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EFFECT OF ETHANOLIC EXTRACT OF *HIBISCUS SABDARIFFA L.* ON 2, 4-DINITROPHENYLHYDRAZINE-INDUCED CHANGES IN BLOOD PARAMETERS IN RABBITS

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ABSTRACT

The effects of *Hibiscus sabdariffa* petal extract on 2, 4 – dinitrophenylhydrazine – induced biochemical and haematological changes in the blood have been examined using blood glucose, and malondialdehyde (MDA) levels as well as RBC, WBC, PCV and Haemoglobin (Hb) concentration as indices of alteration and protection. Relative to the control; 2, 4 – dinitrophenylhydrazine (2, 4 – DNPH) treatment significantly decreased ($P \le 0.05$) blood glucose from 7.25 ± 0.38 mM (control) to 3.53 ± 0.25 mM (2, 4 – DNPH treatment only). The 2, 4 – DNPH treatment significantly increased blood MDA level from $1.09 \pm 0.08 \times 10^{-6}$ units/ml (control) to $10.97 \pm 2.97 \times 10^{-6}$ units/ml (2, 4 – DNPH treatment only). Treatment with 2, 4 – DNPH alone significantly decreased RBC count from $376.33 \pm 4.06 \times 10^{11}$ counts/μL to $284.33 \pm 3.84 \times 10^{11}$ counts/μL, PCV from 35.67 ± 1.45 % to 18.67 ± 0.67 % and Hb from 12.01 ± 0.58 g/dl to 6.63 ± 0.49 g/dl but increased WBC count from $5.20 \pm 0.20 \times 10^{3}$ counts/μL to 9.60 ± 0.31 counts/μL. Relative to the levels of these parameters in the blood of rabbits treated with 2, 4 – DNPH alone, treatment of rabbits with *Hibiscus sabdariffa* extract prior to 2, 4 – DNPH led to significant ($P \le 0.05$) increase in blood glucose (6.94 ± 0.26 mM from 3.53 ± 0.25 mM), decrease in MDA ($1.69 \pm 1.04 \times 10^{-6}$ units/mL from $10.97 \pm 2.97 \times 10^{-6}$ units/ml), increase in RBC count ($375.33 \pm 2.91 \times 10^{11}$ counts/μL from $284.33 \pm 3.84 \times 10^{11}$ counts/μL), PCV (35.87 ± 2.91 % from 18.67 ± 0.67 %) and Hb (12.70 ± 0.38 g/dl from 6.63 ± 0.49 g/dl) but a decrease in WBC count ($5.90 \pm 0.29 \times 10^{3}$ counts/μL from $9.60 \pm 0.31 \times 10^{3}$ counts/μL). These findings indicate that *H. sabdariffa* dried flower extract protects the blood against 2, 4 – DNPH lipoperoxidative and haemolytic effects.

KEYWORDS: 2, 4 – DNPH; Blood glucose; MDA; RBC; WBC; Hb; *Hibiscus sabdariffa* extract.

INTRODUCTION

The plant *Hibiscus sabdariffa L* belongs to the family Malvaceae (Gill, 1992). It is cultivated for its leaf, fleshy calyx, seed or fibre. Some of these parts are used as herbal remedies (Gill, 1992). It is more widely referred to as sorrel by the English, Indians and Jamaicans. In Nigeria a red coloured soft drink which is a hot-water extract of the red flower of this plant is chilled and marketed as "zobo drink". Among the chemical constituents of the flower are the flavonoids, gossypetine, hibiscetine, anthocyanin and sabdaretine (Pietta, 2000). Small amounts of delphinidin – 3 – monoglucoside and cyanidin – 3 – monoglucoside which constitute the anthocyanin are also present (Langenhoven *et al*, 2001). Flavonoids are phenolic compounds (Robinson, 1975). Phenolic substances in red wine have been shown to be potent inhibitors of copper catalysed oxidation of low density lipoproteins (LDL). Hence they are believed to possess antioxidant activity.

There are indications that the extract from the red petals of *Hibiscus sabdariffa L* contains antioxidant principles (Tseng *et al*, 1997; Wang *et al*, 2000). It is, therefore, conceivable that the consumption of the extract may provide natural agents against oxidative tissue damage and other free radical – induced disease conditions (Harman, 1984; Wolff *et al*, 1986). Phenylhydrazine and its derivative 2, 4 – dinitrophenylhydrazine are established haemolytic agents used in toxicological assessments to study the mechanisms of chemically – induced cell damage. They are also used to

Laboratories (US) Glucose oxidase kit was obtained from Randox Laboratories Ltd (UK) and Chow (Growers Mash, BFFM, Ewu, Nigeria).

Treatment of animals and collection of blood samples

Rabbits in groups 3 and 4 were given the extract, 400 mg kg^{-1} body weight by gavage, twice a day for 7 days. For the same duration and in the same manner rabbits in groups 1 and 2 were given 2.5 ml H_2O kg^{-1} body weight. At the end of the 7th day all rabbits were fasted overnight. Following the overnight fast rabbits in groups 2 and 4 received 28 mg 2, 4 – DNPH kg^{-1} body weight in saline. All rabbits were then left for 3 hours with free access to chow and water.

Three hours after 2, 4 – DNPH treatment, the rabbits were anaesthetized in a diethylether saturated chamber. While under anaesthesia the thoracic and abdominal regions were opened to expose the heart. Blood was obtained via cardiac puncture by means of a 5 ml hypodermic syringe and needle and placed in ice-cold heparinized bottles. The heparinized blood samples were centrifuged at 3500 rpm (Uniscope Model SM 902B Bench centrifuge) for 10 minutes each in order to obtain plasma sample. They were collected and left at –20°C until required.

Preparation of extract

Hundred grams of dried *Hibiscus sabdariffa* petals were soaked in one litre absolute ethanol for 12 hours and then filtered to obtain the red coloured extract, the filtrate. The solvent was evaporated in a rotary evaporator and a viscous mass was obtained as residue. This was then reconstituted in 10 % ethanol, put in a bottle, sealed and left at 4°C until required.

Determination of glucose in plasma

Glucose determination was based on the procedure described in Randox Glucose Oxidase Kit assay leaflet (Randox Laboratories Ltd, U.K.). Twenty microlitres of each sample or standard was mixed with 2 ml of the glucose oxidase reagent and incubated for 25 minutes at room

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temperature. The absorbance of the standard and samples were measured at 500 nm against the reagent blank within 60 minutes.

evaluate the mechanism by which red blood cells counteract free – radical mediated cell damage (Clemens *et al*, 1984). The lytic effect of phenylhydrazine has been attributed to its ability to undergo autooxidation and subsequent oxidation of enzymes, membrane proteins and haemoglobin. It is also able to initiate lipid peroxidation in membrane phospholipids (Jain and Hochstein, 1980). These processes of oxidation provide a composite model for testing the claim that *Hibiscus sabdariffa* red petal extract possesses antioxidant action. Hence the purpose of this study is to investigate this claim by administering the extract to rabbits prior to 2, 4 – DNPH treatment. The effect was compared with that in extract – free 2, 4 DNPH – treated rabbits.

MATERIALS AND METHODS

Experimental animals and materials

Rabbits (weight range 800 – 1000 g) used for this study were bred at the Federal College of Agriculture, Akure, Nigeria. They were divided into four experimental groups of 3 rabbits each housed in standard rabbit cages. 2, 4 – Dinitrophenylhydrazine, trichloroacetic acid, sodium chloride and diethylether were purchased from BDH Chemical Company (Poole, England) 2 – Thiobarbituric acid from Koch – Light Laboratories, (England). Hydrochloric acid and absolute ethanol were obtained from WN

Determination of malondialdehyde in plasma

This was based on the method of Buege and Aust (1978). One millilitre of each sample was mixed with 2 ml of TCA – TBA – HCL stock reagent and the mixture heated for 15 minutes in a boiling water bath. After cooling the flocculent precipitate was removed by centrifugation at 3500 rpm for 10 minutes. The absorbance of the sample was measured at 535 nm against reagent blank. The MDA concentration was calculated using extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Haematological analysis

The haematological indices namely red blood cell (RBC) and white blood cell (WBC) counts were estimated by visual counting improved by Neubauer counting chambers. Haemoglobin (Hb) and packed cell volume (PCV) were determined using cyanomethaemoglobin and micro-haematocrit methods respectively (Dacie and Lewis, 1997).

Statistical analysis

The data are presented as means ± SEM. The mean value of the various treatment groups were compared using ANOVA and least square difference test (Lapin, 1978). The significance level was set at $P \leq 0.05$.

RESULTS

Data on the effects of 2, 4 – DNPH and *H. sabdariffa* extract on rabbit blood glucose and MDA are presented in Table 1. 2, 4 – DNPH treatment significantly ($P < 0.05$) reduced blood glucose but increased the MDA level by 51.31 and 906.42 % respectively when compared to the control, group 1 (Table 1). Prior treatment of rabbits (group 4) with the extract before 2, 4 – DNPH administration led to significant ($P < 0.05$) increase in blood glucose but a reduction in MDA level by 96.60 and 84.69 % respectively relative to the values obtained from the group treated with 2, 4 – DNPH alone (group 2; Table 1).

Table 1: Effect of DNPH and *H. sabdariffa* Extract on Blood Glucose and MDA Levels

Group #	Treatment	Glucose Concentration (mM)[a]	% Difference[b] 1	% Difference[b] 2	MDA (units/ml)[a] 10^{-6}	% Difference[b] 1	% Difference[b] 2
1	2.5ml H_2O/kg bd wt (control)	7.25 ± 0.38	–	+105.38	1.09 ± 0.08	–	–109.00
2	28 mg DNPH/kg bd wt	3.53 ± 0.25[c]	–51.31	–	10.97 ± 2.97[c]	+906.42	–
3	400 mg Extract/kg bd wt	7.50 ± 0.17[d]	+3.45	+112.50	0.96 ± 0.32[d]	–11.93	–91.25
4	400 mg Extract/kg bd wt + 28 mg DNPH/kg bd wt	6.94 ± 0.26[d]	–4.28	+96.60	1.69 ± 1.04[d]	+55.05	–84.59

[a] Values are mean ± SEM (n = 3)
[b] % Difference relative to either group 1 or 2
[c] Value significantly different from control (group 1) $P < 0.05$
[d] Value not significantly different from control ($P > 0.05$)

The changes in RBC and WBC counts, % PCV and Hb concentration due to 2, 4 – DNPH and *H. sabdariffa* extract are presented in Table 2. As can be seen treatment with 2, 4 – DNPH (group 2) significantly ($P < 0.05$) reduced rabbit blood RBC, PCV, Hb values but caused an increase in WBC count relative to the water treated control (group 1) by 24.46, 47.66, 44.79 and 84.60 % respectively (Table 2). However, the RBC count, % PCV and Hb concentration of rabbits pretreated with the extract before 2, 4 – DNPH administration (group 4) were significantly greater than those of the rabbit treated with 2, 4 – DNPH alone (group 2) by 28.72, 83.88 and 61.39 % respectively. The WBC count was significantly ($P < 0.05$) lower under this condition by 38.54 % (Table 2).

Table 2: Effect of DNPH and *H. sabdariffa* Extract on RBC, WBC, PCV and Hb Concentration

Group #	Treatment	RBC (counts/μL)[a] x 10^{11}	% Difference[b] 1	% Difference[b] 2	WBC (counts/μL)[a] x 10^3	% Difference[b] 1	% Difference[b] 2	PCV (%)[a]	% Difference[b] 1	% Difference[b] 2	Hb Concentration (g/dl)[a]	% Difference[b] 1	% Difference[b] 2
1	2.5ml H_2O/kg bd wt	376.33±4.06	-	+32.36	5.20 ± 0.20	-	-45.83	35.67 ± 1.45	-	+91.06	12.01 ± 0.58	-	-81.15
2	28 mg DNPH/kg bd wt	284.33±3.84[c]	-24.45	-	9.60 ± 0.31[c]	+84.60	-	18.67 ± 0.67[c]	-47.66	-	6.63 ± 0.49[c]	-44.79	-
3	400 mg Extract/kg bd wt	375.33±2.91[d]	-0.26	+32.01	5.40 ± 0.24[d]	+3.85	-43.75	35.87 ± 2.91[d]	+0.56	+92.13	12.70 ± 0.38[d]	+5.74	+91.55
4	400 mg Extract/kg bd wt + 28 mg DNPH/kg bd wt	366.00±2.53[d]	-2.74	+28.72	5.90 ± 0.29[d]	+13.46	-38.54	34.33 ± 0.88[d]	-3.76	+83.88	10.97 ± 0.18[d]	-8.66	+61.39

a, b, c, d See table 1 footnote for interpretations

DISCUSSION

In this investigation glucose and malondialdehyde levels in blood plasma and some haematological parameters such RBC count and WBC count, PCV and Hb concentration were used to measure both 2, 4 – DNPH – induced tissue damage and protective effect of ethanolic extract of *H. sabdariffa* petal against the same effect in rabbits. In agreement with past reports the results obtained in this study show that DNPH caused a depletion in blood glucose level (Maduka *et al*, 203; Ologundudu and Obi, 2005) increased lipid peroxidation (Ologundudu and Obi, 2005) and significant reduction in the levels of RBC, PCV, Hb and marked increase in WBC count (Jain and Hochstein, 1980; Maduka *et al*, 2003) which are indices of DNPH intoxication in the blood. Treatment of rabbits with 400 mg of *H. sabdariffa* red petal extract kg^{-1} body weight twice a day for 7 days before DNPH treatment caused considerably less haematotoxicity than with DNPH alone (Table 1 and 2) as evidenced by increased blood glucose and decreased blood MDA content (Table 1) as well as increased RBC count, % PCV and Hb concentration and decreased WBC count (Table 2) relative to DNPH – treated extract-free group. Again the results show that the group 3 (extract alone) values in all the parameters examined were not statistically significantly different from the water treated (control) group.

As indicated earlier in this report phenylhydrazine and its derivative 2, 4 – DNPH are capable of inducing haemolysis by initiating lipid peroxidation in membrane phospholipids (Jain and Hochstein, 1980). The lipid oxidation causes disruption of the bilayer and red cell integrity. Also as mentioned previously the above mechanism suggests an underlying process of oxidation. The hypothesis on which the present investigation is hinged is that *Hibiscus sabdariffa* petal extract would prevent lipid peroxidation and by extension red blood cell membrane damage if it possesses antioxidant action. Since the RBC count, % PCV, Hb concentration and MDA level in the blood of rabbits exposed to the extract before 2, 4 – DNPH show that the presence of the extract ensured that the integrity of red blood cells were not compromised following 2, 4 – DNPH administration, it appears that the extract possesses antioxidant action. Evidently this is in consonance with the report of Tseng *et al* (1997) that extract of the dried flower of the plant possesses antioxidant activity.

In our earlier reports (Obi *et al*, 2005; Ologundudu and Obi, 2005) the extract studied was aqueous extract and it showed antioxidant bioactivity. In the present study ethanolic extract was examined and found to possess the same antioxidant effect. This indicates that the principle(s) responsible for this biological action of the extract is both water and alcohol soluble.

The ability of *Sacoglottis gabonensis* stem bark extract and its isolate, bergenin, a polyphenolic isocoumarin (Robinson, 1975) to counteract the haemolytic action of 2, 4 – DNPH has been reported by others (Maduka *et al*, 2003). This is thought to be due to the ability of bergenin to exhibit antioxidant activity on account of its free hydroxyl constituents (Akintonwa and Maduka, 2005).

The extract from *H. sabdariffa* contains flavonoids (Langenhoven, *et al*, 2001) amongst which are two anthocyanins, namely cyanidin – 3 – monoglucoside and delphinidin – 3 – monoglucosides. Like bergenin these anthocyanins are polyphenols and have 4 and 5 free hydroxyl constituents respectively (Strack and Wray, 1989). The mechanism by which *H. sabdariffa* extract impaired the oxidant effects of 2, 4 – DNPH is largely unclear at present. All the same since phenolic hydroxyl groups are potent antioxidant bioactive principles and *H. sabdariffa* extract contains cyaniding – 3 – monoglucoside and delphinidin – 3 – monoglucoside (Langenhoven *et al*, 2001) which are richly endowed with free hydroxyl groups (Strack and Wray, 1989),. they may not be unconnected with the ability of the extract to counter the oxidant effects of 2, 4 – DNPH in the blood of the animals used in this study.

SUMMARY AND CONCLUSION

In this study rabbits have been used as experimental model to ascertain not only whether 2,4 – DNPH is haematotoxic but also to investigate the ability of ethanolic extract of *Hibiscus sabdariffa L* to impair the haematotoxicity. Like earlier reports (Jain and Hochstein, 1980; Clemens *et al*, 1984; Maduka *et al*, 2003) the present study shows that 2, 4 – DNPH, a derivative of phenylhydrazine is haematotoxic. This is evident from the

low RBC count, low PCV, low Hb concentration, increased blood MDA level and increased WBC in 2,4 – DNPH treated rabbits relative to the 2, 4 – DNPH – free group. Exposure of rabbits to the ethanolic extract of *Hibiscus sabdariffa* prior to 2, 4 – DNPH exposure caused remarkably decrease in 2, 4 – DNPH intoxication in the blood.

These results, therefore, show that ethanolic extracts of this flower, by some mechanism that are yet to be established, impair the haematotoxicity of 2, 4 – DNPH. However, since it was effective against lipid peroxidation (measured by MDA level) it evidently possesses antioxidant potential.

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