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*Moringa oleifera* Lamarck (drumstick) Leaf Extract Modulates the Evidences of Hydroxyurea -Induced Testicular Derangement

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**Summary:** Hydroxyurea (HDU) is an antineoplastic agent that is commonly used in the treatment of Sickle cell disease (SCD). However, the therapeutic value of HDU is limited by its organotoxicity including testicular toxicity. It has been shown that free radicals are involved in HDU-induced toxicity. The application of natural phenolic compounds in the prevention of many pathologic diseases has been reported. Herein, the ability of polyphenolic-rich *Moringa oleifera* Leaf Extract (MOLE) to protect rat testis against HDU-induced histomorphometric, spermatogenic, and oxidative status impairments were investigated. Three experimental groups of Sprague-Dawley rats were used; MOLE-alone group that received orally MOLE 50 mg/kg body weight (b.w) daily for 90 consecutive days. HDU-alone group that had 25 mg HDU/kg b.w/day/orally for 90 consecutive days. MOLE plus HDU-group that were treated orally for 90 consecutive days with both 25 mg HDU/kg b.w/day and MOLE 50 mg/kg b.w/day. There was also a corresponding control group which had distilled water 2.5 ml/kg b.w/day/orally for 90 consecutive days. Our results demonstrated that cotreatment with MOLE protected the testis against the morphologic, spermatogenic and oxidative status changes induced by HDU.

**Industrial relevance:** Hydroxyurea is commonly used in management of sickle cell disease. The use of this drug is however limited by its organotoxicity including testicular damage. The present study therefore explores the ability of extract of *Moringa oleifera*. Leaves to prevent testicular damage during HDU therapy with a view of providing base line information on its possible use as an adjunct in future treatment regimes.

**Key words:** Hydroxyurea; *Moringa oleifera*; testis; Sprague-Dawley rats

**Introduction**

Sickle cell disease (SCD) is characterized by a painful vaso-occlusive crisis resulting from the blockage of capillaries by the interaction of sickle erythrocytes, leukocytes, platelets and plasma proteins with vascular endothelium (Smiley *et al.*, 2008). Currently there is no cure for SCD except for stem cell transplantation which is currently largely unavailable in our environment. However, HDU, an antineoplastic agent, is commonly used in the treatment of SCD (King, 2003). HDU increases fetal hemoglobin which has a higher oxygen carrying capacity and does not undergo sickling under low oxygen tension, thus improving some aspects of quality of life in patients suffering from moderate to severe SCD (Charache *et al.*, 1995; Charache *et al.*, 1996).

Hydroxyurea is non-selective, and treatment with HDU is suspected to be associated with side effects including cytotoxicity (or toxicity to cells) and myelosuppression (or reduced production of red blood cells, white blood cells, and platelets), and hydroxyurea can damage DNA (“genotoxic”) (Perreault *et al.*, 2008, Friedrisch *et al.*, 2008).
Evenson and Jost; 1993, Fritz and Hess, 1996; Jorge et al., 2005 and Mojica et al., 2007 have all reported the toxic effects of HDU on the rat testis.

The biochemical mechanism by which HDU causes cytotoxicity is currently unclear. It has however, been postulated that HDU –induced organ toxicity could be due to its metabolites particularly Carbamoyl nitroso. Carbamoyl nitroso is easily oxidized to form nitroxy! and nitric oxide. Carbamoyl nitroso may be involved in electron transfer, reactive oxygen species formation, and oxidative stress (Cokic et al., 2003).

Moringa oleifera leaf extract (MOLE) is from Moringa oleifera Lamark tree which is commonly called ben oil or drumstick tree. Native only to the foothills of the Himalayas, it is now widely cultivated in Africa, Central and South America, Sri Lanka, India, Malaysia and the Philippines. In Nigeria it is mostly grown in the northern part and locally known as Zogeli among the among the Hausa speaking people. The Yoruba of South-West Nigeria call it ewe ile or igi lyaanu (because of its many medicinal uses). M. oleifera is a fast growing deciduous shrub or small tree up to 12 m tall and 30 cm in diameter with an umbrella-shaped open crown (Anjorin et al., 2010).

M. oleifera is considered one of the world’s most useful trees, as almost every part of the tree can be used for food, or has some other beneficial property. Furthermore extracts from all parts of the plant show pharmacological properties, recognized by popular use and corroborated by the scientific community (Oliveira et al., 1999). It is an exceptionally nutritious vegetable tree with a variety of potential uses (Ram, 1994). The leaves can be eaten fresh cooked or stored as dried powder for several months the pods, when young can be cooked; eaten like beans (National Research Council, 2006). Its oil and micronutrients have been reported to contain antitumour, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters (Hsu, 2006).

Scientific research confirms that the humble leaves of this plant are a powerhouse of nutritional value. Gram for gram, Moringa leaves contain: seven times the vitamin C in oranges, four times the Calcium in milk, four times the vitamin A in carrots, two times the protein in milk and three times the Potassium in bananas (Hsu et al., 2006).

In addition, Moringa contains specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids - lutein, alpha-carotene and beta-carotene, xanthins, and chlorophyll; other phytochemicals with known powerful antioxidant ability – kaempferol, quercetin, rutin and caffeoylquinic acids; powerful antioxidant vitamins - C, E, and A (Siddhuraju and Becker, 2003; Aslam et al., 2005). The antioxidant activities of bioflavonoids complement, extend, and sometimes synergize the antioxidant activities of vitamin C, vitamin E, and carotenoids, making them an important nutritional component in the body’s defenses against free radical damage (Ho, 1994).

The realization that HDU- induced testicular toxicity is principally mediated through the oxidative pathway and that MOLE possesses potent antioxidative effect by scavenging free radicals, has prompted us to evaluate in the present study the possible salutary role of MOLE on the histomorphometric, spermatogenic, and biochemical evidences of HDU-induced testicular impairment.

Materials and Methods

Chemicals: Hydroxyurea (® Hydrea, Bristol-Myers Squibb USA) was obtained from Juli Pharmacy, Ikeja, Lagos State, Nigeria in the month of September, 2010.

Plant materials and the aqueous extraction procedure: Samples of leaves were collected from a year old M. oleifera plants located at a plantation in One Man Village, at the outskirts of Abuja (9° 21’ N; 7° 18’ E), Nigeria in the month of September, 2010. They were transported to the laboratory of Anatomy department of Lagos State University College of medicine, Ikeja Lagos. They were authenticated by a staff in the herbarium of the Department of Botany, University of Lagos, Lagos, Lagos state, Nigeria, where a voucher specimen was deposited for reference with specimen no. DSN 32. The leaves were thoroughly washed in sterile water and the water was then drained from the leaves.

Animals: Sprague-Dawley rats were obtained from a breeding stock maintained in the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso and were authenticated by Dr V.A. Togun, Farm Director LAUTECH. The animals were housed in well ventilated wire-wooden cages in the Animal Facility of the Department of Anatomy, Lagos State University College of Medicine (LASUCOM), Ikeja, Lagos. An approval was sought and obtained from the Departmental Ethical Committee on Animal Use. The rats were maintained under standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e. L: D; 12h:12h photoperiod) at room temperature (25ºC - 26ºC) and humidity of 65 ± 5 %. They were allowed unrestricted access to water and rat chow (Feedwell Livestock Feeds Ltd, Ikorodu, Lagos, Nigeria). They were allowed to acclimatize for 28 days before the commencement of the experiments. The weights of the animals were estimated at procurement, during acclimatization, at commencement of the experiments and twice within a week throughout the duration of the experiment, using an electronic analytical and precision balance (BA210S, d= 0.0001 g) (Satorius GA, Goettingen, Germany).
Experimental procedures involving the animals and their care were conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 1985). Further the animal experimental models used conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (American Physiological Society, 2002).

**Acute oral toxicity study of Moringa oleifera leaves extract:** The acute oral toxicity study for *Moringa oleifera* leaves extract was conducted using the Organization for Economic Cooperation and Development (OECD) (2000) Guidance Document on Humane End points that should reduce the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure.

Briefly, 5 animals were weighed and individually identified. The first animal was given the test dose – *Moringa oleifera* leaves extract 2000 mg per kg body weight. The second and third animals were concurrently dosed and the fourth and fifth animals sequentially dosed.

The results were evaluated as follows (S = Survival, X = death). The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a total period of 14 days. All observations were systematically recorded with individual records maintained for each animal.

**Animal Grouping and Experimental Design:** Forty male adult (12 to 14 weeks old) Sprague Dawley rats weighing 190-215 g were used for this research work. The rats were randomly divided into four groups of ten rats each such that the average weight difference between and within groups did not exceed ± 20% of the average weight of the sample population. The first group animals were each given distilled water 2.5 ml/kg body weight/daily/orally for 90 days. These animals served as control. The MOLE- alone group received orally MOLE 50 mg/kg b.w/day/orally for 90 consecutive days. The HDU-alone group received 25 mg HDU/kg b.w/day/orally for 90 consecutive days. This dosage being clinically relevant in the treatment of SCD (Masood et al., 2007). Finally, the MOLE plus HDU-group of rats were each treated orally for 90 consecutive days with both 25 mg HDU/kg b.w/day and MOLE 50 mg/kg b.w. The appropriate quantity of crude aqueous extract and drugs were given orally through an orogastric cannula into the stomach via the esophagus (Prakash, 1981). The extract and drugs were administered once daily by 12 noon for six days (Monday to Saturday) within a week. All the animals were sacrificed 24 hours after the last dosing.

**Animal sacrifice and sample collection:** The rats were at the time of sacrifice first weighed and then anaesthetized by placing them in a closed jar containing cotton wool sucked with chloroform anaesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. Then the testes were excised and trimmed of all fat. The testes volumes were measured by water displacement method. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation.

One of the testes of each animal was fixed in 10% formalin-saline for histological examination. Serum and the remaining testes of each animal were stored at – 25°C for biochemical assays.

**Determination of morphometric parameters:** Histological slides were prepared from the formalin-saline fixed testes. However, prior to embedding, it was ensured that the sections were orientated perpendicular to their long axes, and designated as “vertical sections”.

For each testis, five vertical sections from the polar and the equatorial regions were sampled (Qin and Lung, 2002) and an unbiased numerical estimation of the morphometric parameters was determined using a systematic random scheme (Gundersen and Jenson, 1987).

**Determination of Sperm Characteristics:** The testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues. Epididymal sperm concentration: Spermatozoa in the right epididymis were counted by a modified method of Yokoi and Mayi, (2003). Briefly, the epididymis was minced with anatomic scissors in 5mL physiologic saline, placed in a rocker for 10 minutes, and allowed to incubate at room temperature for 2 minutes. After incubation, the supernatant fluid was diluted 1:100 with solution containing 5 g sodium bicarbonate and 1 mL formalin (35%). Total sperm number was determined by using the new improved Neuber’s counting chamber (haemocytometer). Approximately 10 μL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 minutes. This chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was the calculated multiplied by 5 and expressed as [X] x 10⁶ /ml, where [X] is the number of spermatozoa in a 16-celled square. Sperm progressive motility: This was evaluated by an earlier method by Sonmez.
et al. (2005). The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with Tris buffer solution. A slide was placed on light microscope with heater table, an aliquot of this solution was on the slide, and percentage motility was evaluated visually at a magnification of x 400. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were stored at 35°C. Sperm morphology: The sperm cells were evaluated with the aid of light microscope at x 400 magnification. Caudal sperm were taken from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities (Atessahin et al., 2006). Briefly, in wet preparations using phase-contrast optics, spermatozoa were categorized. In this study a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detached head and was expressed as a percentage of morphologically normal sperm.

**Assay of testicular enzymatic antioxidants**

**Assay of catalase (CAT) activity:** Catalase activity was measured according to the method of Aebi (1983). 0.1 ml of the testicular homogenate (supernatant) was pipetted into cuvette containing 1.9 ml of 50mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H₂O₂). The rate of decomposition of H₂O₂ was measured spectrophotometrically from changes in absorbance at 240nm. Activity of enzyme was expressed as units /mg protein.

**Assay of superoxide dismutase (SOD) activity:** 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 0.067M phosphate buffer, pH 7.8, 0.05 ml of 0.12mM riboflavin, 0.1 ml of 1.5mM NBT, 0.05 ml of 0.01M 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 minutes. Control without the enzyme source was included. The absorbance was measured at 560nm. 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/mg protein.

**Assay of glutathione peroxidase (GPx) activity:** Glutathione peroxidase activity was measured by the method described by Rotruck et al. (1973). The reaction mixture contained 2.0 ml of 0.4M Tris- HCl buffer, pH 7.0, 0.01 ml of 10mM sodium azide, 0.2 ml of enzyme. 0.2 ml of 10mM glutathione and 0.5 ml of 0.2mM H₂O₂. The contents were incubated at 37°C for 10 minutes followed by the termination of the reaction by the addition of 0.4 ml of 10% (v/v) TCA, centrifuged at 5000 rpm for 5 minutes. The absorbance of the product was read at 430nm and expressed as nmol/mg protein.

**Assay of testicular non-enzymatic antioxidants**

**Assay of testicular reduced glutathione (GSH) concentration:** GSH was determined by the method of Ellman (1959). 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5’-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). 0.4 ml of distilled water was added. The mixture was thoroughly mixed and the absorbance was read at 412 nm, expressed as nmol/mg protein.

**Estimation of lipid peroxidation (Malondialdehyde):** Lipid peroxidation in the testicular tissue was estimated colorimetrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust (1978). A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x10⁵ M⁻¹ cm⁻¹ and expressed as nmol/mg protein.

**Statistical analysis:** All data were expressed as mean ± SD of number of experiments (n = 10). The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran 1980. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of p < 0.05 was considered to indicate a significant difference between groups (Duncan, 1957).

**Results**

**Acute oral toxicity studies:** There were no deaths of rats dosed 3000mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD50 was calculated to be greater than 3000mg/kg body weight /orally.
Table 1: Results of Acute Toxicity Test for *M. oleifera* leaves Extract (Up and Down Procedure) in Rats

<table>
<thead>
<tr>
<th>TEST Serial no</th>
<th>Animal Identity</th>
<th>Dose of MOLE mg/kg</th>
<th>Short term results (48 hours)</th>
<th>Long term results (14 days)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>REP</td>
<td>2000</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>LEP</td>
<td>2000</td>
<td>S</td>
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<td>3</td>
<td>TC</td>
<td>2000</td>
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<tr>
<td>4</td>
<td>RLT</td>
<td>2000</td>
<td>S</td>
<td>S</td>
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<tr>
<td>5</td>
<td>I</td>
<td>2000</td>
<td>S</td>
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S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RDC = Right leg tagged; I = Intact rat

**Body Weight Changes:** Figure 1 shows that rats in control and MOLE groups significantly increased in weight when compared to their initial mean live weight. Both HDU-administered groups lost weights when compared with their initial weights. However, the weight loss by the HDU-alone administered rats was significantly higher than the losses by the group received both HDU and MOLE.

![Figure 1: Initial and Final Body Weights of Rats](image1)

**Weights and Volume of testes mean:** The testicular weights, testis weight/body weight ratio and volumes of the HDU-alone rats were the least, being significantly lower compared to the mean testicular weights, testis weight/body weight ratio and volumes of the HDU rats that in addition had MOLE treatment, which in turn were also lower but not significantly lower than those of the control and MOLE-alone rats (Figure 2).

![Figure 2: Testis weight, Testis Volume and Testis Weight/Body Weight Ratio of Rats](image2)
**Testes Histo-morphometry:** The cross-sections of the seminiferous tubules of control rats were fairly circular or oval in outline with normal seminiferous epithelium and numerous spermatozoa within their lumen (Figure 3). Rats that received HDU alone showed destructive changes in their seminiferous tubules and interstitial tissues. This group of animals demonstrated marked testicular atrophy (Figure 4). This contrasted significantly with the testicular profiles of the group of animals that were given only MOLE as these animals showed testicular features that approximated those of the control animals (Figure 5). The rats that were given both HDU and MOLE showed a remarkable preservation of their seminiferous epithelium at autopsy. Degeneration of seminiferous epithelial cells was therefore only minimal in rats that were given combined HDU and MOLE treatment when compared with those animals that had HDU alone treatment (Figure 6).

![Figure 3](image3.png)

**Figure 3:** Cross-section of testis of rat treated with distilled water 2.5 ml/kg b.w/day/orally for 90 days (CONTROLS). Showing the seminiferous tubules; L: Lumen; S: Seminiferous epithelium; I: Interstitium; Stains: haematoxylin and eosin; Mag: x400.

![Figure 4](image4.png)

**Figure 4:** Cross-section of testis of rat treated with hydroxyurea 25 mg/kg b.w/day/orally for 90 days. Showing the seminiferous tubules; L: Lumen; S: Seminiferous epithelium; I: Interstitium; Stains: haematoxylin and eosin; Mag: x400.

![Figure 5](image5.png)

**Figure 5:** Cross-section of testis of rat treated with *Moringa oleifera* leaf extract 50 mg/kg b.w/day/orally for 90 days. Showing the seminiferous tubules; L: Lumen; S: Seminiferous epithelium; I: Interstitium; Stains: haematoxylin and eosin; Mag: x400.
Figure 6: Cross-section of testis of rat treated with both hydroxyurea 25 mg/kg b.w/day and *Moringa oleifera* leaf extract 50 mg/kg b.w/day orally for 90 days. Showing the seminiferous tubules; L: Lumen; S: Seminiferous epithelium; I: Interstitium; Stains: haematoxylin and eosin; Mag: x400.

There was a statistically significant reduction in the tubular diameter, the cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of the animals that had HDU alone. However, the Sprague-Dawley rats that were treated with both HDU and MOLE had only a non-significant reduction in the tubular diameter, the cross-sectional area of the tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules when compared to the controls (Figures 7-10).

Figure 7: The diameter (D) of seminiferous tubules of rats

Figure 8: The Cross-sectional area (Ac) of the seminiferous tubules of rats ($x10^3 \mu M^3$)
Figure 9: The Number of Seminiferous Tubular Profiles per unit area ($N_A$) in rats

Figure 10: The mean numerical density of seminiferous tubules ($N_v$) of rats

Figure 11: Sperm counts of Rats
Sperm parameters: Sperm count and sperm motility: The MOLE-alone Sprague-Dawley rats did not demonstrate any significant difference in their sperm count and motility when compared to the control values. The animals that were given HDU without a co-treatment with MOLE showed a significant reduction in both their sperm count and motility as compared to the control groups and those groups that had a MOLE co-treatment (Figures 11 and 12).

Sperm progressivity and sperm morphology: A similar pattern as that obtained with sperm count and sperm motility was demonstrated with respect to sperm progressivity and morphology. Thus MOLE-alone group showed normal sperm progressivity and morphology. The animals that were given HDU without co-treatment with MOLE showed a significant reduction in both their sperm progressivity and normal sperm morphology rates, respectively as compared to the control group and those groups that had a mole co-treatment (Figures 13 and 14). Combination treatment with MOLE therefore attenuated the impairments in sperm progressivity and percentage of normal sperm morphology.
Figure 14: Testicular SOD of rats

Figure 15: Testicular CAT of rats

Figure 16: Testicular GPx of rats
Testicular oxidative stress: Activities of testicular enzymes- superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx): Treatment with MOLE alone caused no significant change in testicular SOD activity, whereas, HDU alone therapy in rats showed a statistically significant decrease in SOD activity compared to control animals. Treatment combined HDU and MOLE significantly increased the testicular SOD activity compared to animals that received the antineoplastic agent alone (Figure 14). As shown in figure 15, the testicular activities of CAT after MOLE alone were comparable to that of the control values. HDU alone administration, however, resulted in a statistically significant reduction in testicular CAT activity compared to control rats. Co-administration of HDU and MOLE significantly increased the testicular CAT activity when compared to HDU alone-challenged animals. The CAT activity was in this approximately comparable to normal control values. The GPx activities following MOLE administration approximated that of the control animals. HDE, however, markedly decreased the enzyme activity compared to control values. Administration of both HDU and MOLE significantly increased the GPx activity in testicular tissue compared to animals treated with HDU alone (Figure 15). Testicular content of glutathione (GSH) and malondialdehyde (MDA): Following treatment with MOLE, the testicular GSH level was not significantly different from the control group. A notable reduction in GSH content was, however, observed after HDU alone treatment when compared to the control animals. Administration of both HDU and MOLE significantly elevated the testicular content of GSH compared to animals that had the antisickling drug alone (Figure 16). As shown in figure 17, MOLE had no effect on the testicular content of lipid peroxides expressed as MDA when compared to control animals. HDU however, significantly elevated the testicular MDA by several folds as compared
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to the control value. Co-administration of HDU and MOLE exhibited a marked reduction in the testicular MDA level compared to HDU alone treated rats.

Discussion
Currently there is no cure for sickle cell disease (SCD). However, hydroxyurea (HDU), an antineoplastic agent, is commonly used in the treatment of SCD (King, 2003). Nevertheless, the clinical utilization of HDU is greatly limited due to its adverse toxic side effects on various organs especially the testis and the bone marrow ((Perreault et al., 2008, Friedrich et al., 2008). It has been postulated that HDU –induced organ toxicity could be due to its metabolites particularly Carbamoyl nitroso which may be involved in electron transfer, reactive oxygen species formation, and oxidative stress (Cokic et al., 2003). The present study was therefore designed to investigate the potential testiculoprotective effect of Moringa oleifera leaf extract as antioxidant-rich nutraceutical.

The results from the present study indicate that administration of HDU decreased the absolute testicular weights, testicular weight/body weight ratio and testicular volumes of rats. This is in agreement with findings of several investigators ((Evenson and Jost, 1993; Wiger et al., 1995; Zhou et al., 2008) who provide tangible evidences of testicular morphologic derangement following HDU therapy using animal models. The animals that received concurrently HDU and MOLE however, demonstrated largely preserved testis weights, testis weight/body weight ratio and testis volumes.

A qualitative and quantitative evaluation of the testicular histology of all groups of animals that were submitted to HDU indicate derangement in the seminiferous epithelia of their testes Again these findings are similar to earlier reports by Evenson and Jost; 1993, Fritz and Hess, 1996; Jorge et al., 2005 and Mojica et al., 2007. And as was the case with the gross anatomical parameters and for probably similar reasons, co-treatment with MOLE showed a remarkable improvement in the testicular histomorphometric profiles.

Evaluation of lipid peroxidation, GSH content as well as SOD, CAT, GPx and other antioxidant enzyme activities in biological tissue have been always used as markers for tissue injury and oxidative stress (Chularoj montri et al., 2005; Prahalathan et al., 2005; Atessahin et al., 2006; Priestman, 2008). In the present study, testicular impairments and oxidative damage induced by HDU administration are also manifested by a significant increase in the activities of antioxidant enzymes, SOD, CAT, GPx and the testicular content of MDA, and a significant decrease of GSH. Strikingly, co-treatment of rats with leaf extract of M. oleifera markedly attenuated the oxidative damage induced by HDU challenge.

Cokic et al., 2003 has stated that HDU exerts its organ toxicity by generation of free radicals and reactive oxygen species. This increased oxidative stress damages the sperm membranes, proteins and DNA. This explains the reduced sperm concentration and sperm motility with accompanying increase in abnormal sperm rates as seen in HDU alone group of rats. Co-treatment with MOLE containing bioflavonoids and other potent antioxidants resulted in a remarkable amelioration of the deranged sperm parameters.

Numerous scientific reports have shown elevation of a variety of antioxidant enzymes and testicular biomarkers as a result of treatment with M. oleifera or with phytochemicals isolated from M. oleifera (Faizi et al., 1994; Kumar and Pari 2003). This could provide an explanation for the finding in our study that MOLE alone group of animals showed improved sperm parameters. In this respect our results are in consonance with the report of D’cruz and Mathur (2005) which proved that the sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme system levels by Moringa treatment can favor the reproductive process.

Conclusion
We conclude that leaf extract of Moringa oleifera has an attenuating role on hydroxyurea -induced testicular toxicity in Sprague –Dawley rats.

Recommendations
Based on the fact that the leaf extract of M. oleifera exerts a potent protection on the testis of laboratory animals challenged with hydroxyurea, we recommend that further studies be carried out to determine the testiculoprotective potential of this nutraceutical during hydroxyurea chemotherapy in clinical practice.

References
Moringa oleifera L. Extract Modulates Hydroxyurea-Induced Testicular Derangement