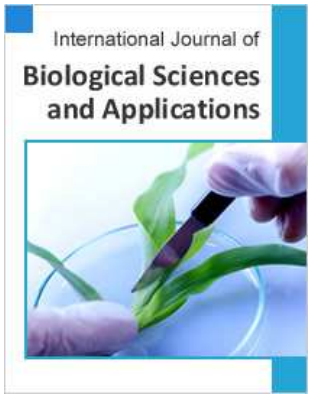
International Journal of Biological Sciences and Applications
2016; 3(1): 15-21
Published online February 24 2016 (http://www.aascit.org/journal/ijbsa)
ISSN: 2375-3811

American Association for Science and Technology



Keywords
Hibiscus sabdariffa,
Aqueous Extract,
Anthocyanin-Rich Extract,
Antioxidant Enzymes,
2,4-Dinitrophenylhydrazine

Received: December 9, 2015
Revised: January 12, 2016
Accepted: January 14, 2016

Effects of *Hibiscus Sabdariffa* Calyx Extracts on 2,4-Dinitrophenylhydrazine-Induced Changes in the Activities of Antioxidant Enzymes in Rabbits

A. O. Olusola

Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Email address
austinolusola@gmail.com

Citation
A. O. Olusola. Effects of *Hibiscus Sabdariffa* Calyx Extracts on 2,4-Dinitrophenylhydrazine-Induced Changes in the Activities of Antioxidant Enzymes in Rabbits. *International Journal of Biological Sciences and Applications*. Vol. 3, No. 1, 2016, pp. 15-21.

Abstract
The effects of aqueous extract and anthocyanin-rich extract of the calyces of *Hibiscus sabdariffa* Linn on the 2,4-dinitrophenylhydrazine (DNPH)-induced changes in the levels of antioxidant enzymes of rabbits were evaluated in this study. The organs examined were the blood, brain and liver. Exposure of rabbits to DNPH (28 mg/kg body weight) caused significant ($P<0.05$) increase in catalase and superoxide dismutase activities relative to the DNPH-free group. The activity of glucose-6-phosphate dehydrogenase was also significantly ($p<0.05$) elevated in the serum while the level of reduced glutathione (GSH) was significantly reduced following DNPH treatment when compared to control. However, pre-treatment with (100 mg/kg body weight) whole aqueous extract of *H. sabdariffa (HS)* and calyx anthocyanins separately provided varying degrees of protection against DNPH-induced biochemical changes. Relative to the controls, the extracts treatments significantly ($P<0.05$) decreased the activities of the antioxidant enzymes. Examined separately and compared, both extracts appeared to have offered effective protection against DNPH-induced oxidative damage, though the anthocyanin isolate appeared to be more effective in this capacity. So *H. sabdariffa* calyx possesses potent antioxidant principles which are likely to be anthocyanins.

1. Introduction

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Protection against free radicals can be enhanced by ample intakes of dietary antioxidants, of which the best studied are vitamins C and E as well as carotenoids (Vertuani et al., 2004; Snell et al., 2012). There is a considerable amount of epidemiological evidence revealing an association between diets rich in fruits and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer (Block, 1992; Hertog and Feskens, 1993; Wang et al., 2000; Aoun and Makris, 2012; Olusola et al., 2012a).

It is generally assumed that the active dietary constituents contributing to these protective effects are antioxidant nutrients such as α - tocopherol and β - carotene. However, recent investigations have revealed that polyphenolic components of plants do

exhibit antioxidant properties and do contribute to the anticarcinogenic or cardioprotective actions brought about by diet (Wang et al., 2000; Stanner et al., 2004). In particular some beverages such as red wine and tea have been shown to elicit antioxidant properties in both *in vitro* and *in vivo* systems (Kanner et al., 1994; Aoun and Makris, 2012; Olusola et al., 2012a). *Hibiscus sabdariffa* Linn (Roselle) belongs to the family of *Malvaceae*, which is native to old World tropics, probably in the East Indies; now cultivated throughout the tropics (Duke and Archley, 1984). The vegetable is widely grown and commonly used as port herb or soup in the northern part of Nigeria. In Nigeria especially in the northern part, the extract of the red calyces is consumed as a beverage known as zobo.

Ethnobotanical information regarding *Hibiscus sabdariffa* reveals the following medicinal uses: diuretic, diaphoretic, antibacterial agent, antifungal agent, mild laxative, sedative, antihypertensive, gastrointestinal disorder treatment, hypercholesterolemia treatment, kidney stone treatment, liver damage treatment, agent for decreasing the viscosity of the blood, and agent for treating the after effects of drunkenness (Morton, 1987; Akindahunsi and Olaleye, 2003; Hirunpanich et al., 2006; Olusola, 2014). Among the chemical constituents of the flower are the flavonoids, gossypetine, hibiscetine, anthocyanin and sabdaretine (Pietta, 2000). Certain amounts of delphinidin-3-monoglucoside and cyaniding-3-monoglucoside which constitute the anthocyanins are also present (Langen-hoven et al., 2001, Olusola et al., 2012a, b; Olusola, 2014).

Some studies have reported that *Hibiscus sabdariffa* is effective for decreasing the levels of total lipids, cholesterol and triacylglycerol, suggesting the possibility that *Hibiscus sabdariffa* functions as hypolipidemic agent (Olatunji et al., 2006; Hirunpanich et al., 2006). Also, we have previously reported the antioxidative potentials of extracts of calyx of *H. sabdariffa* (Ologundudu and Obi, 2005; Ologundudu et al., 2009a, b; Olusola et al., 2012a, b). However, studies on the direct effect of *Hibiscus sabdariffa* calyx extracts on the activities of antioxidant enzymes are scanty. This study was therefore carried out to evaluate the effect of *Hibiscus sabdariffa* extracts on 2,4-dinitrophenylhydrazine-induced changes in the activity of antioxidant enzymes in rabbits.

Phenylhydrazine and its derivatives 2,4-dinitrophenylhydrazine are toxic agents. Their toxic action has been attributed to their ability to undergo auto oxidation. This increased oxidant potential enables them to oxidize enzymes, membrane protein and hemoglobin. Phenylhydrazine is able to initiate lipid peroxidation in membrane phospholipids (Jain and Hochstein, 1980; Olusola, 2014) while 2,4-dinitrophenylhydrazine has been shown to be capable of inducing lipid peroxidation and other oxidative damage in rabbits (Ologundudu and Obi, 2005; Ologundudu et al. 2009a, b; Olusola, 2014) and rats (Maduka et al. 2003). The ability of 2,4-DNP to induce lipid peroxidation and other free radical damage makes it an appropriate model toxicant for testing the claim that the extract of *Hibiscus sabdariffa* Linn calyces can protect tissues from oxidative stress-induced changes and other attendant biochemical changes.

1.1. Plant Material

Fresh calyces of *H. sabdariffa* were harvested from The Botanical Gardens University of Ilorin, Kwara State, Nigeria. They were dried under continuous air-flow maintained at 25°C until constant weight. Identification and taxonomical classifications were done at herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo-State, Nigeria.

1.2. Animals

Thirty (30) rabbits (Oryctolagus cuniculus) used for this research work were obtained from a private breeder in Benin City. The animals weighed 800-1000 g on purchase and were in very good state of health as confirmed by a veterinary physician. The animals were housed in twos (same sex) in improvised rabbit cages composed of wire mesh (100cmX40cmX30cm) under 14 hr/10 hr light/dark regimen. They were fed with growers mash (obtained from Bendel Flours and Feed Mill, Ewu, Edo State, Nigeria) and water ad libitum. The animals were protected from parasite infestation by proper veterinary management throughout the duration of the treatment.

1.3. Preparation of Aqueous Extract

One hundred grams of dried calyces of *Hibiscus sabdariffa* were soaked in one litre of distilled water for 12 hours to obtain a red coloured extract.

1.4. Preparation of Anthocyanin-Rich Extract from Plant Materials

Anthocyanin extract from Hibiscus sabdariffa calyces was prepared according to the method described by Hong and Wrolstad, (1990a). One kilogram of Hibiscus sabdariffa calyces was pulverized by means of Binatone blender and extracted with 10 litres of 0.1% trifluoroacetic acid (TFA) aqueous solution for 12 hours at 20°C on an orbital shaker. The extract was filtered through filter paper (Advantech number 5C). A portion of the filtrate (10 ml) was applied to sepabeads SP-207 resin column (Mitsubishi Chemicals, Japan). The resin was washed with 3 litres of water and then eluted with 50% ethanol solution containing 0.1% TFA.

The eluate was dried under vacuum at 20°C and then freeze-dried. The dried sample obtained was resuspended in distilled water and kept in the refrigerator until required for oral administration and biochemical investigation.

1.5. Experimental Design

Thirty (30) rabbits weighing 800-1000 g were used for this research work. They were randomly selected into six (6) experimental groups as shown below. The experiment lasted for 28 days.

Group 1: Water treated control. Each rabbit was given distilled water, 2.5 ml/kg body weight.

Group 2: Aqueous extract of *H. sabdariffa* was administered at a dose of 100 mg/kg body weight, to each rabbit in this group by gavage.

Group 3: Anthocyanin-rich extract of *H. sabdariffa* was administered at a dose of 100 mg/kg body weight, to each rabbit in this group by gavage.

Group 4: 2,4-Dinitrophenylhydrazine was administered at a dose of 28 mg/kg body weight intraperitoneally to each rabbit in this group during the last 5 days of the 28-day study period before sacrifice.

Group 5: Aqueous extract of *H. sabdariffa* was administered at a dose of 100 mg/kg body weight for 28 days to each rabbit in this group accompanied with 28 mg/kg body weight of 2,4-dinitrophenylhydrazine administered intraperitoneally daily from day 24 (5 days 2,4-DNPH treated) before sacrifice.

Group 6: Anthocyanin-rich extract of *H. sabdariffa* was administered at a dose of 100 mg/kg body weight for 28 days to each rabbit in this group accompanied with 28 mg/kg body weight of 2,4-dinitrophenylhydrazine administered intraperitoneally daily from day 24 (5 days 2,4-DNPH treated) before sacrifice.

1.6. Biochemical Determinations

Catalase activity was determined by the method of Sinha (1971) by following its decomposition of H_2O_2. The superoxide dismutase activity was determined by the method of Misra and Fridovich (1972). GSH in tissue homogenate was determined by the method described by Jollow et al (1974). The activity of glucose-6-phosphate dehydrogenase was determined using assay kit obtained from Randox Laboratories, UK. The method is as described in the manual/leaflet.

1.7. Statistical Analysis

The data obtained were subjected to standard statistical analysis of variance (ANOVA) using the SAS software (SAS Inst. Inc. 1999). Treatment means were compared using the Duncan procedure of the same software. The significance level was set at $P<0.05$.

2. Results

The effects of DNPH, aqueous extract of *H. sabdariffa* (HS) and HS anthocyanin extract (AN) on the specific activities of catalase is presented in Table 1. Treatment with DNPH (Group 4) significantly ($p<0.05$) reduced rabbit serum activity of catalase but caused a significant increase in the liver and brain levels of the enzyme when compared with control (Group 1). The specific activities of catalase in the rabbits that received the whole aqueous extract and anthocyanin extract alone (Groups 2 and 3) and those pretreated with each of the extracts before DNPH administration (Groups 5 and 6) did not show any significant ($p<0.05$) difference (in the serum, liver and brain) when compared with the control.

Table 1. *Effect of DNPH, Aq extract of HS and H. anthocyanins on the specific activities of catalase in the serum, liver and brain of rabbits.*

Group #	Treatment	Specific activity of catalase (n mole H_2O_2 decomposed/min/mg protein)		
		Serum	Liver	Brain
1.	2.5ml H_2O/kg bd wt. (control)*	0.69 ± 0.02	4.81 ± 0.21	4.50 ± 1.21
2.	100 mg AqE/kg bd wt.	0.81 ± 0.12	5.76 ± 0.25	5.05 ± 1.56
3.	100 mg AN/kg bd wt.	0.76 ± 0.16	7.02 ± 0.45	5.58 ± 1.08
4.	28 mg DNPH/kg bd wt.	0.54^a±0.18	18^a.62±0.36	15.89^a ± 2.17
5.	100 mg AqE + 28 mg DNPH/kg bd wt.	0.71± 0.21	6.00 ± 0.54	5.85 ± 1.65
6.	100 mg AN + 28 mg DNPH/kg bd wt.	0.74±0.17	7.19 ± 0.87	6.01 ± 0.76

Results are presented as means ±SEM of five (5) determinations. Statistical comparison is strictly within the same tissue. Values carrying superscripts differ significantly ($p<0.05$) from control (Group 1). Values with same superscript do not differ significantly from each other while values with different superscripts are significantly different from one another. DNPH: 2,4-dinitrophenylhydrazine, AN: anthocyanin, AqE: aqueous extract.

Table 2. *Effects of DNPH, Aq extract of HS and HS anthocyanins on the levels of superoxide dismutase (SOD) in the serum, liver and brain of rabbits.*

Group #	Treatment	Specific activity of SOD (nmol/ mg protein)		
		Serum	Liver	Brain
1.	2.5 ml H_2O/kg bd wt. (control)*	0.18 ± 0.14	4.54 ± 0.21	1.09 ± 0.43
2.	100 mg AqE/kg bd wt.	0.24 ± 0.02	4.96 ± 0.26	1.35 ± 0.26
3.	100mg AN/kg bd wt.	0.26 ± 0.10	6.03^a ± 0.21	1.97^a ± 0.21
4.	28 mg DNPH/kg bd wt.	0.12^a ± 0.01	5.69^b ± 0.15	3.20^b ± 0.26
5.	100 mg AqE + 28 mg DNPH/kg bd wt.	0.22 ± 0.16	5.40 ± 0.23	1.44 ± 0.28
6.	100 mg AN + 28 mg DNPH/kg bd wt.	0.23 ± 0.08	4.86 ± 0.23	1.95^a ± 0.61

Results are presented as means ±SEM of five (5) determinations. Statistical comparison is strictly within the same tissue. Values carrying superscripts differ significantly ($p<0.05$) from control (Group 1). Values with same superscript do not differ significantly while values with different superscripts are significantly different from one another. *See table 1 footnote for interpretation of abbreviations.

Table 2 shows the effects of DNPH, aqueous extract of HS and HS anthocyanins on the specific activities of superoxide dismutase (SOD) in the liver, brain and serum of rabbits. Relative to control (Group 1), treatment with anthocyanin extract (Group 3) significantly ($p<0.05$) increased the activity of the enzyme in the liver and brain but had no significant

effect on its level in the serum when compared with the control. Treatment with DNPH caused a significant ($p<0.05$) reduction in the activity of the enzyme in the serum but increase its activities in the liver and brain. Rabbits that received HS anthocyanin before DNPH administration (Groups 6) showed significant increase in the level of the enzyme in the brain when compared with control.

Table 3. *Effects of DNPH, Aq extract of HS and HS anthocyanins on the activities of Glucose-6-phosphate dehydrogenase (G6PD) in the serum and liver.*

Group #	Treatment	Glucose-6-phosphate dehydrogenase activity (µmol/min/mg protein)	
		RBC	Liver
1.	2.5 ml H_2O/kg bd wt. (control)*	3.20 ± 0.20	23.30 ± 0.15
2.	100 mg AqE/kg bd wt.	3.60 ± 0.10	23.50 ± 0.05
3.	100 mg AN/kg bd wt.	3.10 ± 0.11	23.45 ± 0.50
4.	28 mg DNPH/kg bd wt.	5.65^a ± 0.80	21.80 ± 0.18
5.	100 mg AqE + 28 mg DNPH/kg bd wt.	3.20 ± 0.40	23.80 ± 0.05
6.	100 mg AN + 28 mg DNPH/kg bd wt.	3.25 ± 0.12	22.45 ± 0.30

Results are presented as means ±SEM of five (5) determinations. Statistical comparison is strictly within the same tissue. Values carrying superscripts differ significantly ($p<0.05$) from control (Group 1). Values with same superscript do not differ significantly while values with different superscripts are significantly different from one another. *See table1footnote for interpretation of abbreviations.

Table 3 shows the effects of DNPH, aqueous extract of HS and HS anthocyanin extract on the activities of glucose-6-phosphate dehydrogenase (G6PD) in the liver and blood. The data show that the activity of glucose-6-phosphate dehydrogenase was significantly ($p<0.05$) elevated in the blood following DNPH treatment when compared with control. Pretreatment with the extracts prior to DNPH administration (Groups 5 and 6) however, prevented the DNPH-induced changes.

Table 4. *Effects of DNPH, Aq extract of HS and HS anthocyanins on the levels of reduced glutathione (GSH) in the liver and brain of rabbits.*

Group #	Treatment	GSH (nmol/mg protein)	
		Liver	Brain
1.	2.5 ml H_2O/kg bd wt. (control)*	24.84 ± 1.33	24.48 ± 0.21
2.	100 mg AqE/kg bd wt.	26.27 ± 0.59	25.54 ± 0.5
3.	100 mg AN/kg bd wt.	26.98^a ± 0.25	24.71 ± 0.69
4.	28 mg DNPH/kg bd wt.	16.39^b ± 0.92	17.48^a ± 0.19
5.	100 mg AqE + 28 mg DNPH/kg bd wt.	23.55 ± 0.72	25.32 ± 0.27
6.	100 mg AN+ 28mg DNPH/kg bd wt.	23.53 ± 0.26	24.10 ± 0.38

Results are presented as means ±SEM of five (5) determinations. Statistical comparison is strictly within the same tissue. Values carrying superscripts differ significantly ($p<0.05$) from control (Group 1). Values with same superscript do not differ significantly while values with different superscripts are significantly different from one another. *See table 1 footnote for interpretation of abbreviations.

The effects of DNPH, aqueous extract of HS and HS anthocyanins on the levels of reduced glutathione (GSH) in the liver and brain of rabbits is presented in Table 4. DNPH treatment caused significant depletion of GSH in the liver and brain (Group 4). Treatment with anthocyanin extract significantly increased the level of GSH in the liver (Group 3). The groups that received each of the extracts prior to DNPH treatment did not show any significant reduction in their liver and brain GSH levels relative to the control (Groups 5 and 6).

3. Discussion

The cell is always under the threat of oxidative injury inducible by hydrogen peroxide (Chiu et al., 1982). Hydrogen peroxide can be produced in the cell by wide varieties of metabolic pathways, by both endogenous and exogenous substrates (Chiu et al., 1982). During the catabolism of purines, deamination of L and D-amino acids by L and D-amino acid oxidases, incomplete transfer of electrons, in the complex IV of electron transport chain and from iron III in heme to oxygen, during reversible oxygen transport of hemoglobin and subsequent action of superoxide dismutase on the superoxide generated and the bio-conversion of ribonucleotides to deoxyribonucleotides; hydrogen peroxide is produced. Exogenous factors inducing hydrogen peroxide formation include drugs, chemicals, and radiation. The cells are shielded from the oxidative effect of hydrogen peroxide by the enzymatic activity of catalase and glutathione peroxidase.

The groups treated with aqueous extract of *H. sabdariffa* and anthocyanin extract of *H. sabdariffa* showed no significant ($P<0.05$) difference in the specific activity of catalase compared to the water control in the three tissues. Treatment of rabbits with DNPH alone, resulted in significant ($P<0.05$) decrease in the activity of catalase in serum while it's increased in the liver and brain, relative to water control. The bioaccumulation of DNPH in the tissues sequel to intraperitoneal administration must have caused the generation of superoxide free radicals which were converted to hydrogen peroxide. Catalase must be synthesized and react appropriately to detoxify the hydrogen peroxide. It is noteworthy that comparing the activities of the enzyme in the three tissues, the serum showed the lowest value. This low

activity could possibly be due to other available agents for detoxifying hydrogen peroxide. These include the presence of dietary antioxidants and bilirubin which is a product of heme degradation (Jain et al., 1983) aptly present in the blood cells and circulation. Prior administration of aqueous extract of HS and anthocyanin-rich extract separately followed by treatment with DNPH resulted in significant decrease in the serum specific activity of catalase but caused a significant decrease in its level in the liver and brain, compared with those treated with DNPH alone. ANOVA showed no significant ($P<0.05$) difference between the means of the two groups. Pretreatment with anthocyanins, however, proved more efficient in protecting the tissues from DNPH-induced oxidative stress.

Superoxide dismutase is widely distributed in tissues and its role as one of the early enzymes catalyzing detoxification reactions of superoxide has been well documented (Das and Nair, 1980). It does not only represent the first major enzyme of superoxide metabolizing system, its deficiency has been linked to life-threatening pathologic conditions (Flynn et al., 1983). Its determination in this research was significant because it represents the chief enzyme that prevents peroxidation initiated when superoxide radicals attack the unsaturated fatty acyl groups of phospholipids; a reaction which heralds other downstream reactions like hemolysis in red blood cells and necrosis of tissues (Winterbourg et al., 1976; Olusola, 2014).

Again, while treatment with DNPH resulted in significant decrease in the serum activity of superoxide dismutase compared with water control, it significantly increased the activity of the enzyme in the liver and brain. Necrotic properties of DNPH on blood cells and other tissues must have accounted for the reduced activity of superoxide dismutase in the serum (Orringer et al., 1973; Olusola, 2014). Also, the increased activity of the enzyme in the liver and brain is a toxic response arising from the need to detoxify the increased free radicals being generated from the DNPH. The accumulation of DNPH in the liver must have significantly increased the rate of superoxide generation possibly higher than what the basal enzyme activity could cope with. Therefore, to salvage the cells from the peroxidative action of superoxide and tissue necrosis, an SOS response is induced to increase the cellular expression of superoxide dismutase thus, accounting for the significant increase in the superoxide dismutase activity of liver homogenate under DNPH administration. The presence of superoxide radical is responsible for the increased synthesis of superoxide dismutase. Given that the groups administered with water represented the basal rate of enzyme synthesized in normally metabolizing cells, the presence of the extracts in the tissues of the rabbits must have been responsible for the lowered specific activity of superoxide dismutase. This is essentially true because anthocyanins, anthocyanidins, phenols, polyphenols and ascorbate have the tendency of furnishing electrons for the unpaired and reactive radicals. On the effect of the different treatments on the brain, these data either indicate that the brain has a reduced dependence on exogenous antioxidants and prefers to engage free radicals generated during metabolism strictly by antioxidant enzymes or the antioxidant molecules do not appreciably cross the blood-brain barrier. The former argument may be particularly true because of the fast rate of oxidative reactions in the brain especially oxidation of nutrient molecules and oxidative phosphorylation which have been characterized as important in free radical generation in cells.

Glucose-6-phosphate dehydrogenase is a widely distributed enzyme in tissues and its role as the anchor of reductive metabolism is well established. It does not only represent the major regulatory enzyme of the pentose phosphate pathway but also its metabolic products: NADPH and phospho-ribose are involved in reductive anabolism and nucleotide synthesis respectively. The deficiency of this enzyme has been implicated in favism and hemolytic anemia. Its determination in this research was necessitated by the fact that it represents the chief enzyme that provides the reduced glutathione with hydride ion (H^-) in form of NADPH for sustenance of free radical detoxification (Bergmeyer et al., 1974, Ologundudu et al., 2009 a, b).

Treatment of rabbits with aqueous extract of *H. sabdariffa* and anthocyanin isolate showed no significant ($p<0.05$) difference in the activities of the enzyme in both the erythrocytes and liver when compared with the water control group. The group treated with aqueous extract showed higher but statistically insignificant specific activity of this enzyme in the serum when compared with the anthocyanin extract group. When values recorded for aqueous extract group in the blood was compared with the values reported in anthocyanin isolate treatment, the value obtained for aqueous extract group was slightly higher than that of anthocyanin treatment although, not statistically significant. The possible explanation for this observation is that aqueous extract of *H. sabdariffa* is not homogenous but contains heterogeneously distributed phytochemicals such that while polyphenolic constituents protect these tissues, other constituent compounds might have delirious effect on the cellular integrity of different tissues, the balance between these opposing factor would likely result in the observed values of the enzyme (Youdim et al., 2002, Mazza et al., 2002). Also, since most red blood cells in circulation are matured types, they tend to have a constant cytosolic pool of glucose-6-phosphate dehydrogenase (Flynn et al., 1983). Therefore, an increased specific activity would mean sample contains more red blood cell and reduced hemolysis. Tissue toxicity was induced by administering DNPH and the toxicity was established by the significantly increased specific activity of glucose-6-phosphate dehydrogenase in the erythrocytes (Flynn et al., 1983). This property of DNPH has been well characterized and it stems from its cellular disruption and oxidative damage resulting in hemolysis. The integrity of erythrocyte membrane is maintained by reduced glutathione whose level in turn depends on the cellular level of NADPH, a metabolic product of the reaction of glucose-6-phosphate dehydrogenase in pentose phosphate pathway. Therefore the observed significant increase in the specific activity of

glucose-6-phosphate dehydrogenase under the condition of DNPH administration was a toxic response which usually happens in the presence of xenobiotics (Orringer and Parker, 1977; Ologundudu et al*.,* 2010).

Pretreatment of rabbits with each of the extracts before DNPH administration revealed that each of the preparations offered protection as evidenced by the significant reduction in the blood level of specific activity of glucose-6-phosphate dehydrogenase when compared with group administered with DNPH alone. This is in consonance with earlier reports (Winterbourn et al., 1976; Orringer and Parker, 1977; Ologundudu et al., 2010). This indicated that the antioxidant preparations exhibit prophylactic-type of protection against chemical damage in the blood cells.

Glutathione peroxidase is a seleno-enzyme which requires the continuous supply of reducing agent in the form of glutathione (a tripeptide consisting of glutamate, cysteine and glycine). The sulfhydryl group of cysteine ultimately supplies the needed hydride ion for the reaction. An increased synthesis of the glutathione peroxidase and depleted reduced glutathione in tissues and cells usually occurs as a result of the presence of hydrogen peroxide, which in turn is produced from the conversion of superoxide by superoxide dismutase (Calviello et al., 2005).

Administration of anthocyanin isolate and aqueous extract of *H. sabdariffa* showed a significant increase in the level of reduced glutathione in the liver, indicating the sparing effect of these extracts on the glutathione pool of the cell (Stadtman, 1980). Treatment of rabbits with DNPH resulted in the significant reduction of reduced glutathione levels in the liver and brain. This finding is well documented and stemmed from the free radical generating potential of DNPH (Jain and Hochstein, 1979; Clemens et al., 1984; Olusola, 2014). It is well established that GSH, the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation or by direct free radical quenching (Wang et al*.,* 2000; Ologundudu et al*.,* 2009a, b). This study showed that DNPH reduced GSH levels in the liver and brain, but *H. sabdariffa* extracts blocked the phenomenon effectively.

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