reduced upon the administration of the plant extracts (Table 5). Figure 2(a) shows the canonical correspondence biplot in the 3rd trimester. It was observed that HDL positively influenced Ext-B (med) and Ext-B (high) owing to its close relationship, while proteinuria influenced Ext-A (high) on Axis 2.

Table 5: Presentation of lipid ratios in induced test animals after exposure to plant extracts

	CRI,I	CRI,II	nonHDL	AC	AIP	VLDL	CRI,I	CRI,II	nonHDL	AC	AIP	VLDL
	3rd trimester						***Post-partum***					
Control	2.48	1.89	25.73	1.48	0.47	10.28	2.96	2.31	26.40	1.96	0.54	9.42
Only Ext-A (No induced PreEc)	2.32	1.81	24.17	1.32	0.41	9.32	2.77	2.16	27.60	1.77	0.39	7.64
Only Ext-B (No induced PreEc)	1.86	1.49	23.65	0.86	0.18	8.29	3.05	2.39	31.10	2.05	0.42	8.06
Only Ext-C (No induced PreEc)	1.44	1.04	14.40	0.44	0.28	12.24	1.09	0.89	3.20	0.09	0.01	7.68
Induced PreEc, no treatment provided	4.37	3.49	55.21	3.37	0.61	13.23	6.58	5.65	63.00	5.58	0.72	11.94
Induced PreEc + 100 mg/kg StdD	3.76	2.97	43.43	2.76	0.52	10.42	3.22	2.59	32.60	2.22	0.47	8.68
Induced PreEc + 50 mg/kg Ext-JC	3.62	2.93	50.44	2.62	0.49	11.92	2.66	2.84	22.90	1.66	0.38	6.62
Induced PreEc + 100 mg/kg Ext-JC	3.79	3.33	37.07	2.79	0.44	7.34	3.43	2.91	39.20	2.43	0.42	8.48
Induced PreEc + 200 mg/kg Ext-JC	2.67	2.29	30.14	1.67	0.34	7.84	1.18	0.97	6.90	0.18	0.03	8.06
Induced PreEc + 50 mg/kg Ext-AC	2.71	2.21	21.28	1.71	0.40	6.24	3.07	2.43	33.50	2.07	0.44	8.86
Induced PreEc + 100 mg/kg Ext-AC	1.32	1.07	11.27	0.32	0.07	8.26	2.23	1.89	19.00	1.23	0.29	6.06
Induced PreEc + 200 mg/kg Ext-AC	1.24	1.05	8.34	0.24	0.04	7.64	4.01	3.35	43.00	3.01	0.52	9.44
Induced PreEc + 50 mg/kg Ext-SA	3.10	2.70	25.89	2.10	0.39	6.02	3.71	2.95	41.50	2.71	0.40	7.74
Induced PreEc + 100 mg/kg Ext-SA	4.05	3.35	29.63	3.05	0.54	6.75	3.42	2.72	37.80	2.42	0.49	9.54
Induced PreEc + 200 mg/kg Ext-SA	2.97	2.50	36.89	1.97	0.37	8.80	1.24	1.02	7.80	0.24	0.08	7.88
LSD(0.05)	1.32	1.51	12.31	1.07	0.23	5.42	3.03	1.22	19.03	1.96	0.16	2.03
p-value	0.586	0.449	0.093	0.409	0.832	0.334	0.239	0.139	0.181	0.311	0.110	0.213

Key: *CRI,I=Castelli risk ratio (CRI, 1); AC= Antherogenic coefficient CRI,II= Castelli risk ratio (CRI, 11); AIP=Antherogenic index of plasma;; nonHDL= nonHDL cholesterol; VLDL=very low density lipoprotein*

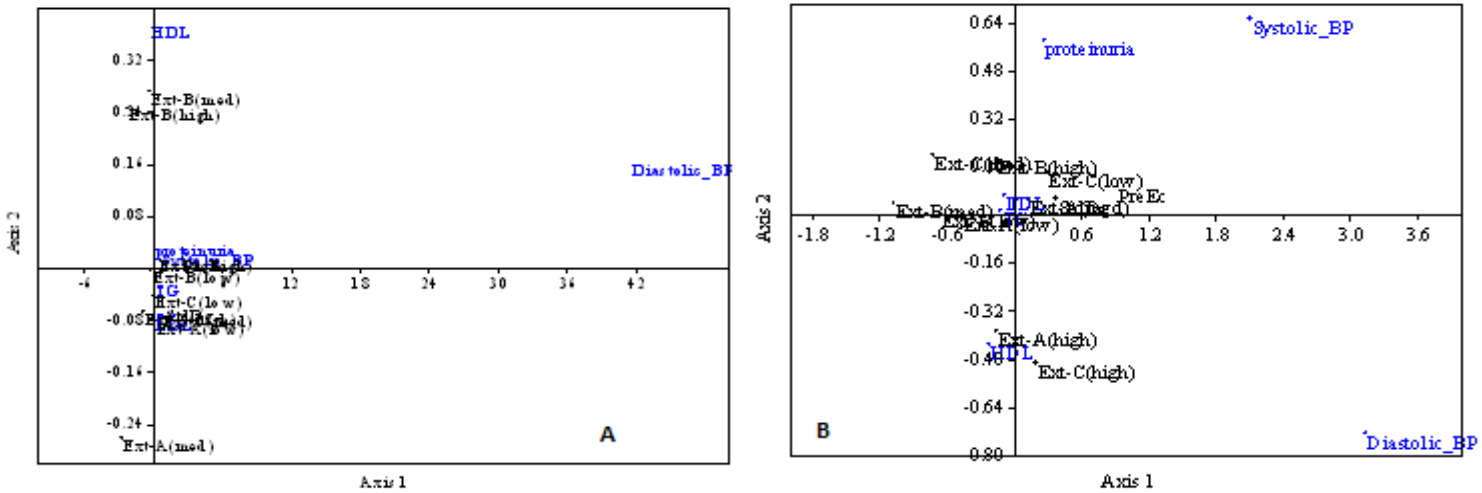

Fig 2: Canonical correspondence ballot showing association between measured parameters and the plant extracts during (a) 3rd trimester and (b) postpartum. Ext-A, Metholic leaf extract of *Jatropha cacus;* Ext-B, Metholic leaf extract of *Alchonnea cordifolia;* Ext-C, Metholic leaf extract of *Secamone afzelii.* Standard drug was methyl DOPA (Aldomet®)

However, TG, HDL negatively influenced other Although systolic bp as well as proteinuria were negatively influenced during 3rd trimester, implying the impact of treatments on improving the outcome of preeclampsia (Figure 2a), blood pressure and proteinuria were not influenced by the intervention of plant extracts during postpartum (Figure 2b).

However, the outcome of this study is in contrast to Tanir *et al*. (2005), who found a rise in blood pressure in preeclampsia rats after giving them plant extract. Many studies have been conducted to investigate the relationship between preeclampsia complications and the level of proteinuria. Several studies have found that moderate or severe proteinuria increases the likelihood of poor maternal and fetal outcomes (Ferrazzani *et al*., 1990; Chan *et al*., 2005; Thangaratinam *et al*., 2009). Other research has found that the severity of proteinuria in women with preeclampsia is a poor predictor of either maternal or fetal problems (Airoldi *et al*., 2000; Thangaratinam *et al*., 2009). Therefore, the management of proteuria with herbal remedies is imperative. There was significant reduction in proteinuria upon administration of various plant extracts. In a study by Jaiswal et al. (2009) to assess the effect of *Moringa oleifera* leaves aqueous extract therapy on urine protein, significant reduction was found in urine protein levels from +2 to trace. Methanolic extracts of *Jatropha cacus*, *Alchonnea cordifolia*, and *Secamone afzelii* were found to be a promising source of preeclampsia management in this study. The normotensive pregnant Wistar rats was the control group, whereas, preclampsia pregnant Wistar rats were test group. The study showed positive relationship between dyslipidemia and preeclampsia. This is in keeping with other studies (Gractacose *et al*., 2003; Winkler *et al*., 2003; Enquobahrie *et al*., 2004; Toescu *et al*., 2004, Ray *et al*., 2006). In normal pregnancy, studies have shown that TC, TG, HDL, and LDL levels are significantly increased. However, in preeclampsia, instead of an increase in HDL, which is cardioprotective, there is a decrease in HDL (see Table 4), which makes the preeclamptic subject more susceptible to atherogenicity and the risk of cardiovascular heart disease. In a study by Maruthappan and Shree (2010), aqueous extract of *Phyllanthus reticulates* (250 mg and 500 mg/kg) caused a significant reduction triglyceride, VLDL-cholesterol, total cholesterol (TC), and LDL-cholesterol levels, while increasing HDL-cholesterol in atherogenic diet-induced hypercholesterolemic rats (45 days). Ighodaro and Omole (2012) also reported reduction in lipid profiles using *Piliostigma thonningii* leaf extract in wistar rats. Similar to the present study, the reduction in the aforesaid parameters were reported. The lipid ratios, which include the artherogenic coefficient (AC), the artherogenic index of plasma (AIP), and Castelli risk ratios (CRI), were found to be significantly high (see Table 5) in this study. The higher the lipid ratio, the greater the risk of atherosclerotic heart disease; however, when the plant extracts were administered, the lipid ratios significantly decreased.

Conclusion: Methanolic leaf extracts of *Jatropha cacus, Alchonnea cordifolia,* and *Secamone afzelii* were found to have a significant impact on preeclampsia treatment by lowering blood pressure, proteinuria, lipid profile parameters, and lipid ratios, in line with the findings of this study.

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