

EFFECT OF ETHANOLIC EXTRACT OF *HIBISCUS SABDARIFFA* L. ON 2, 4-DINITROPHENYLHYDRAZINE-INDUCED LOW GLUCOSE AND HIGH MALONDIALDEHYDE LEVELS IN RABBIT BRAIN AND LIVER

A. OLOGUNDUDU, A. O. LAWAL, O. G. ADESINA and F. O. OBI

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ABSTRACT

The effect of *Hibiscus sabdariffa* calyx extract on 2, 4 - dinitrophenylhydrazine (2, 4 - DNPH) - induced low glucose and high malondialdehyde levels in rabbit brain and liver have been examined. Relative to the control, 2, 4 - DNPH treatment significantly decreased ($P \leq 0.05$) brain glucose from 1.93 ± 0.63 mM (control) to 0.14 ± 0.03 mM (2, 4 - DNPH treatment only) but increased malondialdehyde level significantly ($P \leq 0.05$) from $0.88 \pm 0.47 \times 10^{-6}$ units/mL (control) to $4.02 \pm 0.61 \times 10^{-6}$ units/mL (2, 4 - DNPH treatment only). Liver glucose was decreased from 8.31 ± 0.11 to 3.53 ± 0.06 mM while its malondialdehyde level was increased from $0.91 \pm 0.23 \times 10^{-6}$ to 14.20 ± 2.10 units/mL. Compared to the level of these parameters in the brain and liver of rabbits treated with 2, 4 - DNPH alone, treatment of rabbits with *Hibiscus sabdariffa* calyx extract prior to 2, 4 - DNPH led to significant ($P \leq 0.05$) increase in brain glucose (1.72 ± 0.20 mM from 0.14 ± 0.03 mM) and liver glucose (7.83 ± 1.19 mM from 3.52 ± 0.06 mM) but a decrease in brain MDA ($1.15 \pm 0.52 \times 10^{-6}$ units/mL from $4.02 \pm 0.61 \times 10^{-6}$ units/mL) and liver MDA ($2.43 \pm 1.01 \times 10^{-6}$ units/mL from $14.20 \pm 2.10 \times 10^{-6}$ units/mL). These findings indicate that ethanolic extract of *H. sabdariffa* calyx protects the brain and liver from 2, 4 - DNPH - induced glucose depletion and lipoperoxidation.

KEYWORDS: 2, 4 - DNPH; Brain glucose; Liver glucose; MDA; Ethanolic extract; *Hibiscus sabdariffa*.

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 calyx 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 as well as other free radical - induced disease conditions (Harman, 1984; Wolff *et al*, 1986) and biochemical changes. Phenylhydrazine and its derivative 2, 4 - DNPH are toxic agents. Their toxic action has been attributed to their ability to undergo autooxidation. This increased oxidant potential enables them to oxidize enzymes, membrane proteins and haemoglobin. Phenylhydrazine is able to initiate lipid peroxidation in membrane phospholipids (Jain and Hochstein, 1980) while 2, 4 - dinitrophenylhydrazine has been shown to be capable of inducing lipid peroxidation and low glucose level in rabbit (Ologundudu and Obi 2005) and rat (Maduka *et al*, 2003) brains. The ability of 2, 4 - DNPH to induce lipid peroxidation and cause low glucose concentration makes it an appropriate model toxicant for testing the claim that the extract of *H. sabdariffa* L calyx can protect tissue from oxidative stress -

induced damages and other attendant biochemical changes. 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 Laboratories (US). Glucose oxidase kit was obtained from Randox Laboratories Ltd (UK) and chow (growers mash) from BFFM, Ewu, Nigeria.

Preparation of extract

Hundred grams of dried *Hibiscus sabdariffa* calyx 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 % aqueous ethanol, put into a bottle, sealed and left at 4°C until required.

Treatment of animals and collection of brain and liver 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 H₂O kg⁻¹ body weight. At the end of the 7th day all rabbits were fasted overnight. Following the

A. Ologundudu, Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.
A. O. Lawal, Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.
O. G. Adesina, Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.
F. O. Obi, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin-City, Edo State, Nigeria.

overnight fast rabbits in groups 2 and 4 received 28 mg 2, 4 – DNPB 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 – DNPB treatment, the rabbits were anaesthetized in a diethylether saturated chamber. While under anaesthesia the abdominal region was opened to expose and excise the liver. To excise the brain, each rabbit was laid on the dissection board dorsal part up. The cranium was slit and opened from the base of the brain near the atlas, the first cervical bone, after the skin and fur over the head have been removed. The exposed brain was then excised. Twenty percent (20%) homogenates of the liver and brain of each rabbit were prepared separately in ice-cold saline. Each homogenate was centrifuged at 3500 rpm (Uniscopo Model SM 902 B Bench centrifuge) for 10 minutes in order to obtain the supernatant of each homogenized brain and liver. The supernatants were collected and left at – 20°C until required.

Biochemical Assay Protocol and Statistical Analysis

Determination of brain and liver glucose

Glucose determination was based on the procedure described in Randox Glucose Oxidase Kit assay leaflet (Randox Laboratories Ltd, U.K.). Twenty microlitres of each homogenate supernatant or standard was mixed with 2 ml of the glucose oxidase reagent and incubated for 25 minutes at room temperature. The absorbance of the standard and samples were measured at 500 nm against the reagent blank within 60 minutes.

Determination of brain and liver malondialdehyde

This was based on the method of Buege and Aust (1978). One millilitre of each homogenate supernatant 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 x 10⁵ M⁻¹ cm⁻¹.

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 ≤ 0.05.

RESULTS

Results of the effects of 2, 4 – DNPB and *H. sabdariffa* extract on rabbit brain glucose and MDA as well as on the liver glucose and MDA are presented in figures 1 and 2 respectively. The data show that 2, 4 – DNPB treatment significantly (P ≤ 0.05) reduced brain glucose but increased the MDA level when compared to the control, group 1 (Fig. 1 A and B). Prior treatment of rabbits (group 4) with the extract before 2, 4 – DNPB administration led to significant (P ≤ 0.05)

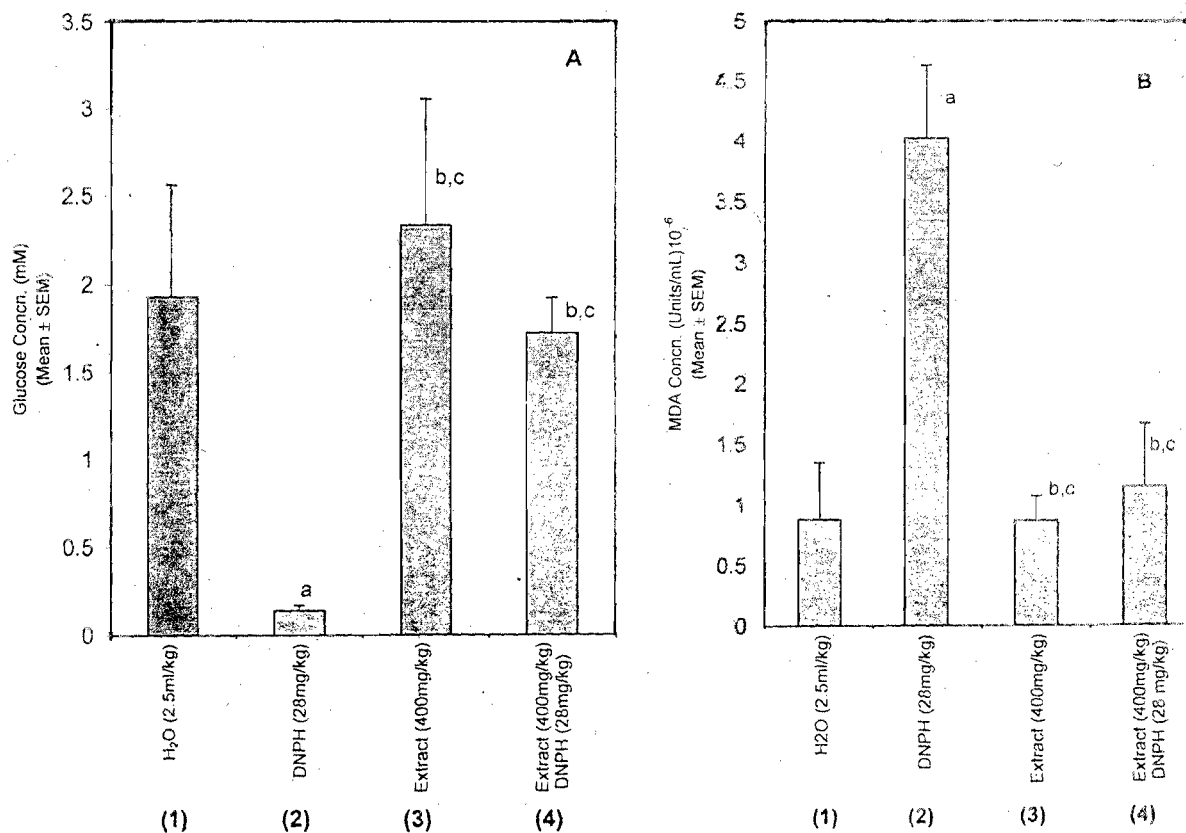


Fig. 1: Effects of DNPB and *H. sabdariffa* Extract on Brain Glucose (A) and MDA (B) Concentrations

- ° Value significantly different from group 1 value (P ≤ 0.05)
- Value not significantly different from group 1 value (P ≥ 0.05)
- Value significantly different from group 2 value (P ≤ 0.05)

increase in brain glucose but a reduction in MDA level when compared to the values obtained from the group treated with 2, 4 – DNPB alone (group 2; Fig. 1). The results presented in figure 2, show that 2, 4 – DNPB when administered alone caused significant (P ≤ 0.05) reduction in the level of glucose in the liver but a significant (P ≤ 0.05) increase in MDA when

compared to the water treated controls. When 2, 4 – DNPB treatment was preceded by the ethanolic extract of *H. sabdariffa* if the 2, 4 – DNPB – induced liver glucose reduction and MDA increase were impaired relative to the water treated control.

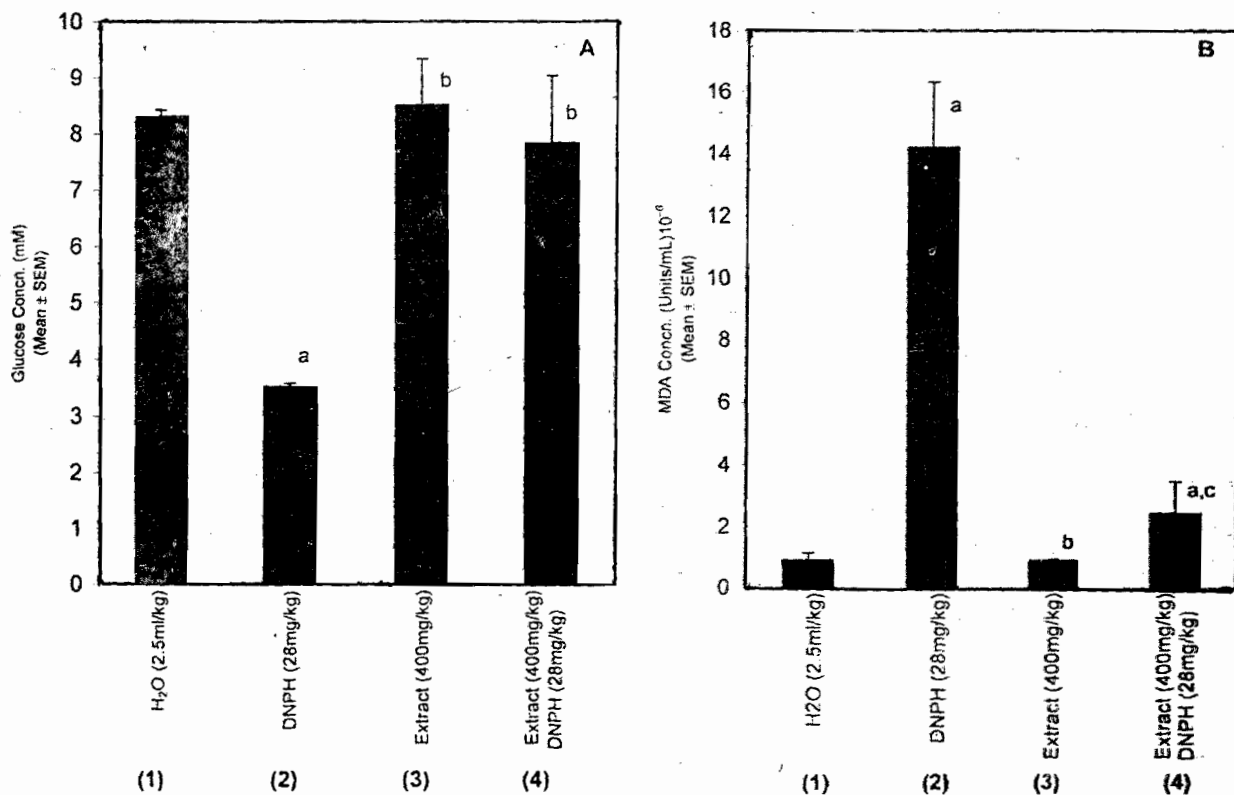


Fig. 2: Effects of DNP and *H. sabdariffa* Extract on Liver Glucose (A) and MDA (B) Concentrations.

- ^a Value significantly different from group 1 value ($P \leq 0.05$)
^b Value not significantly different from group 1 value ($P \geq 0.05$)
^c Value significantly different from group 2 value ($P \leq 0.05$)

DISCUSSION

In this study, glucose and malondialdehyde levels in brain and liver have been used as indices of 2, 4 – DNP – induced tissue damage and prevention by ethanolic extract of *H. sabdariffa* calyx against the same effect in rabbits. In harmony with past reports (Maduka *et al*, 2003; Ologundudu and Obi, 2005) the results obtained in this study show that 2, 4 – DNP caused depletion in the concentration of brain and liver glucose. The current report also shows that this toxic agent caused increased brain and liver lipid peroxidation, which is also in consonance with earlier reports (Maduka and Okoye, 2002; Ologundudu and Obi, 2005). Treatment of rabbits with 400 mg of *H. sabdariffa* calyx extract kg⁻¹ body weight twice a day for 7 days before DNP treatment caused considerably less brain and liver intoxication than with 2, 4 – DNP alone (Figures 1 and 2) as evidenced by increased brain and liver glucose and their decreased MDA contents. Again the results show that the values obtained from the group of rabbits treated with the extract alone were not statistically significantly different from the water treated (control) group (see figures 1A & B; 2A and B).

As indicated earlier in this report phenylhydrazine and its derivative 2, 4 – DNP are capable of inducing lipoperoxidation in membrane phospholipids (Jain and Hochstein, 1980), while the derivative has been shown to be both blood and brain glucose depletor (Maduka *et al*, 2003; Ologundudu and Obi, 2005). Lipid peroxidation causes disruption of lipid bilayer and cellular integrity. This mechanism of action suggests an underlying process of oxidation. The hypothesis on which this investigation is hinged is that *Hibiscus sabdariffa* calyx extract would prevent lipid peroxidation if it possesses antioxidant action. Since the MDA levels in brain and liver of rabbits exposed to the extract before 2, 4 – DNP show that the presence of the extract ensured that the toxicant – induced MDA production was considerably reduced, it is evident that the extract possesses antioxidant

principles. This finding agrees with that of Tseng *et al* (1997) who found that the extract has protective effect against oxidative stress in rat primary hepatocytes. Again it agrees with that of Wang *et al* (2000) who found the extract protective against tert – butyl hydroperoxide – induced hepatotoxicity. Tert – butyl hydroperoxide is a strong oxidant and an oxidative damage inducer (Tseng *et al*, 1997).

The mechanism by which DNP causes glucose depletion in tissues remains to be clearly understood. Maduka *et al* (2003) have however proposed that tissue damage by DNP stimulates hormonal responses which likely culminates in glucose mobilization. The mobilized glucose molecules are believed to be subsequently catabolized for the provision of extra energy required for the repair of damaged tissues.

The mechanism by which *H. sabdariffa* prevents DNP – induced changes in MDA level is also yet to be sufficiently understood. It is likely that the extract impaired DNP – induced free radical formation and/or propagation. It is also possible that the extract boosts or complements the antioxidant defense system *in vivo*. These processes singly or in combination may therefore, be responsible for the ability of the extract to prevent DNP – induced cellular damage via lipid peroxidation (Jain and Hochstein, 1979; Maduka *et al*, 2003).

The ability of *Sacoglottis gabonensis* stem bark extract and its isolate, bergenin, a polyphenolic isocoumarin (Robinson, 1975) to counteract DNP – induced brain glucose depletion and MDA increase has been reported (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 – monoglucoside. Like bergenin these anthocyanins are polyphenols (Figure 3) and have 4 and 5 free hydroxyl constituents respectively (Strack and Wray, 1989). So although the mechanism of action of the

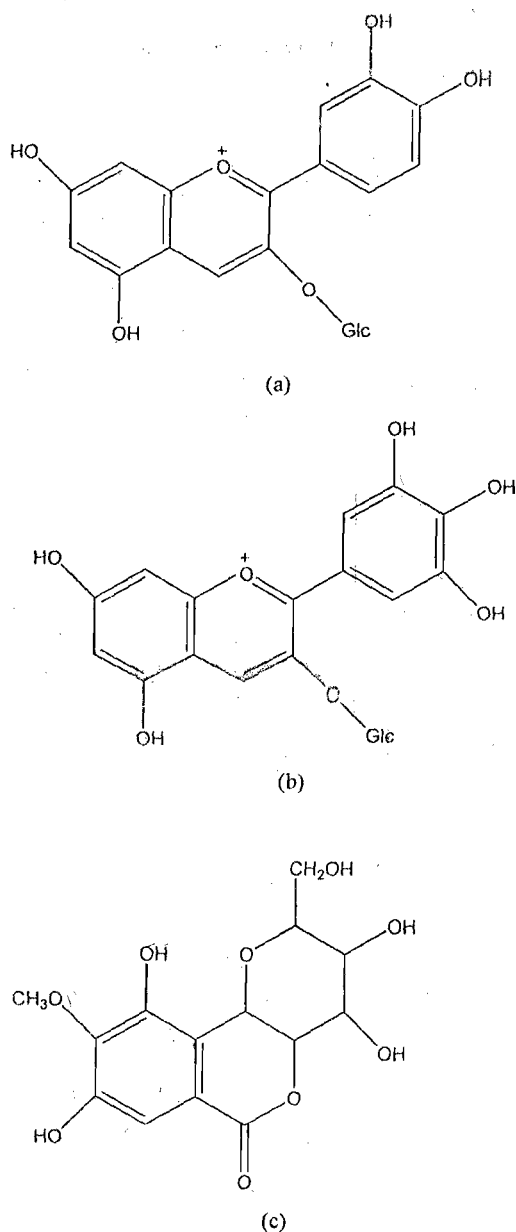


Fig. 3: Structure of cyaniding - 3-monoglucoside (a), delphinidin - 3 - monoglucoside (b) and bergenin (c). Structures of a and b were adapted from Strack and Wray (1989) while the structure of c was adapted from Robinson (1975).

constituents of the extract is yet to be understood the presence of the phenolic hydroxyl groups may not be unconnected with the potency of the extract to counter the oxidant effects of 2, 4 - DNPH in the brain and liver of the rabbits used in this study.

SUMMARY AND CONCLUSION

In this study rabbits have been used as experimental model to ascertain that 2, 4 - DNPH is able to induce low glucose and high malondialdehyde levels in both brain and liver. Also established is the ability of ethanolic extract of *Hibiscus sabdariffa* L to impair these toxic effects of 2, 4 - DNPH. Like earlier reports (Maduka *et al*, 2003) the present study shows that 2, 4 - DNPH, a derivative of phenylhydrazine is a glucose depletor and lipoperoxidation enhancer. These are evident from the low brain and liver glucose as well as the increased malondialdehyde levels 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 remarkable decrease in 2, 4 - DNPH intoxication in the brain and liver.

These results, therefore, show that ethanolic extracts of this flower, by some mechanisms that are yet to be understood impair the neurotoxic and hepatotoxic aspects of 2, 4 - DNPH highlighted in this study. Furthermore, since it was effective against lipid peroxidation (measured by MDA level) it evidently possesses antioxidant bioactive principles.

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