Afr J Med med. Sci. (1990) 19, 5–9.

Effects of cows' urine concoction with and without tobacco leaves on plasma glucose concentration in fasted rats

D. D. O. OYEBOLA* AND A. A. FASANMADE
Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Summary

The effects of a full preparation (FP) and a modified preparation (MP) of cows' urine concoction (CUC) on the level of plasma glucose in fasted male Wistar rats were investigated. The results showed that FP caused significant hyperglycaemia; while the MP and a control injection using 0.9% saline had no effect on plasma glucose. Hexamethonium, prazosin, propranolol and combined prazosin and propranolol did not abolish the hyperglycaemic response to FP. The mechanism of the hyperglycaemic response to FP and the reason why MP did not produce hypoglycaemia are obscure. The result of this study did not agree with the findings of CUC-induced hypoglycaemia reported by earlier workers.

Résumé

On a étudié les effets de la préparation intégrale (PI) et de la préparation modifiée (PM) de la mixtion d'urine de vache (MUV) sur le niveau du plasma de glucose chez les rats de Wistar mâles. Les résultats ont montré que PI a causé une hyperglycémie significative alors que PM et une piqûre de contrôle avec une saline de 0.9% n'ont pas eu d'effet sur le plasma ni le propranolol, ni une combinaison de prazosin et de propranolol n'a éliminé la réponse hyperglycémique de la PI. Le mécanisme de la réponse hyperglycémique à la PI et la raison pour laquelle la PM n'a pas produit de la hypoglycémie restent inconnus. Le résultat de cette étude n'était pas en accord avec celui de l'étude avec MUV rapportée par les chercheurs précédents.

*To whom correspondence should be addressed.

Introduction

Cows' urine concoction (CUC) is commonly used in the treatment of convulsive seizures in children in some parts of Nigeria. Its use has resulted in severe poisoning and death in some children [1–3]. Onadeko and Adetuyibi [4] identified CUC as the leading cause of acute poisoning in children in western Nigeria. The very toxic nature of CUC had been confirmed in experimental animals [5–7]. Recently, Ayorinde *et al.* [8] analysed two samples of CUC by direct application of gas chromatography–mass spectrometry (GC–MS). Gas chromatographic traces from their studies indicated over fifty compounds in CUC, the major constituents include nicotine, *p*-cresol and benzoic acid.

Hypoglycaemia is a feature of CUC poisoning in children [3]. Ojewole and Olusi [9] demonstrated hypoglycaemic effect due to CUC in fasted rats, while Grange [10] demonstrated CUC-induced hypoglycaemia in rabbits. However, Oyebola [11] did not find hypoglycaemia following CUC administration to fasted dogs. The reason for this difference is obscure and the discrepancies in the results of these studies seemed sufficiently important to warrant further studies on the glycaemic effect of CUC.

The design of this study is based on the following information on some of the components of CUC in relation to blood glucose. Three components of CUC have been reported to affect blood glucose level. These are tobacco leaves (*Nicotiana tabacum*), onions (*Allium cepa*) and garlic (*Allium sativum*). The principal alkaloid in leaves of *N. tabacum* is nicotine which accounts for 95% of the total alkaloid [12]. The remaining 5% is made up of secon-

dary alkaloids, the most important of which are nornicotine, anabasine and anatabine [12]. Nicotine causes hyperglycaemia when administered to fasted dogs and rats [13], while onions and garlic cause hypoglycaemia in rabbits [14–16]. Apart from the alkaloids, no other component of tobacco leaves has been reported to affect blood glucose. From this the effect of CUC on blood glucose may therefore be determined by the relative concentration of the hyperglycaemic agents in *N. tabacum* and the hypoglycaemic agents in onion and garlic in the concoction. A possible explanation for the hypoglycaemia reported by Ojewole and Olusi [9] and Grange [10] may therefore be that the CUC these workers used contained more hypoglycaemic agents than hyperglycaemic agents; while the two glycaemic agents may be about equal in the CUC used by Oyebola [11]. If this explanation is correct, then we hypothesized that a CUC preparation without *N. tabacum* leaves (modified preparation) will produce marked hypoglycaemia, while a CUC preparation containing *N. tabacum*, onions and garlic (full preparation) may produce hyperglycaemia, normoglycaemia or hypoglycaemia depending on the relative concentration of the hyper- to the hypoglycaemia agents. The present study was designed to test this hypothesis.

Materials and methods

A full preparation of CUC (FP) and a modified preparation of CUC without leaves of *Nicotiana tabacum* (MP) were prepared in the laboratory according to the method of Oyebola and Adetuyibi [17]. The effects of FP and MP on plasma glucose concentration were studied using male albino Wistar rats weighing 350–380 g. Our method was similar to that described by Ojewole and Olusi [9]. The rats were selected randomly into three groups of eight. Each rat was fasted for 18–24 h before use. Anaesthesia was induced with pentobarbitone sodium (50 mg/kg i.p.). After cannulating the trachea, the left femoral vein and artery were cannulated for drug administration and blood sampling. Each animal was heparinized (100 units heparin/100 g body weight) to prevent blockage of the cannulae by blood clot. A 1-h recovery period was allowed post-surgery.

Experimental procedure

A resting (control) blood sample was obtained from each animal before it was injected. Rats in group 1 were injected with FP, 0.05 ml/100 g body weight intravenously, while group 2 rats were injected with MP, 0.05 ml/100 g body weight intravenously. The injected CUC was washed in with 0.05 ml of 0.9% saline on each occasion. Blood samples (50 µl per sample) were withdrawn through the arterial cannula at 10, 20, 30, 60 and 90 min post-injection, and the plasma glucose was determined using a Beckman glucose analyser. The third group of rats was given 0.9% saline i.v. (0.05 ml/100 g body weight plus 0.05 ml saline used for flushing) and samples for plasma glucose analysis were taken as above. As normal saline has no significant pharmacological activity, this served as a control against which the effects produced by FP and MP could be compared.

The preliminary results with FP showed that it caused marked hyperglycaemia. In order to investigate the mechanism of this hyperglycaemia, four groups of six rats (350–380 g) were selected randomly. One group was pretreated with hexamethonium bromide (400 µg/100 g body weight i.v.); the remaining three groups were pretreated with prazosin (20 µg/100 g i.v.) and propranolol (50 µg/100 g). The experiment was then repeated by injecting FP, 0.05 ml/100 g body weight after each treatment.

The mean glucose values at 0, 10, 20, 30, 60 and 90 min for groups 1, 2 and 3 were compared using analysis of variance. A similar analysis was carried out on the glycaemic response to FP before and after administering the different drugs.

Results

The results are shown in Fig. 1 and Table 1. In the group given FP, after CUC injection, plasma glucose rose from a resting value of 113.1 ± 6.9 mg/dl (mean ± s.d.) to a peak of 199.1 ± 26.7 mg/dl attained 20 min post-injection. The plasma glucose level remained elevated throughout the 90 min, post-injection observation period. All post-injection plasma glucose levels were significantly higher than the control value for the group ($P < 0.0001$).

In the group given MP, there was a moderate increase in plasma glucose from a resting value

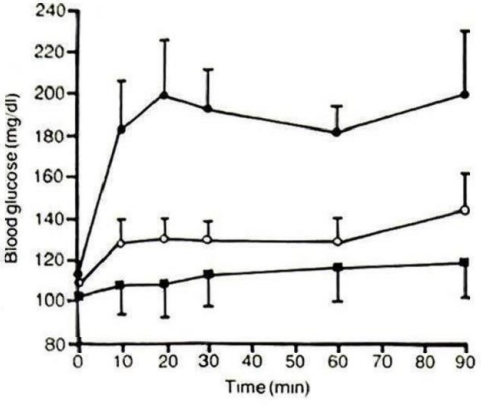

Fig. 1. Plasma glucose levels (mg/dl) in rats given FP of CUC (●), MP (○) and normal saline (■).

of 108.3 ± 6.2 mg/dl to a peak of 128.2 ± 11.5 mg/dl attained 10 min post-injection. Plasma glucose remained slightly elevated throughout the post-injection observation period. The increases in plasma glucose were, however, not statistically significant when compared with the control value for the group ($P = 0.10$).

In the control group, there was no change in plasma glucose from the resting value of 102.6 ± 7.2 mg/dl throughout the period of observation.

A comparison of corresponding plasma glucose levels in groups 1 and 2 showed that except for the resting values, all other values showed that the glycaemic response in group 1 was significantly higher than in group 2 ($P < 0.0001$). A comparison between groups 1 and 3 gave similar results. A similar comparison between groups 2 and 3 showed that the glycaemic response in group 2 was not significantly higher than in group 3 ($P = 0.07$).

Table 1 shows the glycaemic response in the untreated rats given FP and in rats treated with hexamethonium, prazosin, propranolol or a combination of prazosin and propranolol before being challenged with FP. The results showed that none of these treatments abolished the hyperglycaemic response to CUC. It should also be noted that the control plasma glucose level in the prazosin treated rats rose to 140.5 ± 14.8 mg/dl compared to a control value of 113.1 ± 6.9 mg/dl in the untreated group. This

Table 1. Plasma glucose levels (mg/dl) in untreated, ganglion blocked or adrenoceptor-blocked rats given FP of CUC

		Post-injection period (min)				
	Control	10	20	30	60	90
Untreated group and FP	113.1 ± 6.9	182.7 ± 24.5	199.1 ± 26.7	191.4 ± 19.4	181.4 ± 12.3	199.2 ± 31.5
Hexamethonium (400 μg/100 g) and FP	126.5 ± 15.9	173.6 ± 21.5	186.6 ± 27.4	184.8 ± 29.2	166.8 ± 30.7	172.5 ± 38.7
Prazosin (20 μg/100 g) and FP	140.5 ± 14.8	201.8 ± 13.6	218.5 ± 16.1	226.8 ± 18.6	214.3 ± 23.7	242.3 ± 44.3
Propranolol (50 μg/100 g) and FP	108.1 ± 14.1	179.1 ± 29.1	185.3 ± 33.9	174.0 ± 39.2	148.1 ± 30.8	162.6 ± 31.1
Combined prazosin (20 μg/100 g) and propranolol (50 μg/100 g) and FP	116.7 ± 11.9	197.7 ± 25.5	209.5 ± 23.4	213.5 ± 24.0	212.2 ± 38.3	215.2 ± 48.9

Values represent means ± s.d.

increase was highly significant ($P = 0.003$). An increase in control plasma glucose was not observed in the propranolol-treated rats and in the group given combined prazosin and propranolol injections. In the latter it is important to note that the propranolol injections were given before the prazosin injections in all cases.

Discussion

The hyperglycaemia reported in this study is the first report of hyperglycaemia following CUC administration. Previous authors reported either hypoglycaemia [3,9,10] or normoglycaemia [11] following CUC administration. The difference in the present results and those of Ojewole and Olusi [9] is most probably not due to a species or dosage difference because both studies used Wistar rats and comparable doses of CUC. Although the CUC used by Ojewole and Olusi [9] was prepared by a herbalist while that used by Oyebola [11] was prepared in the laboratory, the recent studies of Ayorinde *et al.* [8] have shown that there was no quantitative difference between the herbalist-prepared CUC and the CUC prepared in the laboratory according to the method of Oyebola and Adetuyibi [17].

We are unable to explain the difference in our results and those of Ojewole and Olusi [9] and Grange [10]. On the basis of our hypothesis, it is tempting to suggest that the reason for the hyperglycaemia observed with FP in the present study is that FP contains a higher concentration of hyperglycaemic agents than hypoglycaemic agents. The absence of hypoglycaemia in the MP however, makes this suggestion untenable. An important point to note is that the CUC samples used by Ojewole and Olusi [9] and Grange [10] were prepared by traditional healers, whereas the CUC samples used by Oyebola [11] and that used in the present study were prepared in the laboratory. Although Ayorinde *et al.* [8] could not demonstrate any quantitative difference in the compounds contained in a herbalist-prepared CUC and CUC prepared in the laboratory according to the method of Oyebola and Adetuyibi [17], a difference may well exist in the pharmacological action of the herbalist-prepared CUC and the laboratory-prepared CUC, at least, with respect to its action on blood glucose. This possibility deserves a more detailed investigation because the results may have important implications for attempts to standardize traditional medicinal preparations in the laboratory. It is relevant to add, however, that the action of a herbalist-prepared CUC on the central nervous system in mice and in rats [5] and on the cardiorespiratory systems in cats and dogs [6,7] are not different from the action of a laboratory-prepared CUC on the CNS in mice and rats [Oyebola and Ariwodola, unpublished observations] and on the cardiorespiratory systems in rats [18].

An unexpected finding in the present study is the failure of MP to produce hypoglycaemia. This was a reversal of our expectation and is difficult to explain.

Also, the failure of hexamethonium, prazosin, propranolol or a combination of prazosin and propranolol to prevent the hyperglycaemic response to CUC suggests that this response is not mediated via the autonomic nervous system and/or the adrenal catecholamines, and that adrenoceptors are not involved in the response. This conclusion is at variance with the results of Oyebola and Ariwodola [18] which showed that the cardiorespiratory effects of CUC are mediated via the autonomic nerves and the adrenal catecholamines. It may be informative to investigate the role of other hyperglycaemic factors such as glucagon, thyroxine, growth hormone or the adrenal glucocorticoids in this response. Until additional information is available, the mechanism of the hyperglycaemic response to CUC in the study will remain obscure.

The elevation of resting plasma glucose in animals pretreated with prazosin suggests that the alpha-adrenoceptors provide a tonic damping effect on blood glucose level in rats. Removal of this damping effect by an alpha-adrenoceptor blocker results in the significant increase in resting plasma glucose. Beta-adrenoceptor blocker alone did not have such an effect. The absence of this increase in blood glucose in animals first given a beta-blocker before the alpha-blocker suggests that the increase in the alpha-blocked animals is due to unopposed beta-adrenoceptor activity. This finding is consistent with the recent findings of Grayson and Oyebola [19] on the role of beta-adrenoceptors in glucose handling in the dog gastrointestinal tract.

Acknowledgment

We thank Professor J. Grayson of the Department of Physiology, University of Toronto, Toronto, Canada for provision of animals and laboratory facilities for this study.

References

1. Atalabi O. 'Cow's Urine' poisoning. Dokita 1964;6:1–4.
2. Osuntokun BO, Odeku EL, Sinnette CH. Convulsive disorders in Nigerians: the febrile convulsions. E Afr Med J 1969;46:385–94.
3. Familusi JB, Sinnette CH. Febrile convulsions in Ibadan children. Afr J Med Med Sci 1971;2:135–49.
4. Onadeko BO, Adetuyibi A. Acute poisoning in Nigerians: a review of cases managed over a five years period (1968–1972) at U.C.H., Ibadan. West Afr J Pharm Drug Res 1979;5:63–9.
5. Oyebola DDO, Elegbe RA. Cow's urine poisoning in Nigeria: experimental observations in mice. Trop Geogr Med 1975;27:194–202.
6. Elegbe RA, Bamgbose SOA, Oyebola DDO. Cow's urine poisoning in Nigeria: the cardiotoxic effects of cow's urine concoction in anaesthetized cats. J Trop Paediatr Environ Child Hlth 1976;22:232–5.
7. Elegbe RA, Oyebola DDO. Cow's urine poisoning in Nigeria: cardiorespiratory effects of cow's urine in dogs. Trans R Soc Trop Med Hyg 1988;71:127–32.
8. Ayorinde FO, Avery JW, Adekile AD, Ojewole JA, Odebiyi OO. Chemistry of a Nigerian herbal preparation (Cow's urine concoction). J Trop Paediatr 1982;23:13–17.
9. Ojewole JAO, Olusi SO. Effects of cow's urine concoction on plasma glucose concentration in fasted rats. J Trop Med Hyg 1976;79:241–3.
10. Grange A. Experimental studies on the 'Cow's urine mixture'. Ann Trop Paediatr 1981;1: 175–9.
11. Oyebola DDO. Effect of cows' urine concoction on blood flow, oxygen consumption and glucose uptake in the canine small intestine. Afr J Med Med Sci 1982;11:183–9.
12. Kurn H. Tobacco alkaloids and their pyrolysis products in smoke. In: Von Euler, US, ed. Tobacco Alkaloids and Related Compounds. Oxford: Pergamon Press, 1964:37–49.
13. Tsujimoto A, Tanimo S, Kurogochi Y. Effect of nicotine on serum potassium and blood glucose. Jpn J Pharmacol 1964;15:415–22.
14. Brahmachari HD, Augusti KT. Hypoglycaemic agent from onions. J Pharm Pharmacol 1961; 13:128.
15. Jain RC, Vyas CR. Hypoglycaemic action of onion on rabbits. Br Med J 1974;2:730.
16. Jain RC, Vyas CR. Garlic in alloxan-induced diabetic rabbits. Am J Clin Nutr 1975;28:684–5.
17. Oyebola DDO, Adetuyibi A. Toxicity of modified preparation of 'cow's urine' concoction in mice. Trans R Soc Trop Med Hyg 1977;71:349–50.
18. Oyebola DDO, Ariwodola JO. The effects of ganglionic blockade and adrenalectomy on the cardio-respiratory response to cows' urine concoction in rats. Afr J Med Med Sci 1984;13: 61–70.
19. Crayson J, Oyebola DDO. The effect of catecholamines on intestinal glucose and oxygen uptake in the dog. J Physiol (Lond) 1983; 343:311–22.

(*Accepted* 18 *March* 1988)