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Effect of feeding organic acid treated maize (*Zea mays* L.) on growth and reproduction of albino rats (*Rattus norvegicus albinicus*) and Indian desert gerbils (*Meriones hurrianae*, jerdon)

AMEETA BANERJEE, YASHODA L URS* and S K MAJUMDER*
Department of Studies in Applied Botany, University of Mysore, Manasagangothri, Mysore 570 006, India
*Discipline of Infestation Control and Protectants, Central Food Technological Research Institute, Mysore 570 013, India

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Abstract. The organic, acetic and sorbic acids at 1% level were fed to albino rats and Indian desert gerbils in whole and broken maize corn. The gerbils were highly sensitive to maize treated with organic acids. The reproduction studies showed that the gerbils had more number of abnormalities with regard to the young ones. The sorbic acid treated maize possessed a teratogenic potential and it induced cannibalism. In case of albino rats no such abnormalities were found. However, abortion, delayed gestation period and cannibalism were common. Based on the data obtained it was postulated that the gerbils could be used as alternatives to albino rats in toxicological studies.

Keywords. Organic acids; maize; reproduction; albino rats; desert gerbils.

1. Introduction

The antimicrobial food additives such as sorbic acid and acetic acid are known to have an antimicrobial control potential (National Academy of Sciences 1973). The use and official regulation of acid treated grains in food and feed products is limited partly by lack of data on behaviours of the acids in food systems and effect on animal nutrition. Sorbic and acetic acids have been approved by the food and drug administration (FDA) as generally recognised as safe (GRAS). While, no report exists on the chronic toxicity of acetic acid or acetates when used as food preservatives (Lueck 1980) much work has been carried out with sorbic acid. Sorbic acid is known to possess low mammalian toxicity (Gaunt *et al* 1975) properties with ovicidal action (Dunkel *et al* 1982; Dunkel and Read 1986). Hence, it has been approved by the environmental protection agency (EPA) for use on whole grain and other raw agricultural commodities (Federal Register 1982). Hence, the present study was undertaken to study the effect of acetic and sorbic acids on the growth and reproduction of albino rats and Indian desert gerbils.

2. Materials and methods

2.1 *Animals and diet*

Fifteen adult female and male albino rats (*Rattus norvegicus albinicus*, Wistar-CFT strain) and Indian desert gerbils (*Meriones hurrianae*, jerdon) bred and reared in the CFTRI animal house, weighing between 180–250 g were selected and grouped randomly.

2.2 *Preparation of experimental diet*

Freshly harvested maize grain (50 kg) was determined for its moisture content and the presence of aflatoxins B_1, B_2, G_1 and G_2 (Pons *et al* 1966). Maize was ground in a laboratory mill and 1% sorbic acid (Sorbinosoüre, E Merck, Germany) was incorporated (10 g/kg) into the maize flour and sieved through a wire mesh (20 mesh size) for even mixing. Similarly 1% acetic acid (10 ml/kg) was added to the grain mixed thoroughly for even coating and then passed through the mill. This flour containing the organic acids were fed to the albino rats.

The 1% dose of acids was selected from the pilot studies during which 0·05–3% acid doses were used. Dose of both the acids (1%) controlled all the mycoflora (mostly storage fungi) and also significantly suppressed the F_1 progeny of the test insects (Ameeta Banerjee 1986). For the gerbils, the grain was treated with the organic acids as mentioned above except that the grain was broken into small pieces in the mill and then fed. The control diets were maize without any acid treatment.

2.3 *Embryotoxic studies in albino rats and Indian desert gerbils*

2.3a *Experimental design*: Fifteen pairs of albino rats and gerbils (1 : 1 ratio) were caged in pairs for mating and divided into 3 groups. The first group received the control diet. The second group was fed with maize diets treated with 1% acetic acid, while the third group received maize diets treated with 1% sorbic acid. The diets were fed *ad libitum* for a period of 60 days and the daily food consumption was monitored by weighing the residues. Drinking water was provided all the 24 h. Adult animals were studied for body weight gain and haematological parameters. After day one of pregnancy the males were separated and the females were allowed to litter and rear their young ones upto weaning. The offsprings were observed for survival, physical and functional development. At the end of 60 days, the animals were autopsied using a diethyl ether anaesthesia. Liver, kidney, ovary and testis were excised, blotted to remove the adhering blood and weighed on a mettler balance nearest to mg and the organ weights were determined.

2.5 *Measurement of haematological parameters*

Blood was drawn by cardiac puncture and aliquot was taken in tubes containing EDTA for haemoglobin (Hb) and white blood counts (WBC). WBC's were estimated by a haemocytometer (Bharucha *et al* 1976). Differential count of WBC was made by wright's staining technique. Hb was determined by the cyanmethamoglobin method using the commercial kit (Bharat Laboratories, Thane).

3. Results and Discussion

The moisture content of the grain was found to be 13·5% and the maize sample was found to be negative for any of the aflatoxins tested. There was no consistent differences between the treated and control albino rats and gerbils in food consumption. The rate of body weight gain of both the species of rats given 1% sorbic acid

and 1% acetic acid did not differ significantly from that of the controls. However, observations of a slight increase or decrease in the body weight of the animals could be due to the food intake pattern as there were inconsistencies in food intake. The increase in the body weight with sorbic acid has been observed by other workers (Deuel *et al* 1954; Gaunt *et al* 1975).

There were no significant alterations in the relative organ weight of the animals (table 1). The only organ showing an increase in terms of absolute weight was the ovaries in female gerbils and albino rats fed 1% sorbic acid. A statistically significant decrease ($P < 0.05$) in the testis of the male albino rats fed 1% acetic acid was also evident. The slight increase in the weight of the liver observed could be interpreted as a functional hypertrophy.

The findings of the haematological parameters are shown in table 2. As evident from the table, there was a statistically significant decrease ($P < 0.05$) in the WBC count accompanied by a statistically significant decrease ($P < 0.01$) in the neutrophils of the male albino rats fed with 1% sorbic acid. A statistically significant increase ($P < 0.01$) in the differential count of the neutrophils was also evident in the female albino rats fed with 1% acetic acid. Though, a decrease in the white blood cells accompanied by the decrease in lymphocytes of the Indian desert gerbils fed with 1% acetic acid and sorbic acid treated maize was also evident, the values obtained were not statistically significant from those of the controls.

The above changes suggest the possibility of the chemicals especially the acetic acid and sorbic acid to disrupt the immunological responses of the gerbils as reflected by the decreased count of the lymphocytes. Roitt (1977) reported such changes in lymphocytes in albino rats with certain pesticides. The increased neutrophil count might be related to the response of infectious agents or xenobiotics in the host environment as postulated by Wintrobe (1976). However, the exact mechanism by which the changes in the haematological parameters were evoked could not be known.

The data on reproduction studies in albino rats and gerbils are presented in table 3. The reproduction performance of the rats was affected as evident from the abnormalities found in the young ones. In the sorbic acid treated group two out of five female albino rats gave birth to one pup each and the rest of the foetus aborted. The animals on autopsy showed resorption of the embryo which were still formed. In the gerbils too the same effect was observed. It was noted that two out of the five female gerbils gave birth to malformed pups. The malformations were of the fore, the hind limbs and the tail. In another the female gerbil gave birth to a pup which survived for 14 days and later died. X-ray scanning of the 14 day old pup revealed gross growth retardation, microcephaly with spina bifida and teratoma in the abdomen. Sorbic acid also induced cannibalism. With 1% acetic acid treated maize no such abnormalities were found in the rats but delayed gestation period, resorption of the embryos and abortion were observed. Demaree *et al* (1955) reported that 10% sorbic acid in the diets of albino rats had no effect on the reproduction. While, O'Dell *et al* (1948) demonstrated that a deficiency of folic acid was the chief cause of congenital abnormalities. Later O'Dell *et al* (1951) found that a high proportion of the offsprings are afflicted with abnormalities if the mothers are severely depleted of vitamin B_{12}. The present study indicated that sorbic acid treated maize could induce congenital abnormalities. As the mode of action of sorbic acid and acetic acid is not known, the abnormalities found could not be directly related to the organic acids nor

Table 1. Organ weight of rats fed with maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

		Male			Female		
Animals (rats)	Organs	C	AA	SA	C	AA	SA
Meriones hurrianae (Jerdon)	Terminal body weight	101·75 ± 7·31	107·25 ± 13·41	108·00 ± 8·89	96·6 ± 3·45	96·00 ± 6·37	94·5 ± 7·57
	Liver	2·97 ± 0·10	3·47 ± 0·75	2·846 ± 0·30	3·12 ± 0·14	3·11 ± 0·26	3·03 ± 0·14
	Kidney	0·55 ± 0·026	0·52 ± 0·05	0·45 ± 0·05	0·94 ± 0·28	0·52 ± 0·02	0·58 ± 0·04
	Testis/ovary	0·630 ± 0·033	0·525 ± 0·04	0·607 ± 0·06	0·027 ± 0·008	0·027 ± 0·006	0·073* ± 0·009
Rattus norvegicus albinicus	Terminal body weight	236·33 ± 11·33	245·66 ± 2·02	228·66 ± 10·7	238·00 ± 27·38	231·00 ± 5·567	241·00 ± 13·79
	Liver	3·433 ± 0·19	3·278 ± 0·02	3·712 ± 0·12	3·276 ± 0·37	3·204 ± 0·09	3·531 ± 0·29
	Kidney	0·572 ± 0·02	0·508* ± 0·01	0·562 ± 0·01	0·489 ± 0·03	0·484 ± 0·03	0·472 ± 0·02
	Testis/ovary	1·245 ± 0·03	0·946* ± 0·10	1·233 ± 0·07	0·045 ± 0·06	0·041 ± 0·003	0·058* ± 0·05

Values are mean ± SE of 4 animals; Students '*t*' test *$P < 0·05$.
C, Control; AA, acetic acid; SA, sorbic acid.

Table 2. Blood picture of rats fed with maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

Animals (rats)	Blood parameters	Male			Female		
		C	AA	SA	C	AA	SA
Ruttus norvegicus albinicus (Albino rats)	Hb (g/dl)	13·16 ± 1·09	13·5 ± 0·06	12·0 ± 1·04	14·0 ± 0·8	13·0 ± 0·86	13·16 ± 0·44
	WBC (μl)	14,550 ± 1,841	10,750 ± 1,970	8,950[a] ± 781	11,433 ± 1,186	10,100 ± 1,985	13,500 ± 1,719
	Differential count (%)						
	Lymphocytes	67·33 ± 2·40	71·0 ± 3·51	61·66 ± 23·66	76·66 ± 6·38	65·33 ± 7·26	78·0 ± 1·15
	Neutrophils	32·33 ± 2·33	28·33 ± 3·38	13·66[b] ± 1·76	23·0 ± 6·08	33·66[b] ± 7·62	21·66 ± 0·88
	Monocytes	—	0·66 ± 0·33	—	0·33 ± 0·3	1·0 ± 0·57	0·33 ± 0·3
Meriones hurrianae (Jerdon) (Desert gerbil)	Hb (g/dl)	13·25 ± 0·43	14·12 ± 0·65	14·25 ± 0·59	13·25 ± 0·25	13·37 ± 0·55	12·87 ± 0·12
	WBC (μl)	9,450 ± 2,810	6,362 ± 528	5,865 ± 5,489	9,500 ± 1,934	5,775 ± 1,214	9,300 ± 3,403
	Differential count (%)						
	Lymphocytes	74·5 ± 2·62	71·25 ± 6·56	68·5 ± 1·70	82·25 ± 1·75	81·25 ± 4·95	68·75 ± 8·48
	Neutrophils	24·25 ± 2·78	28·75 ± 6·56	30·5 ± 1·5	16·75 ± 1·54	18·0 ± 5·04	30·75 ± 8·66
	Monocytes	1·25 ± 0·47	—	1·0 ± 0·57	1·00 ± 0·44	0·15 ± 0·75	0·5 ± 0·5

Values are Mean ± SE of 4 animals each; Students '*t*' test [a]$P < 0·05$; [b]$P < 0·01$.

C, Control; AA, acetic acid; SA, sorbic acid; Hb, Haemoglobin; WBC, white blood cells.

Table 3. Results of reproduction study in rats fed maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

Animals (rats)	Dietary level (%)	No. of females pregnant/ No. mated	No. of litters born	Mean pup wt (g) at day	No. of dead pups	No. of live pups	No. of resorp-tions/ pregnant female	No. of sterile males
Meriones hurrianae (Jerdon)	Control	5/5	12	3·0	0	12	Nil	0/5
	1% Acetic acid	3/5	9	2·38	0	8	5	2/5
	1% Sorbic acid	5/5	6	2·7	8	Nil	8	0/5
Rattus norvegicus albinicus	Control	5/5	35	4·37	2	33	Nil	0/5
	1% Acetic acid	4/5	15	3·73	0	15	23	1/5
	1% Sorbic acid	4/5	12	2·87	12	0	10	1/5

to the mycotoxins as the sample was found to be free from it. However, it can be stated that the sorbic acid treated maize possessed a teratogenic potential.

It is therefore, essential that further studies be carried out for evaluating the safety of these much used antimicrobial food additives. It should be noted that there are very few published reports on the effect of sorbic acid on reproduction (Demaree *et al* 1955) which suggested that sorbic acid at 10% level had no effect on reproduction. The present findings are in disagreement with the earlier reports of Demaree *et al* (1955). The present study revealed that sorbic acid in combination with some low protein and vitamin diet like maize exerts an adverse effect on reproduction of gerbils and albino rats. The teratogenic potential of the sorbic acid treated maize could be due to the above mentioned fact. Such studies on gerbils seem to have not been carried out earlier.

The response with respect to embryo toxicity under the influence of dietary organic acids like the sorbic and acetic acids seem to have opened a new field of future research for using Indian desert gerbils as alternatives to albino rats as laboratory animals.

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