ANTI-OXIDANTS LEVELS IN FEMALE RATS ADMINISTERED PRO-FERTITILITY ETHANOLIC LEAVE EXTRACT OF BYRSOCARPUS COCCINEUS

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Abstract

OBJECTIVE: The etiology of female infertility and recurrent pregnancy loss, in some women remains unclear and a scientific challenge. This study was carried out to investigate the qualitative phytochemical analysis, in-vitro and in-vivo antioxidant property of the ethanolic extract of *Byrsocarpus coccineus* leaves. This was based on the traditional claims, that it is used for the treatment of idiopathic infertility case in females with high body temperature which most times does not favors successful pregnancy.

DESIGN: Qualitative phytochemical screening was performed using standard procedures as described by Dipali and Vilas, (2013). In-vitro antioxidant assay of DPPH- 2,2 -Diphenyl-1-picrylhydrazine hydrate radical scavenging activity, Hydrogen Peroxide Scavenging Activity (HPSA) and Total Antioxidant Capacity (TAC) were determined. In-vivo antioxidant activity investigated was catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation. Ethanolic extract of *Byrsocarpus coccineus* were administered orally for 14 days to the Wister rats in the groups, including female rats induced with temporary infertility with Micronor (MIC) or N-acetylcysteine (NAC) for 7days before the administration of the extract. Results obtained showed that the ethanolic extract of *B. coccineus leaves* possesses effective antioxidant properties both in-vitro and in-vivo.

RESULT / OUTCOME: The phytochemical analysis of the extracts showed the presence of alkaloids, terpenoids, saponins, tannins, flavonoids, resins, phenols and reducing sugars. The extract showed 78.28% inhibition of DPPH, 71.57% of HPSA and 97.95% TAC at 250µg/ml concentrations. Also, the extract significantly causes reduction of the activities of CAT *(0.207 ± 0.060 U/ml),* SOD *(3.83 ± 0.268 U/ml)* and lipid peroxidation *(1.11*

± *0.027 nmol/ml)* compared with control group. The results clearly demonstrated that *Byrsocarpus coccineus* possess good antioxidant activity as evidenced by the free radical scavenging property in augmenting antioxidant defense mechanisms.

Keywords: *Byrsocarpus coccineus,* Antioxidants, Reactive Oxygen Species (ROS), phytochemical screening, Micronor (MIC), N-acetylcystein (NAC)

1.1 INTRODUCTION

In an healthy biological system, free radical species are maintained in a balance with the body's scavenging ability (antioxidants). When this balance is impaired in favour of the abundance of these species, it results in oxidative stress (Nordberg and Amer, 2001). ROS have been implicated in over hundreds of diseases state including pathological conditions affecting re-productivity and causes infertility cases in human. In treatment of these diseases, antioxidants therapy have gained immense importance (Nordberg and Amer, 2001; Ray and Husain, 2002). Antioxidants have been reported to prevents oxidative damage by free radicals and hence prevent the occurrence of diseases, cancer and ageing (Halliwell and Gutteridge, 1999; Daniel et al 1998). There is an increasing interest to examine the role of oxidative stress in female reproduction because it may be a major link in the "idiopathic infertility" puzzle in married couples as well as in some reproductive organs diseases such as endometriosis (Argawal et al, 2004).

Current researches are now directed towards finding naturally occurring antioxidants of plant origin, as plant products which are used as a source of medicine, just as from the earliest time of human existence. The medicinal properties of plants have been investigated due to their potent antioxidant activities, possible side effects and economic viability (Lee, et al, 2004). Poly-phenolic compounds such as flavonoids are widely distributed in plants which have been reported to exert multiple biological effect, including anti-tumour, anti-inflammatory abilities etc (Irshad and chaudhari 2002; Haug et al, 2005). They were also suggested to be a potential iron chelator. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties (Lee, et al, 2004). Byrsocarpus coccineus is a shrub of the connaracea family and it is a wide plant mostly found in west and central Africa in countries like Nigeria, Ghana, Togo, Ivory Coast, Cameroon, Central African Republic and Congo. The plant usually grows as a scandent shrub of savanna thickets and secondary jungle with delicate pink-tingled foliage and sweet-scented flowers (Burkill 1995). The plant is usually used for beautification, due to its pinked tingled foliage and sweet-scented flowers; however, it is used in traditional African medicines for the treatment of diverse ailments

(Newinger, 1996). The decoction or infusion of the leaf of the plants has been used for skin and mouth disorders, German measles, jaundice, gonorrhea, urinary problems, impotence, anaemia, primary and secondary sterility in both male and females, blemorrhagia, tachycardia, and as an abortifacient (Neuwinger, 1996). The plant has also been used for swelling and tumors, hemmorhage and as an emetic (Burkil 1985; Adjanohoun et al, 1986; Newinger 1996).

However, traditional or local claims have proven that aqueous ethanolic extraction of the plant leaves can be used as remedy for infertility problems in females. While in female, the plant extract is taken to reduce oxidative stress and excessive heat (or high blood temperature) which could affect fertilization processes and even improper implantation of the embryo or foetus in the womb leading to abortion (Jauniaux et al 2003, Jauniaux et al, 2004).

In this study, we hypothesized that the pro-fertility effect of Byrsocarpus coccineus may be due to the combined effects of various phytochemicals present in it which may act as free radical scavengers, and help in modulating various in-vivo antioxidants activities that are involved in normal redox state of cells especially during pregnancy in female. To test this hypothesis, experiment was conducted to determine the in-vitro antioxidant activities of the ethanolic leaves extract and the corresponding modulatory effects on in-vivo antioxidant activities in both fertile and infertile female rat model.

1.2. RESEARCH METHODS:

1.2.1 Drugs and Chemicals:

N-acetylcysteine (NAC) and Micronor (MIC) were purchased from Alpha pharmacy in Ikeja. DPPH (2,2-Diphenyl-1-picrylhydrazine hydrate), Folin C reagent, hydrogen peroxide and thiobabituaric acid (TBA) reagent were purchased from Sigma-aldrich USA. All other chemicals used in the study were of analytical grades and procured from local suppliers.

1.2.2 Collection of Plant Materials:

The leaves of Byrsocarpus coccineus were collected from Igbesa town in Ado-odo Ota Local Government Area in Ogun state. The plant parts were identified and authenticated at the University of Lagos Herbarium, where a voucher specimen (Ref No: LUH 5655) was deposited for future references.

1.2.3 Preparation of Plant Extract:

The plant leaves were washed in water and air dried at room temperature for 4weeks and thereafter reduced to powdered form using

electric blender. The blended powdered form was stored in an air-tight container. A total of 150g powdered leaves were socked with 1.5Litres of 50% ethanol for 72hours with occasional shaking. The resultant extract was filtered using white muslin cloth and the filtrate was thereafter concentrated (freeze dried) using lyophilizer (model: FD 5518). The yield obtained was 15.6g (10.4% w/w) and was kept frozen in a refrigerator until the time of use.

1.2.4 Phytochemical Screening:

The ethanolic extract of B. coccineus was subjected to qualitative phytochemical analysis using standard procedures (Dipali and Vilas, 2013). This was to identify the major secondary metabolites like Alkaloids, Terpenes, Flavonoids, Saponins, Steroids, Phenolic Compounds, Tannins and Amino Acids.

1.2.5 Toxicity Test

Acute toxicity test was performed according to Guessom, *et al.*, (2013). Nine (9) rats were randomly selected (three per group) and starved overnight. Extract doses of 150, 250 and 400 mg/kilogram body weight were administered to each animal group respectively. The animals were kept under the same natural condition and observed for toxicity signs and mortality for 72 hours. All dosage administered were found to be non-lethal as earlier determine as the safe doses reported by Guessom, *et al.,* (2013).

1.2.6 Experimental Protocols:

A total of 42 female (100g) albino rats were used in the study obtained from University of Ibadan and were acclimatized for 4weeks until their average weight reached 120g. The rats were randomly divided into seven groups of 6 rats each (n=6). N-acetylcysteine (NAC) or Micronor (MIC) was used to induce infertile state. Oral administration was done using oral cannula and all rats were fed on standard diet and had water *ad libitum.* while the treatments for each group were as follows:

Group 1 (CONTROL): rats were orally fed with 1ml distilled water only, throughout the experiment period.

Group 2 (MIC-only): rats were administered 1ml of micronor at a dose of 20µg/kilogram body weight (kg.bw) orally for 7days only. To test for the effectiveness of micronor in inducing infertility, one rat was separated from the group and mated with a normal male rat in a separate cage and observed for conception throughout the period of experiment.

Group 3 (NAC-only): rats were administered NAC at a dose of 1000mg /kg.bw (0.74ml; 5%w/v) orally for 7 days only. To test for the effectiveness of NAC in inducing infertility, one rat was separated from the

group and crossed with a normal male rat in a separate cage and observed for conception throughout the period of experiment.

Group 4 (EXTRACT-only): rats were administered with 1ml of extract only for 14days at a dose of 100mg/kg.bw.

Group 5 (MIC + EXTRACT-L): rats were administered micronor at a dose of 20µg/kg.bw (1ml) for 7days orally and thereafter administered with plant extract at a dose of 100mg/kg.bw (1ml) for 14days orally.

Group 6 (MIC + EXTRACT-H): rats received micronor at a dose of 20µg/kg.bw (1ml) for 7days orally and thereafter administered with plant extract at a dose of 200mg/kg.bw (2ml) for 14days orally.

Group 7 (NAC + EXTRACT): rats received NAC at a dose of 1000mg/kg.bw (0.74ml) for 7days orally and thereafter administered with plant extract at a dose of 100mg/kg.bw (1ml) for 14days orally.

Animal Sacrifice and Sample collection:

24 hours after the last administration, the animals were sacrificed by jugular puncture and blood samples collected in both plain and heparinized well labelled bottles. These were later centrifuged for 15mins at 1000rpm. The supernatant obtained was stored frozen until used for biochemical analysis.

1.2.7 Assay of ROS Scavengers level (in-vitro) Using Various Methods:

DPPH radical scavenging activity:

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability by using stable free radical, 2,2 -Diphenyl-1-picrylhydrazine hydrate (DPPH) in methanol as described by Prabhakar et al.,(2007).

Hydrogen peroxide scavenging activity (HPSA):

Hydrogen peroxide scavenging activity was determined following the procedure described by Subhashini et al., (2001).

Total Antioxidant Capacity (TAC):

The total antioxidant capacity of the extract was determined using phosphomolybdate method as described by Prieto, et al., (1999).

Assessment of In-vivo Enzymatic Antioxidant Activity

Catalase (CAT) Assay:

Catalase activity was determined by using hydrogen peroxide (H_2O_2) as a substrate (Chance and Maehly, 1955). Briefly, 0.1ml of the supernatant of tissue homogenate was mixed with 2.5ml of 50mM phosphate buffer (pH 5.0), 0.4ml of 5.9mM H_2O_2 and change in absorbance was recorded at

240nm after one minute of reaction. One unit of CAT activity was defined as an absorbance change of 0.01 as unit/ml.

Superoxide Dismutase (SOD) Assay:

Superoxide dismutase activity was measured according to the method of Winterbourn *et al*. (1975) as described by Rukmini *et al*. (2004). The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). Briefly, the reaction mixture contained 2.7 mL of 0.067 M phosphate buffer, pH 7.8, 0.05 mL of 0.12 mM riboflavin, 0.1 mL of 1.5 mM NBT, 0.05 mL of 0.01 M methionine and 0.1 mL of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10 min. Control without the enzyme source was included. The absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. It was expressed as U/ml

Estimation of Lipid Peroxidation (TBARS):

The assay for lipid peroxidation was carried out following the modified method of Iqbal et al. (1996). One milliliter of 20percent TCA aqueous solution and 1.0 ml of 0.67percent TBA aqueous solution was added to 0.6 ml of phosphate buffer (0.1 M; pH 7.4) and 0.4 ml of homogenate sample. The reaction mixture was heated in a boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500 × g for 10 min. The amount of TBARS (being malondialdehyde, MDA) formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. The results were expressed as nM TBARS/ml at 37 ºC using molar extinction coefficient of 1.56 ×105 M-1cm-1.

Statistical Analysis:

For in-vitro antioxidant assay, all tests were carried out in triplicates (n=3). Mean values were calculated using EXCEL from MS office package and recorded as mean ± SD. The amount of extract needed to inhibit free radicals concentration by 50% (IC_{50}) was graphically determined by linear regression algorithm.

For in-vivo antioxidant assays all data were expressed as Mean ± SEM (n=3). The level of significance among the groups was tested using Analysis of Variance (ANOVA) followed by Turkey,s multiple comparison test from prism 5 graph pad.

1.3 RESULTS

1.3.1 Results of Phytochemical screening of ethanolic leave extract of *B. coccineus:*

The phytochemical analysis of ethanolic leave extract of B. coccineus showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, resins, and reducing sugars. However, the presence of amino acid was not detected.

1.3.2 Results of the In-vitro Reactive Oxygen Species Scavengers Level by Various Methods:

DPPH radical scavenging activity:

Table 1 shows the significant scavenging ability of extracts of the ethanolic extract of B. coccineus leaves in a dose-dependent manner. The extract shows maximum scavenging activity at 250µg/ml (78.24%) which is comparable to that of ascorbic acid (81.71%) and IC_{50} value was found to be 0.010mg/ml (table 2).

Hydrogen Peroxide Scavenging Activity (HPSA):

The ethanolic extract of B. coccineus leaves is also capable of mopping up H_2O_2 in a dose-dependent manner. The scavenging ability of the extract is shown in table 3. The scavenging ability of the extract is moderate and slightly closer to that of α- tocopherol at doses of 200 and 250µg/ml with IC_{50} value of 0.138mg/ml (table 2).

Total Antioxidant capacity (TAC):

The reduction of ammonium molybdate by ethanolic extract of B. coccineus leaves was studied. Ascorbic acid was taken as 100%. It has been found that the extract showed a significant level of total antioxidant capacity as assessed by reduction of ammonium molybdate when compared to that of ascorbic acid at doses of 10 - 200µg/ml (table 4).

1.3.3 In-vivo Enzymatic Antioxidant Activities and Inhibition of Lipid Peroxidation:

Table 5 show the effects of the plant extract on the activities of catalase (CAT), superoxide dismutase (SOD), and inhibition of lipid peroxidation (TBARS expressed as MDA) respectively.

Catalase (CAT) Activity:

Administration of the extract alone (EXTRACT-only) at a lower dose of 100mg/kgb.w reduces CAT activity significantly ($P < 0.01$), when compared to control and MIC- only animals. Similarly, the animals that received MIC followed by treatment with the extract at lower (100mg/kg.bw, MIC+EXTRACT-L) and higher (200mg/kg.bw, MIC+EXTRACT-H) doses

respectively shows significant antioxidant activity ($P < 0.01$) by reducing the activity of catalase drastically when compared to the MIC-only and control groups. Also the NAC + EXTRACT group shows statistical reduction ($P < 0.05$) in CAT activity compared to the NAC-only and control animals (Table 5).

Super Oxide Dismutase (SOD) Activity:

The activities of SOD for EXTRACT-only and MIC+EXTRACT-L groups were both similar and statistically show significant reduction ($p < 0.01$) in SOD activity when compared to the control and MIC-only groups. But MIC+EXTRACT-H animals provoke a statistical decrease ($P < 0.01$) in SOD activity than the MIC+EXTRACT-L group when compared to the control and MIC-only animals. In a likewise manner, the NAC+EXTRACT group showed statistical reduction ($P < 0.05$) in SOD activity compared to the control and NAC-only groups (Table 5).

Inhibition of Lipid peroxidation:

Administration of the extract only (EXTRACT-only group), significantly inhibited lipid peroxidation as there is statistical reduction ($p < 0.01$) in the level of MDA when compared to the control group. Also, both MIC+EXTRACT-L and MIC+EXTRACT-H groups had similar activity which showed significant reduction ($p < 0.01$) in the level of MDA compared to the control group that received distilled water only. Nevertheless, NAC+EXTRACT animals markedly shows significant reduction ($p < 0.05$) in MDA level compared to control and NAC-only groups (Table 5).

TABLE 1: DPPH Radical Scavenging Activity of Ethanolic Extract of *Byrsocarpus Coccineus* Leaves

Concentration	Absorbance	% Scavenging
10µg/ml	0.386 ± 0.014	47.64
20µg/ml	0.250 ± 0.013	63.08
50µg/ml	0.201 ± 0.016	70.30
100 µg/ml	0.187 ± 0.007	72.32
200µg/ml	0.166 ± 0.013	75.57
250µg/ml	0.149 ± 0.010	78.24
Control	0.678 ± 0.002	0.00

Data are expressed as mean ± SD (n = 3).

TABLE 2: % Inhibition of Ethanolic Extract of *Byrsocarpus coccineus* leaves Data are expressed as mean ± SD (n = 3).

In-vitro assay methods	% Inhibition at different concentrations						IC $_{50}$ (mg/ml)
	10µg/ml	20µg/ml	50µg/ml	100µg/ml	200µg/m	250µg/ml	
DPPH	47.64	63.08	70.30	72.32	75.57	78.02	0.010
Ascorbic Acid	45.72	67.40	74.04	77. 58	79.50	81.71	
HPSA	25.00	29.90	32.35	41.67	62.25	71.57	0.138
α-Tocopherol	48.53	54.41	63.24	70.59	77.94	85.29	

TABLE 3: Hydrgen Peroxide Scavenging Activity (HSPA) of Ethanolic Extract of *Byrsocarpus Coccineus* Leaves

Concentration	Absorbance	% Scavenging
10µg/ml	0.051 ± 0.007	25.00
20µg/ml	0.048 ± 0.005	29.00
50µg/ml	0.046 ± 0.023	32.35
100 µg/ml	0.039 ± 0.008	41.67
200µg/ml	0.026 ± 0.008	62.25
250µg/ml	0.019 ± 0.004	71.57
Control	0.068 ± 0.010	0.00

Data are expressed as mean ± SD (n = 3)

TABLE 4: Total Antioxidant Capacity (TAC) of Ethanolic Extract of *Byrsocarpus Coccineus* Leaves

Concentration	Absorbance	% Scavenging
10µg/ml	0.522 ± 0.010	59.32
20µg/ml	0.733 ± 0.003	83.30
50µg/ml	0.825 ± 0.003	93.79
100 µg/ml	0.848 ± 0.016	96.36
200µg/ml	0.862 ± 0.072	97.95
Ascorbic acid	0.880 ± 0.006	100

Data are expressed as mean ± SD (n = 3).

TABLE 5: In-vivo Effects of Ethanolic Extract of *Byrsocarpus coccineus* on Enzymatic Antioxidant Activity and Inhibition of Lipid Peroxidation.

Treatment Groups	CAT (U/ml)	SOD (U/ml)	MDA (nmol/ml)
Control (Distilled H_2O)	3.531 ± 0.019 [ab]	17.80 ± 1.770	12.40 ± 2.570
MIC-only	7.348 ± 0.081*	13.50 ± 1.270	20.90 ± 3.210*
NAC-only	0.784 ± 0.011*	20.10 ± 3.690	3.33 ± 0.815*
EXTRACT-only	0.207 ± 0.060*[a]	3.83 ± 0.268*[a]	1.11 ± 0.027*[a]
MIC+EXTRACT-L	0.480 ± 0.120*[a]	4.43 ± 0.432*[a]	2.74 ± 0.018*[a]
MIC+EXTRACT-H	0.205 ± 0.047*[a]	0.72 ± 0.018*[a]	2.24 ± 0.027*[a]
NAC+EXTRACT	0.333 ± 0.017*[b]	2.89 ± 0.831*[b]	1.15 ± 0.041*

Values are expressed as Mean ± SEM (n = 6). **Values on the same column represent significant difference (p< 0.01) when compared to the control group (distilled H_2O). [a]Values on the same column represent significant difference (p<0.01) from the group that was*

treated with MIC-only. [b]*Values on the same column represent significant difference (p<0.05) from the group that was treated with NAC-only.*

1.4 Discussion

In recent years, the studies on "oxidative stress" and its adverse effects on human health have become a subject of considerable interest. It is well documented fact that exposure of organism to exogenous and endogenous factors generates a wide range of reactive oxygen species resulting in homeostatic imbalance (Halliwell et al, 1992).

The free radical scavenging activity of ethanolic extract of *Byrsocarpus coccineus* was evaluated by various in vitro assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of the leaves extract. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl- 1-picrylhydrazyl (DPPH). As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm (Kumar et al, 2007). Table 1 and 2 presented a significant decrease in the concentration of DPPH radical due to the scavenging ability of extracts of *B. coccineus* leaves, when Ascorbic acid was used as standard. The scavenging effect of ethanolic extract of B. coccineus on the DPPH radical was high. These results indicated that extract has a noticeable effect on scavenging the free radicals.

Removal of excess H_2O_2 had earlier been shown to be very important for protection of physiologic cellular environment (Subhashini et al, 2011). H_2O_2 itself is not very reactive but highly important because of its ability of penetrate biological membranes; it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Table 3 showed the H_2O_2 scavenging activity of the ethanolic extract of *B. coccineus* and compared with α-tocopherol. The percentage of H_2O_2 scavenging activity of *B.coccineus* and α-tocopherol was found respectively high. The H_2O_2 scavenging ability of the ethanolic extract of *B. coccineus* leaves may be attributed to the presence of phenolics, which was earlier indicated could donate electrons, thereby neutralizing it into water (Haraguchi et al, 2002).

It is also shown from total antioxidant capacity of ethanolic extract of *B. coccineus* assessed by reduction of molybdate which showed high potential to scavenge free radicals when compared to standard ascorbic acid (table 4). Preliminary phytochemical analysis of the plant revealed the presence of phenolic compounds, terpenoids, tannins, alkaloids, flavonoids and reducing sugars. All these Phenolic compounds are a class of antioxidant agents which may act individually or synergistically as free radical terminators. Hence, it was therefore expected that the ethanolic extract of *B. coccineus* has potent antioxidant and radical scavenging ability. This activity

is believed to be mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxide. Therefore, present study revealed the antioxidant properties of ethanolic extract of *B. coccineus* leaves which agree with the research work carried out by Oke and Hamburger,(2002).

ROS scavenging properties of *B. coccineus* leaves extract in-vivo was evaluated in such a way to see the ameliorating antioxidant effects of the extract on induced infertile female rats as a model.

Assessment of CAT, SOD, lipid peroxidation and other antioxidant enzyme activities in biological tissue have been used as markers for tissue injury and oxidative stress (Chularojmontri et al., 2005; Prahalathan et al., 2005; Atessahin et al., 2006; Priestman, 2008).

Administration of the extract alone (EXTRACT-only) reduces CAT activity significantly ($P < 0.01$), when compared to control and MIC- only animals. Similarly, the animals that received MIC followed by treatment with the extract at lower (100mg/kg.bw, MIC+EXTRACT-L) and higher (200mg/kg.bw, MIC+EXTRACT-H) doses respectively shows significant antioxidant activity ($P < 0.01$) by reducing the activity of catalase drastically when compared to the MIC-only and control groups. Also the NAC + EXTRACT group shows statistical reduction ($P < 0.05$) in CAT activity compared to the NAC-only and control animals (table 5).

The activities of SOD for EXTRACT-only and MIC+EXTRACT-L groups were both similar and statistically show significant reduction ($p < 0.01$) in SOD activity when compared to the control and MIC-only groups. The MIC+EXTRACT-H animals provoke a statistical decrease ($P < 0.01$) in SOD activity than the MIC+EXTRACT-L group when compared to the control and MIC-only animals. In a likewise manner, the NAC+EXTRACT group showed statistical reduction ($P < 0.05$) in SOD activity compared to the control and NAC-only groups (table 5).

Administration of the extract only (EXTRACT-only group), significantly inhibited lipid peroxidation as there is statistical reduction ($p < 0.01$) in the level of MDA when compared to the control group. Also, both MIC+EXTRACT-L and MIC+EXTRACT-H groups had similar activity which showed significant reduction ($p < 0.01$) in the level of MDA compared to the control group that received distilled water only. Nevertheless, NAC+EXTRACT animals markedly shows significant reduction ($p < 0.05$) in MDA level compared to control and NAC-only groups (table 5). The results obtained from the extract markedly shows the ability to ameliorate the effect of MIC as there was observable and significant reduction in the activities of CAT, SOD and reduced level of lipid

peroxidation in the groups that received the extract following induction of infertility (Table 5).

The active component (Northisterone) is a synthetic progestone (hormone) and is known to alter the cervical mucus and lining of the womb. Similarly, with respect to excessive ROS present within the reproductive organs, the normal physiologic environment and integrity of the uterus (womb) may be compromised. As a result, it also makes it harder for any fertilized egg to become attached to the wall of the womb and therefore less likely for pregnancy to occur. Therefore, the results suggested that MIC proves to be an effective oral contraceptive and most likely may have the side effect of inducing cellular oxidative stress within the reproductive system. This is confirmed by the mating test carried out between MIC-only and NAC-only groups of rats. NAC mated rat did not possess foetus in its uterus at the time of sacrifice, while MIC crossed rat possessed.

According to Akindele et al., (2010), the most important activity of this plant species is the antioxidant properties. The results obtained here, agrees with the study which reveals that the species Byrsocarpus coccineus, showed a significant antioxidant activity by reducing the activity of catalase, SOD, peroxidase and GSH in vivo. These activities may be linked to the presence of natural antioxidants (phytochemicals) in B. coccineus which may act individually or synergertically in scavenging free radicals. However, further studies should be undertaken to isolate and identify the active compounds.

1.5 Conclusion

Based on the various in-vitro and in-vivo assays, it can be concluded that the ethanolic extract of *Byrsocarpus coccineus* possess good antioxidant activity as evidenced by the free radical scavenging property in augmenting antioxidant defense mechanisms. Overall, the plant is a source of natural antioxidant that may be important in the therapeutic role of free radical-mediated disease prevention and health preservation.

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