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Short Communication

Effects of Some Oral Hypoglycaemic Drugs on Erythrocyte Nicotinamide Adenine Dinucleotide Hydrogen Diaphorase (E.C.1.6.4.3) Activity of Wistar Albino Rats (*Rattus rattus*)

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Abstract. The *in vivo* effects of three oral hypoglycaemic drugs, daonil (a glubenclanude), diabenes (a sulphonylurea) and glucophage (a metformun) on erythrocyte nicotinamide adenine dinucleotide hydrogen (NADH) activity of Wistar albino rats *(Rattus rattus*) were monitored at drug concentrations of 0.00, 0.01, 0.02 and 0.03 mg/200 g body weight. The effects of the drugs were monitored at a pH 8.0 and 37 °C for 14 days at intervals of 1, 2, 6 and 14 day(s) following administration of each drug. Three rats were used per each drug concentration per time interval (days). Daonil significantly ($P<0.05$) activated NADH-diaphorase activity in a concentration dependent manner with an optimal activation (11.44 ± 0.82) obtained at a concentration of 0.03 mg/200 g body weight and on the sixth day of drug administration. The increase in enzyme activity following drug administration was progressive with time duration (days); maximum effect was obtained on the 6^{th} day with a decline on the 14^{th} day. At 0.03 mg/200 g body weight, NADH activities (iu/L) of 20.53 ± 0.57 were obtained on the 6^{th} day with glucophage. Comparatively, the activation of the erythrocyte enzyme by the drug (on the 6^{th} day of administration) was in the order: Glucophage >Daonil>Diabenese. Diabenese had no significant effect.

Keywords: daonil, diabenese, glucophage, erythrocyte, NADH, hypoglycaemia

The red cell nicotinamide adenine dinucleotide hydrogen (NADH) dependent methaemoglobin reductase or diaphorase I enzyme (Breaking *et al.,* 1951; Gibson and Harrison, 1947), protects the erythrocyte from an accumulation of methaemoglobin. Methaemoglobin is haemoglobin in which the group (iron II) is oxidized to iron III (Sauve, 2008). Small amounts of methaemoglobin are produced continuously but the proportion of total haemoglobin that is present as methaemoglobin is maintained at about 1% by the action of an NADH dependent methaemoglobin reductase; also called NADH diaphorase II (Neuwrit and Ponka, 1977) and NADH ferricynide reductase (Board, 1981) and NADH cytochrome 65 reductase.

A methaemoglobin reductase concentration that is greater than 1% occurs if the rate of methaemoglobin formation exceeds its rate of reduction. Also methaemoglobin is not just incapable of binding oxygen, the oxidation of one or more of the heme iron atoms in the tetramer distort the tetramers structure (Ajamar *et al.,* 2012; Haymond *et al.,* 2005). As a result, the remaining non-oxidized heme sub-units bind oxygen avidly and also release it less efficiently.

The deficiency of NADH methaemoglobin reductase is genetically transmitted as an autosomal recessive characteristic chromosome (Jaffe, 1959). Also biochemical variants of this enzyme has been reported (West *et al.,* 1967).

Oral hypoglycaemic drugs vary in their mode of excretion and duration of action. Chloropropamide (diabenase) has a fairly prolonged biological action and increased potency/ weight ratio (Kaln and Schechter, 1993). The biguanides are oral hypoglycaemic drugs and their effects are also directed on oxidative phosphorylation (Ellenhorn and Barceloux, 1994).

The oral hypoglycaemic drugs, diabenese, daonil and glucophage used in this study were obtained from Nigeria-German drugs, Plc (Lagos, Nigeria). Other chemicals used for the *in vitro* analysis were from BDH (Poole Dorset, U.K) and Sigma Chemical Company (St. Louis, Missouri, USA).

Wistar albino rats, (*Rattus rattus*) 12-14 weeks, weighing

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between 200-220 g and derived from a colony maintained at the animal house unit of the Department of Biochemistry, University of Port Harcourt, were used for the experiment. The animals which were kept in cages (within a temperature of 25±2 °C) were fed with standard laboratory chow (Pfizer feeds Plc, Nigeria) and water *ad libitum*. The animals were allowed to acclimatize for two weeks.

For the *in vivo* test, a total of 144 rats (with average weight of 210.15 ± 10.2 g) were used. Rats were divided into three groups; diabenese group, daonil group and glucophage group. Each group had 48 test rats while 12 rats served as control. Each of the drugs was administered to the rats at four different concentrations of 0.00 g/mg (control), 0.01 mg, 0.02 mg and 0.03 mg per 200 g body weight. The administration of the drugs to the rats was orally by intubations. The drugs at each of the concentrations were administered to the rats at day one, two, six and fourteen. Since water was used for the solubization of the drugs, the control rats were administered the equivalent volume (0.2 mL) of water in each case.

On each of the day(s) interval and at 3 h after the administration of the drug, three rats from each of the drug concentration groups were sacrificed after blood collection. Rats from the 0.00 mg concentration group served as control. Blood from the rats were collected by cardiac puncture into heparinized anticoagulant bottle and used for analysis as required.

NADH determination. The technique used for NADH assay is based on the method described by Board (1981). The assay mixture of 2 mL contained 0.1m tri HCl with 0.5 mM EDTA, PH 8.0, 0.2 mM NADH, 0.2 mM k3Fc (CN)6 and an aliquot (0.02 mL) of haemolysate; A tris buffer NADH mixture was first incubated for 10 min at 30 °C. The reaction was then initiated by the addition of 0.22 mL of ferricyanide-haemolysate mixture (in the ratio 10:1) pre-mixed a minute before addition. The rate of decrease in optical density of the system at 340 nm was measured for 10 min at 30 sec intervals against the blank containing the reaction mixture without haemolysate. The reaction was carried out at 30 °C rather than the usual 37 °C because the enzyme is unstable at higher temperature (Beutler, 1984).

Statistical analysis. Results (Tables 1-3) of Biochemical estimations were reported as mean ± SD and statistical analysis was performed using the students t-test of statistical significance at 95% confidence level (P=0.05) (Brookes *et al.,* 1979). Data were also analyzed by one-way analysis of variance (ANOVA) using SPSS/PC package and difference between means were compared using Duncan's (1955) multiple range test.

The *in vivo* study showed that rat erythrocyte NADH diaphorase activity was significantly elevated in the

Table 1. *In vivo* effect of Daonil on erythrocyte NADH diaphorase activity of rat at pH 8.0 and 30 °C NADH (iu/L)

Daonil (mg/200g body weight)	Day 1 X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	6.19 ± 0.81	6.70^{a} ± 0.65	6.91^{b} ± 0.42
0.01	6.66^{a} ± 0.83	7.83^{b} ± 0.59	10.17^{e} ± 0.69	10.35^{e} ±0.96
0.02	7.00^{b} ± 0.02	8.50^{c} ± 0.01	10.35^{e} ± 0.97	11.01^{f} ± 1.41
0.03	7.43^{b} ± 0.01	9.41^{d} ± 0.14	11.44^{f} ± 0.82	7.04^{b} ± 1.03

Table 2. *In vivo* effect of Diabenese on erythrocyte NADH diaphorase activity of rat at pH 8.0 and 30 °C.

Diabenes (mg/200g body weight)	Day 1 X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	5.70^{a} ±0.81	6.70^{b} ±1.16	6.91^{b} ±0.42
0.01	7.00^{b} ± 1.40	6.91^{b} ±0.09	7.40^{c} ± 0.01	7.39^{e} ±0.22
0.02	6.95^{b} ±1.04	8.33^{d} ± 0.54	7.34^{e} ± 0.45	8.91^{d} ± 0.62
0.03	7.09^{b} ± 0.05	9.41^{d} ± 0.14	7.71^{c} ± 0.98	10.66^{f} ± 0.52

Table 3. *In vivo* effect of Glucophage on erythrocyte NADH diaphorase activity of rat at pH 8.0 and 30 °C.

Glucophage (mg/200 g body weight)	Day I X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	5.70^{a} ± 0.81	6.70^{a} ± 1.16	6.91^{b} ± 0.42
0.01	8.18^{b} ± 0.23	12.06^{e} ± 0.69	14.74^{f} ± 0.04	17.57^{g} ± 0.08
0.02	9.07^{b} ± 0.06	14.71^{f} ± 0.60	19.50^{h} ± 0.15	17.87^{g} ± 0.09
0.03	10.56^{b} ± 0.67	18.20^{g} ± 0.43	20.55^{h} ± 0.57	17.92^{g} ± 0.15

Values with the same superscript letters (in Table 1-3) are not statistically significant at 95% confidence level (P<0.05).

presence of the oral hypoglycaemic drugs, daonil, glucophage and diabenese. The maximal *in vivo* effect of the drugs on rat erythrocyte NADH diaphorase activity was obtained on the sixth day of drug administration with a significant ($P<0.05$) decline on the fourteenth day (Tables 1-3). Comparatively, the effect of the three drugs on the enzyme was in the order: Glucophages>Daonil>Diabenese. The result of this work has shown that NADH diaphorase activity was enhanced following the administration of glucophage and daonil at concentration dependent manner. The effect of glucophage on NADH diaphorase activity was greater than that of daonil for the various concentrations. Also the effect was significantly different than that of the control.

On the other hand, diabenese had no significant effect on the erythrocytes enzyme activity. The activation of the enzyme by daonil and glucophage confirms the work of Chasseaud (1979) in which he proposed that erythrocyte enzyme functions to detoxify red cell xenobiotics. The increase in the rate of oxidation of haemoglobin leads to the accumulation of methaemoglobion which forms granules. This leads to an increase in the rate of its destruction by the spleen. As a result, the patient becomes anaemic (Mc Gilvery, 1979; Neutwrit and Ponka, 1977). Thus NADH diaphorase helps in curbing this effect as has been demonstrated by this work. This observation perfectly agrees with the suggested role of erythrocyte NADH diaphorase in xenobiotics detoxication (Mc Gilvery, 1979).

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