*Nigerian Journal of Paediatrics 1996; 23 (4): 96-101*

# *Effects of Routes of Administration on the Toxicity of Cow's Urine Concoction (CUC) in Mice*

**DDO Oyebola+**

**Summary**

**Oyebola DDO. Effects of Routes of Administration on the Toxicityof Cow's Urine Concoction (CUC) in Mice.** *Nigerian Journal of Paediatrics* 1996; 23:96. A study of the effects of different routes of administration on the acute toxicity of cow's urine concoction (CUC) in mice was undertaken. The response to CUC was measured as the rate of convulsion and/or death of the animals. The study showed that the subcutaneous (sc) route was the least toxic, while the oral, intramuscular (im) and intraperitoneal (ip) routes had toxicity ratios of 1.51, 2.89 and 4.95 when compared with the sc route. CUC acted faster when given by the im and ip routes compared with the oral and sc routes. Since CUC is usually given orally in man, the results underscore the need for caution in extrapolating results of animal experiments in which CUC had not been administered orally to what might happen in man.

## Introduction

COW'S urine concoction (CUC) is a local remedy for seizures in children in some parts of Nigeria. Its use has resulted in severe poisoning in man[1-5] and in experimental animals.[6-8] The concoction is administered orally in man.[1-6] In most of the animal experiments, oral, intraperitoneal (ip) and intravenous (iv) routes were used. There is, to my knowledge, no studies on the effects of CUC when administered by the subcutaneous (sc) and intramuscular (im) routes respectively. Similarly, there is no information on the relative toxicity of CUC when it is administered by different routes in the same animal species. Yet, nicotine, a major component of CUC,[9] has been reported to be fifteen times more toxic when administered by the iv route compared with its administration by the sc route.[10] The present study was therefore undertaken to compare the toxicity of CUC administered by the sc, ip, im and oral routes respectively.

College of Medicine, University of Ibadan

Department of Physiology

+Professor

## Materials and Methods

Male mice, weighing 19 - 26gm were used. Full preparation of CUC was undertaken as earlier described.[11] The concoction, administered by

sc, ip, im and oral routes respectively, was tested for acute toxic effects, using convulsion/death rates as the measure of response. The assay design was the same as that used in an earlier study.[11] The effect of CUC for each route of administration was observed at three-dose levels, except in the oral route where four-dose levels were used. Six mice were used at each dose level. All injections were undiluted preparation of CUC. For the ip routes, effects of 1.5ml, 2.0ml and 2.5ml of CUC per kg body weight respectively, were studied. Subcutaneous doses of 6.0ml, 8.0ml and 10.0ml per kg body weight respectively, were administered at the nape of the neck of the animals, while im doses of 3.0ml, 4.0ml and 5.0ml per kg body weight respectively, were given into the gluteal muscles. For the oral routes, doses of 4.0ml, 5.0ml, 10.0ml and 15.0ml per kg body weight respectively, were administered using a specially adapted gastric tube similar to that described by Olusi and Ojewole. [12] The three-dose levels for the ip, sc and im routes were determined by first establishing the zero and 100 percent mortality doses. The three-dose levels that will produce mortality rates between these two extremes were determined by trial and error. In the oral route, a similar procedure was followed, but four dose levels were used before a spread of mortality similar to those of the other routes was obtained. The number of mice that convulsed and the number that died at each dose level were recorded for each route of administration. The time of onset of convulsion and the time of death after each injection were also noted. The animals were observed for a maximum of 30 minutes in all cases. Usually, death, when it occurred, was within two to seven minutes of injection. It is important to note that although the doses of CUC given are quoted per kg body weight, each mouse weighed 25gm or less. Thus, a 25gm mouse receiving 10ml CUC per kg actually received 0.25ml of CUC.

The responses (death rates) were converted into probits, using a table of probits and the regression lines of the empirical probits for all the routes of administration against the $\log_{10}$ of the dose-volumes administered were plotted. The actual dose-volumes used were multiplied by 100 so as to convert all $\log_{10}$ dose-volume to positive logarithms. From the regression lines, the expected probits (Y) were determined. The data were then taken through the successive calculations for the probit transformation for quantal response assays.[13] The LD50 and standard error values for each route were obtained from these calculations. In order to compare relative toxicities of the different routes, the toxicity ratios based on a comparison of LD50 values were calculated. The least toxic route was arbitrarily assigned the toxicity ratio of 1.0 and the toxicity ratio of the other routes were expressed with reference to this. Statistical assessment of significance of differences between toxicities was by comparison of LD50 values, using Student's t-test to assess the statistical significance of difference between the means of two samples. P values of 0.05 or less were taken as statistically significant.

## Results

The results are shown in the Table as well as in the Figure. The Table shows the time between the administration of CUC and the onset of convulsion and the occurrence of death. It will be observed that while all the mice at the doses used convulsed, only a fraction of them eventually died.

Similarly, the lowest doses that produced 100 percent convulsion in the ip, im, oral and sc routes were 0.03, 0.06, 0.08 and 0.12ml, respectively. This showed that the ip route was the most potent and the sc route was the least potent to induce convulsive seizures. Furthermore, it will be observed that convulsion preceded death of the animals that died by two to seven minutes (Table).

**Table**

*Routes and Doses of Administration, onset of Convulsions and times of Death in 78 mice given CUC (n for each dose-level is 6)*

| *Route* | *Dose* (ml)* | *Onset of Convulsion** (Sec ± SEM)* | *No of Convulsions*** | *Time of Death (Min ± SEM)* | *No of Deaths* |
|---|---|---|---|---|---|
| Intraperitoneal (ip) | 0.03 | 46.17 ± 6.95 | 6 | 3.71 ± 0.29 | 2 |
| | 0.04 | 41.67 ± 3.07 | 6 | 3.0 ± 0.28 | 4 |
| | 0.05 | 24.83 ± 1.14 | 6 | 2.54 ± 0.46 | 5 |
| Intramuscular (im) | 0.06 | 40.0 ± 6.54 | 6 | 0.96 ± 0.13 | 2 |
| | 0.08 | 25.83 ± 2.39 | 6 | 7.42 ± 3.75 | 3 |
| | 0.10 | 26.67 ± 1.67 | 6 | 1.28 ± 0.25 | 5 |
| Subcutaneous (sc) | 0.12 | 40.83 ± 4.7 | 6 | | 0 |
| | 0.16 | 49.67 ± 3.38 | 6 | 5.72 ± 3.65 | 2 |
| | 0.20 | 45.83 ± 2.71 | 6 | 3.82 ± 2.74 | 5 |
| Oral | 0.08 | 60.83 ±10.91 | 6 | 3.79 ± 0.71 | 2 |
| | 0.10 | 46.33 ±10.96 | 6 | 2.92 ± 1.07 | 3 |
| | 0.20 | 57.17 ±12.62 | 6 | 3.65 ± 0.99 | 4 |
| | 0.30 | 36.33 ±11.72 | 6 | 3.15 ± 0.60 | 5 |

* The doses are those given per mouse

** All the 78 mice tested convulsed, with or without subsequent death. Hence, the number of convulsions is equal to "n" for each of the dose-levels used.

SEM = Standard Error of Mean

CUC = Cow's Urine Concoction

Although the higher doses caused convulsion and death in a shorter time than the lower doses, there was no consistent relationship between the doses and the time of onset of convulsion, or of death.

From the probit analysis, the LD50 doses for the subcutaneous, oral, im and ip routes were 0.19ml, 0.12ml, 0.07ml and 0.04ml respectively. The Figure shows the relative toxicity for the various routes of administration. The probit regression line to the extreme right of the Figure (sc route) showed the least potent route, while the regression line to the extreme left (ip) was the most potent route. The subcutaneous route was thus the least potent and the ip route the most potent with respect to mortality effect. The potency ratios of the oral, im and ip routes were 1.51, 2.89 and 4.95 respectively when compared with the sc route whose potency ratio was taken as 1.0. When the LD50 doses were subjected to statistical analysis as stated earlier, the differences in toxicity between the oral, im and ip routes of administration compared with the sc route were statistically significant ($P < 0.002$, 0.001 and 0.001 respectively).

When potency ratios were compared, the im and ip routes were two and three times respectively, more toxic than the oral route. The mortality rates for all the routes studied were dose-related.

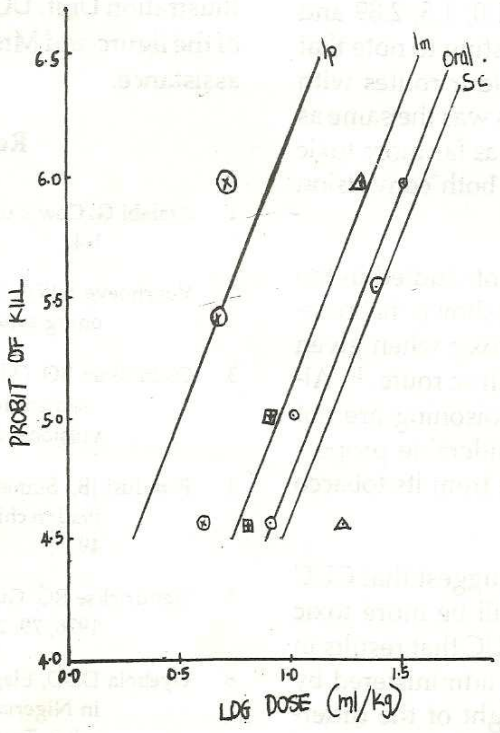


Fig: Probit regression lines showing the relative toxicity for the various routes of administration of CUC. Note that the line to the extreme right (sc) was the least potent route, while the line to the extreme left (ip) was the most potent route.

## Discussion

Previous studies have established that cow's urine concoction is pharmacologically active when administered orally,[12] intraperitonially[6] and intravenously.[7,8] The present study has shown that apart from the oral, ip and iv routes, cow's urine concoction is also pharmacologically active when administered subcutaneously and intramuscularly. The study has also provided an opportunity to compare toxicity of CUC when administered to the same animal species by the four routes used in the study. The results have established that the toxicity ratios with respect to mortality for the subcutaneous, oral, intramuscular and intraperitoneal routes were 1.0, 1.5, 2.89 and 4.95, respectively. It is interesting to note that the order of toxicity for the four routes with respect to convulsive seizures was the same as for mortality. The ip route was far more toxic than all the other routes for both convulsion and death responses.

The intravenous route was not studied in the present series, but it had been shown that nicotine was fifteen times more toxic when given by the iv route compared with sc route.[10] Although CUC and nicotine poisoning are not the same, CUC owes a considerable proportion of its toxicity to nicotine from its tobacco leaves component.[11]

It is therefore reasonable to suggest that CUC by the intravenous route will be more toxic than CUC by the ip route. CUC that results in human poisoning is usually administered by the oral route.[1,4,5] In the light of the differences in toxicity of CUC when administered by different routes as shown in the present study, it is important that care be exercised in extrapolating dose-effects obtained in animal experiments in which CUC had not been administered orally to what might happen in man, species variation aside. The absence of a consistent dose-response pattern in the time of onset of convulsion and time of death after CUC administration at all the dose-levels tested suggests however, that individual susceptibility of the mice used at each dose level most probably determined how long it took to convulse or die.

## Acknowledgements

I thank Prof Olusola Ayeni, formerly of the Department of Preventive and Social Medicine (PSM), University of Ibadan, for assistance with the statistical analysis, the Medical Illustration Unit, UCH, Ibadan for production of the figure and Mrs. Bose Pratt for secretarial assistance.

## References

1 Atalabi G. Cow's urine poisoning. *Dokita* 1964; **6**: 1-4.

2 Voorhoeve HWA, Smith J. Post-Cow's urine poisoning syndrome. *Dokita* 1966; **8**: 22-5.

3 Osuntokun BO, Odeku EL, Sinnette CH. Convulsive disorders in Nigerians: The febrile convulsions. *E Afr Med J* 1969; **46**: 385-94.

4 Familusi JB, Sinnete CH. Febrile convulsions in Ibadan children. *Afr J Med Sci* 1971; **2**: 135-49.

5 Hendrickse RG. Guest Editorial. *J Trop Med Hyg* 1976; **79**: 237-40.

6 Oyebola DDO, Elegbe RA. Cow's urine poisoning in Nigeria: experimental observations in mice. *Trop Geogr Med* 1975; **27**: 194-202.

7 Elegbe RA, Bamgbose SOA, Oyebola DDO. Blood pressure and electrocardiographic effects of cow's urine concoction in rats. *J Trop Paediatr Environ Child Hlth* 1977; **22**: 232-5.

8 Elegbe RA, Oyebola DDO. Cow's urine poisoning in Nigeria: cardiorespiratory effects of cow's urine in dogs. *Trans Roy Soc Trop Med Hyg* 1977; 71: 127-32.

9 Ayorinde FO, Avery JW, Adekile AD, Oyewole JA, Odebiyi OO. Chemistry of a Nigerian herbal preparation (cow's urine concoction). *J Trop Paediatr* 1982; 23: 13-7.

10 Larson PS, Haag HB, Silvette H. In: Tobacco: experimental and clinical studies. Larson PS, Haag HB, Silvette H, eds. Baltimore: Williams and Wilkins, 1961: 41-58.

11 Oyebola DDO, Adetuyibi A. Toxicity of modified preparation of cow's urine concoction in mice. *Trans Roy Soc Trop Med Hyg* 1977; 71: 349-50.

12 Olusi SO, Oyewole JAO. Evidence for complement activation following oral administration of cow's urine concoction in rats. *Afr J Med Sci* 1978; 7: 79-83.

13 Finney DJ. Statistical Methods in Biological Assay. New York: *Hafner Publishing Co.* 1964: 630-41.