

Acute Toxicity and Cardio-Respiratory Effects of 2-Deoxy-D-Glucose: A Promising Radio Sensitiser

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Objective To evaluate the acute toxicity of 2-deoxy-D-glucose (2DG) by oral (p.o.) and intravenous (i.v.) routes, and also the cardio-respiratory effects following high doses of 2DG in animal models. **Methods** The LD₅₀ of 2DG (in water) was determined in rats and mice by p.o. route and in mice by i.v. route. The effect of 2-DG (250 mg/kg, 500 mg/kg, and 1000 mg/kg, i.v.) was studied on various cardio-respiratory parameters viz., mean arterial blood pressure, heart rate and respiratory rate in anaesthetised rats. The effect of 2DG (500 mg/kg, 1000 mg/kg, and 2000 mg/kg, p.o.) was also studied on various respiratory parameters viz., respiratory rate and tidal volume in conscious rats and mice using a computer program. **Results** The p.o. LD₅₀ of 2DG was found to be >8000 mg/kg in mice and rats, and at this dose no death was observed. The LD₅₀ in mice by i.v. route was found to be 8000 mg/kg. At this dose 2 out of 4 mice died and the death occurred within 6 h. A significant increase in the body weight was observed after p.o. administration of 2DG in rats at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg doses. There was no significant change in the body weight at 4000 mg/kg and 8000 mg/kg by the p.o. route in rats and up to 8000 mg/kg by p.o. as well as i.v. routes in mice. Intravenous administration of 2DG (250 mg/kg, 500 mg/kg, and 1000 mg/kg) in anaesthetised rats showed a time-dependent decrease in the mean arterial blood pressure. There was no change in the heart rate in any of the treatment groups. The tidal volume was not changed significantly by p.o administration in conscious rats, but a significant decrease in the respiratory frequency at 500 mg/kg and 1000 mg/kg doses was observed. In the mice also there was no change in the tidal volume after p.o, administration, but the respiratory frequency decreased significantly at 2000 mg/kg dose. **Conclusion** 2DG is a safe compound but can cause a fall in the blood pressure and a decrease in respiratory frequency at high doses.

Key words: 2-deoxy-D-glucose; Glucose analogue; Radio sensitizer; Acute toxicity; Blood pressure; Heart rate; Respiration

INTRODUCTION

The glucose analogue, 2-deoxy-D-glucose (2DG) is a competitive inhibitor of glucose transport and a well known inhibitor of aerobic and anaerobic glycolysis. 2DG interferes with cellular mechanisms by competing with glucose for key enzymes in glycolysis^[1]. The inhibition in glucose utilisation and glycolytic pathway results in metabolic stress in the form of cellular glucopenia leading to significant reduction in the cellular contents of high energy phosphates like ATP and GTP^[2]. In a variety of conditions 2DG has also been shown to be beneficial^[3-9]. 2DG is beneficial to several other instances viz., ageing, Alzheimer's disease, Parkinson's diseases, Huntington's disease and stroke^[10].

2DG is effective in animal models for the

treatment of sarcomas, adeno-carcinomas, leukaemia, melanomas and bladder, colon and breast cancers when administered continuously^[11]. However, initial attempts to treat cancer patients with 2DG as an anticancer agent are largely unsuccessful since continuous administration of 2DG is found to be toxic^[12]. Subsequently, it has been observed that the presence of 2DG following irradiation inhibits the cellular systems with high rates of glycolysis like the cancer cells under euoxic as well as hypoxic conditions^[13-15]. 2DG can enhance radiation-induced cell killing in Ehrlich ascites tumour cells and also DNA double strand break repair^[16]. 2DG has also been administered as an adjunct to radiation therapy in neuroblastomas^[17]. It has been shown that the cytotoxic effect of tumour necrosis factor is potentiated by 2DG in human lymphoma U937 cells^[18]. 2DG also potentiates the effect of topotecan and cisplatin on cervical cancer cells, and also the

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effect of sublethal doses of radiation^[19]. Altered thiol metabolism has been suggested as a possible mechanism of radiosensitisation by 2DG particularly in slow growing radioresistant tumours^[20]. Slow growing cells in the solid tumours are resistant to conventional cytotoxic agents which target the rapidly dividing cells and 2DG is expected to selectively target them. 2DG significantly increases the efficacy of chemotherapeutic agents like adriamycin and paclitaxel in animal models of osteosarcoma and lung cancer^[21]. 2DG is also known to stimulate the apoptotic pathway and is beneficial to the multidrug resistant human carcinoma cell lines^[22-23].

The toxicity profile of 2DG has not been the subject of intense investigation although metabolic, physiologic and functional disturbances have been reported. Doses up to 400 mg/kg, i.v., in humans are well tolerated although few side effects have been reported at lower doses^[24]. Inhibition of glycolysis by 2DG causes profound hypothermia in rats^[25]. Glucoprivation induced by 2DG increases the level of growth hormone and prolactin levels in humans^[26]. Inhibition of glycolysis by 2DG reduces neuronal function in rat hippocampal slices in the presence of glutamate^[27]. Metabolic stress induced by 2DG influences the capacity of the immune system to resist infection by certain classes of microbial pathogens. 2DG can alter immune cell functions, including the enhancement of normal patterns of cytokine production *in vivo*^[28].

Glucoprivation by 2DG is an effective method of enhancing the efficacy of chemotherapeutic agents and radiation therapy. In most of these instances the drug is given through parenteral routes. The oral safety of 2DG in animal models is not available in the literature. Hence this study was planned to evaluate the acute toxicity of 2DG by oral and intravenous routes and also the cardio-respiratory effects following high doses of 2DG.

MATERIALS AND METHODS

Animals

Wistar male rats (150-200 g) and Swiss male mice (25-30 g), randomly bred and maintained in the Defence Research and Development Establishment (DRDE) animal facility, were used for the study. The animals were housed in polypropylene cages on dust free rice husk as the bedding material, and were provided with pellet diet (Amrut Ltd., India) and water ad libitum. The animals were fasted overnight prior to the drug treatment. The care and maintenance of the animals were as per the approved guidelines of

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). This study had the approval of the Establishments Animal Ethical Committee.

Chemicals

2DG was synthesised in the pilot plant (300 g batch process) of DRDE, with D-glucose as the starting material. The product was characterised by elemental analysis, IR, ¹H NMR and mass spectral analysis. The purity of 2DG was estimated to be 99.5% by HPLC. 2DG was dissolved in double distilled water for oral and intravenous administrations.

LD₅₀ Determination

The LD₅₀ of 2DG was determined in male rats and male mice by oral route, and in male mice by intravenous route. The volume given by oral and intravenous routes was not more than 10 mL/kg. Food and water were given 3 hours after 2DG administration. The animals were weighed daily and observed for mortality for 14 days. For all LD₅₀ determinations, 3 to 4 log doses were used and for each dose 4 animals were used. The LD₅₀ and confidence intervals were determined by the moving average method^[29].

Cardio-respiratory Effects of 2DG Through Intravenous Route in Rats

Mean arterial blood pressure, heart rate and respiratory rate were recorded using an 8 channel polygraph (model 7D, Grass Instruments, USA). The rats were anaesthetised with urethane (1.6 g.kg⁻¹ i.p.) and the neck region was dissected and exposed. Trachea was cannulated and connected to a pneumotachograph (Hugo Sachs Elektronik, Germany). Right jugular vein was cannulated and a syringe was connected for intravenous administrations. Left carotid artery was cannulated with a thin polypropylene tube connected to a pressure transducer (Statham P23Dc) containing heparinised normal saline. The pressure transducer was connected to a preamplifier (low-level DC, Grass Instruments, USA) and arterial blood pressure was recorded on the polygraph. Mean arterial pressure was calculated from the recorded blood pressure. The signals from DC driver amplifier recording blood pressure were taken from J6 output and fed into the external triggering (High) input of EKG tachograph preamplifier (Grass Instruments, USA) for the recording of heart rate. The pneumotachograph was connected to a differential pressure transducer (SWEMA, Germany) and connected to a pre-amplifier (Low level DC, Grass Instruments, USA), and inspiration was recorded as

an upward deflection and expiration as a downward deflection. The animals were allowed to stabilise for 60 min after the surgical procedures. Three doses of 2DG (dissolved in distilled water) viz., 250 mg/kg, 500 mg/kg, and 1000 mg/kg were given intravenously and mean arterial blood pressure, heart rate and respiratory rate were recorded for a period of 4 h after administration. For each dose 4 rats were used and 4 rats served as control, administered with distilled water only.

Respiratory Effects of 2DG Through Oral Route in Rats and Mice

Four rats or four mice at a time were restrained in body plethysmographs for recording the respiratory signals. Glass plethysmographs of length 140 mm and diameter 45 mm that can accommodate rats weighing between 150 g to 250 g and of length 100 mm and diameter 28 mm that can accommodate mice weighing between 24 g to 32 g were used. A volumetric pressure transducer (model PT5, Grass Instrument, USA) was used for sensing respiratory flow signals. A continuous airflow of 170 mL·min⁻¹ was maintained into each body plethysmograph using a critical orifice (27 gauge needle). The signals from the individual transducers were amplified using universal amplifiers (Gould, USA). The amplified signals were digitised using an analogue to digital converter (Metrabyte, Taunton, USA) and stored and analysed in a personal computer. The amplified signals from the amplifiers were also fed into an oscillograph for recording the breathing pattern (WindoGraf, Gould, USA). The animals were acclimatised in the body plethysmographs for 30 min. After the initial acclimatisation a control recording of respiratory variables was carried out for 30 min. Three doses of 2DG (dissolved in distilled water) viz., 500 mg/kg, 1000 mg/kg, and 2000 mg/kg were given using an oral feeding cannula (20 gauge for rats and 22 gauge for mice, Harvard Instruments, USA) and the respiratory variables were recorded for a period of 4 h after administration. For each dose 4 rats or 4 mice were used and 4 rats and 4 mice served as control, administered with distilled water only. A computer programme developed in the University

of Pittsburgh, USA for characterisation of respiratory pattern in small animals in the conscious state was used for recording various respiratory variables^[30]. Various respiratory variables viz., respiratory frequency (f), tidal volume (VT), inspiratory time (TI), expiratory time (TE), time of brake (TB), time of pause (TP) and flow at 0.5 VT during expiration (VD) were measured. The computer program is also capable of recognising the effects of chemicals as normal breath (N) sensory irritation (S), airway obstruction (A) or pulmonary irritation (P).

Statistical Analysis

All the data were represented as percent change of the control values. The data were analysed by one way ANOVA, and Dunnett's multiple comparison procedure was used for finding the difference between the control group and the 2DG administered group. A probability less than 0.05 was taken as statistically significant. The statistical analyses were carried out using SigmaStat (SPSS Inc., Chicago, IL, USA).

RESULTS

The rats and mice were given a maximum dose of 8000 mg/kg by the oral route and none of the animals died at any of the doses (500 mg/kg to 8000 mg/kg). The LD₅₀ was more than 8000 mg/kg. The animals appeared less active and otherwise no apparent effect was observed. Two mice died following intravenous administration of 8000 mg/kg and hence a further dose of 16 000 mg/kg was administered. All the mice in this group died and the estimated LD₅₀ was 8000 mg/kg (Table 1). The death in the animals occurred within 6 h. A significant increase in the body weight was observed after p.o. administration of 2DG in rats at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg doses. There was no significant change in the body weight at 4000 mg/kg and 8000 mg/kg by the p.o. route in rats and up to 8000 mg/kg by p.o. as well as i.v. routes in mice (Table 2).

TABLE 1
LD₅₀ of 2DG in Rats and Mice

Species	Sex	Route	LD ₅₀ (mg/kg)	Confidence Limits (mg/kg)
Rat	Male	Oral	> 8000	
Mouse	Male	Oral	> 8000	
Mouse	Male	Intravenous	8000	5100 - 12600

TABLE 2
Percent Body Weight Change Following 2DG Administration in Rats and Mice

Species and Sex	Route	Dose (mg/kg) 2DG	% Body Weight After 7 Days	Statistical Significance
Rat	Control	-	106.3±2.5	
Male	Oral	500	115.5±1.2*	
		1000	116.7±2.8*	
		2000	113.5±1.7*	
		4000	110.3±0.3	F = 7.1
		8000	104.8±1.8	P < 0.001
Mouse	Control	-	102.3±0.9	
Male	Oral	500	103.8±0.8	
		1000	104.0±2.0	
		2000	105.3±1.7	
		4000	114.3±5.3	F = 2.1
		8000	108.3±4.2	NS
Mouse	Control	-	103.3±0.6	
Male	Intravenous	1000	102.3±3.8	
		2000	101.5±3.0	
		4000	102.8±2.6	F = 0.2
		8000	99.0±0.0	NS

Note. *Significant from control.

The control values ($\bar{x} \pm s$, $n=16$) of mean arterial blood pressure, heart rate and respiratory rate in the anaesthetised rats were 119 ± 2 mmHg, 373 ± 13 per minute and 102 ± 10 per minute. During the four-hour monitoring period the control animals showed a steady response. Intravenous administration of 250 and 500 mg/kg of 2DG showed a time-dependent decrease in the mean arterial blood pressure. A dose of 1000 mg/kg did not show any change initially but at 4 h period showed a decrease in the mean arterial blood pressure. The decrease in the mean arterial blood pressure was not dose-dependent. There was no change in the heart rate in any of the dose groups. The respiratory rate also did not show any significant change in any dose groups compared to the control.

The respiratory flow of four rats is shown in Fig. 2a, which showed an uniform breathing pattern. The control values ($\bar{x} \pm s$, $n=16$) of respiratory frequency and tidal volume in the conscious rats were 110 ± 6 breaths/min and 0.54 ± 0.06 mL. The pattern was not changed one hour after oral administration of 1000 mg/kg of 2DG (Fig. 2b). The time response analysis

of breath classification and measured variables for a group of four rats, before and after oral administration of 1000 mg/kg of 2DG is shown in Fig. 3. There was no significant change in any of the respiratory parameters, but the respiratory pattern classified as normal breath (N) declined and airway obstruction (A) increased after 2 h. Similarly there was no significant change in the other dose groups in rats as well as in mice. The respiratory pattern classified as normal breath, the respiratory frequency and tidal volume following oral administration of 2DG is shown in Fig. 4 for rats and Fig. 5 for mice. The percent normal breath and the tidal volume were not significantly changed in rats, but a significant decrease in the respiratory frequency at 500 mg/kg and 1000 mg/kg doses was observed. The decrease in respiratory frequency was not dose-dependent. The control values ($\bar{x} \pm s$, $n=16$) of respiratory frequency and tidal volume in the conscious mice were 241 ± 10 breaths/min and 0.084 ± 0.007 mL. In the mice also there was no change in the normal breath and tidal volume, but the respiratory frequency decreased significantly in the 2000 mg/kg dose group.

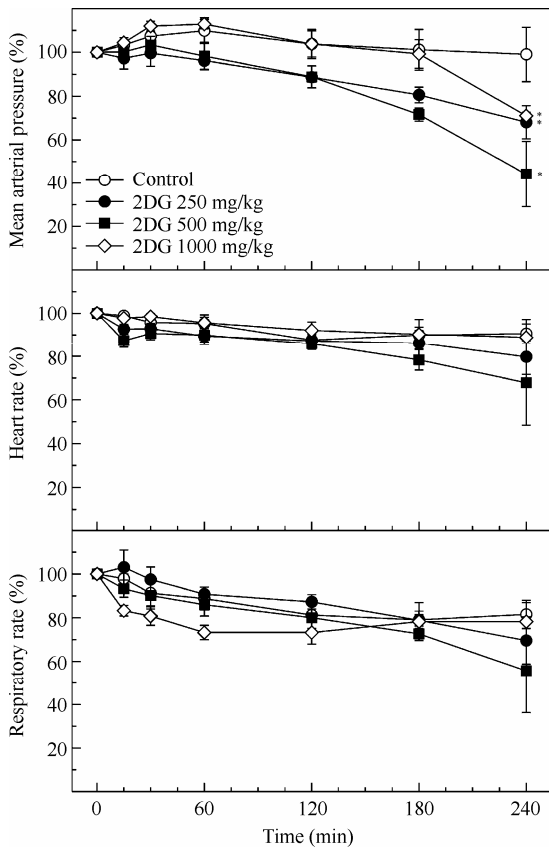


FIG. 1. Effect of intravenous administration of 2DG on mean arterial blood pressure, heart rate and respiratory rate in anaesthetised rats. Each point is a mean of 4 animals. Normal values ($n=16$) of mean arterial blood pressure = 119 ± 2 mmHg, heart rate = 373 ± 13 per min and respiratory rate = 102 ± 10 per min. Values are expressed as $\bar{x} \pm s$. *Significantly different from control for the corresponding time period.

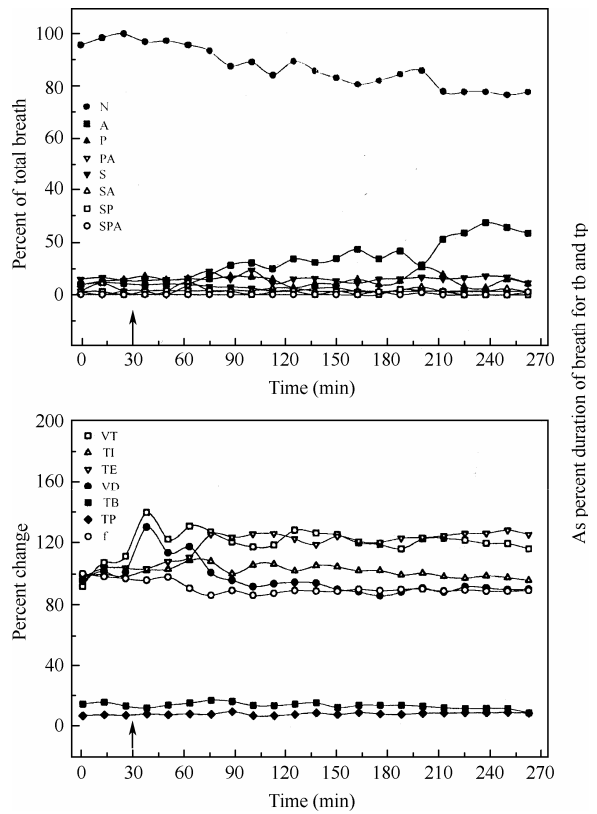


FIG. 3. Time response analysis for breath classification and measured variables for a group of four rats: before and after oral administration of 2DG, 1000 mg/kg. The arrow indicates the time of administration. N: normal; S: sensory irritation; A: airway obstruction; P: pulmonary irritation; SA, SP, PA and SPA: combinations of breath classification; VT: tidal volume; TI: time of inspiration; TE: time of expiration; VD: mid expiratory flow; TB: time of brake; TP: time of pause; f: respiratory frequency.

DISCUSSION

2DG is a structural analogue of D-glucose and is metabolised very slowly compared to the parental sugar D-glucose. Like D-glucose it is converted by hexokinase to 2-deoxyglucose-6-phosphate (2DG6P) at a rate similar to D-glucose. Due to enhanced glycolytic metabolism in cancerous cells the formation of 2DG6P is increased and inhibits hexokinase by negative feedback^[31]. The dephosphorylation of the trapped 2DG6P to 2DG is extremely slow due to low levels of glucose phosphatase in cancer cells^[32]. The accumulated 2DG6P inhibits phosphoglucoisomerase and is oxidised by glucose-6-phosphate dehydrogenase at a rate 1000 times slower than glucose-6-phosphate^[33]. The inhibition of glycolysis at these sites by 2DG and the reduced activity of the

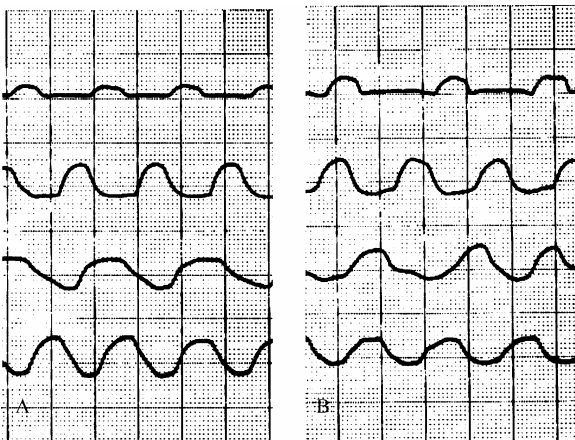


FIG. 2. Effect of oral administration of 2DG (1000 mg/kg) on the respiratory flow of rats before (A) and 1 h after 2DG administration (B).

As percent duration of breath for tb and tp

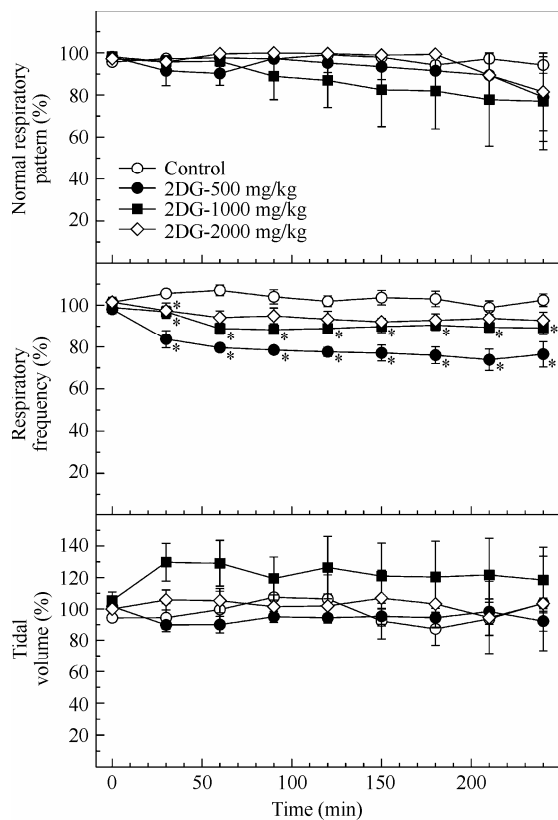


FIG. 4. Effect of oral administration of 2DG on percent normal respiration, respiratory frequency and tidal volume. Each point is a mean of 4 animals. The normal respiration is the computer output based on the breathing pattern of the rats. The normal ($n=16$) values of respiratory frequency= 110 ± 6 breaths/min and tidal volume= 0.54 ± 0.06 mL. Values are expressed as $\bar{x} \pm s$. *Significantly different from control for the corresponding time period.

tricarboxylic acid cycle add to the reduced production of ATP in cancerous cells compared to normal cells, decreasing the cell viability. The preferential killing of multidrug-resistant cancerous cell lines by 2DG is due to apoptotic pathway and is different from normal cell lines^[22, 34].

Though 2DG induces metabolic stress, the systemic toxicity appears to be very low. The *in vivo* half life of 2DG in murine system is found to be nearly 70 minutes and in humans it is 100 minutes^[17, 35]. Since brain depends mainly on glucose for its energy production, 2DG-induced metabolic inhibition leading to CNS disturbances may be a concern in the systemic administration of 2DG. Fall in body temperature, followed by warmth and sweating has been reported due to glucoprivation of CNS^[36]. In the present study it was found that a dose of 8000 mg/kg was not lethal to the animals by the oral route. By

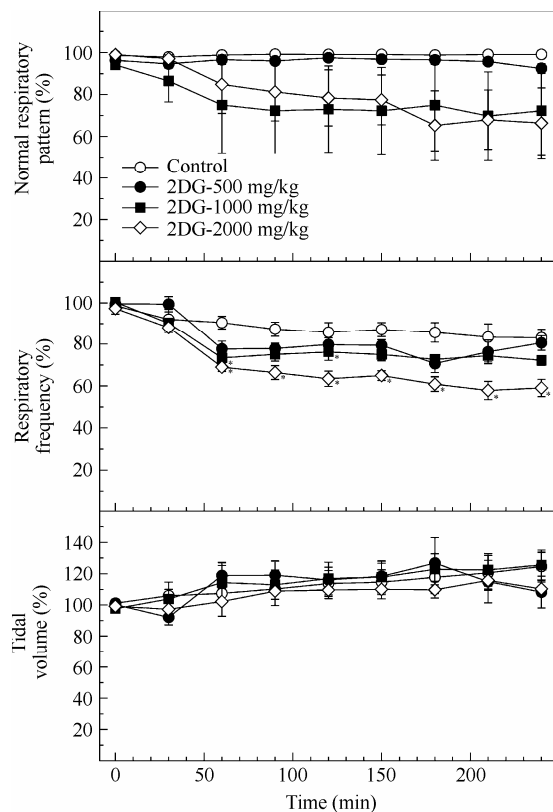


FIG. 5. Effect of oral administration of 2DG on percent normal respiration, respiratory frequency and tidal volume. Each point is a mean of 4 animals. The normal respiration is the computer output based on the breathing pattern of the mice. The normal ($n=16$) values of respiratory rate= 241 ± 10 breaths/min and tidal volume= 0.084 ± 0.007 mL. Values are expressed as $\bar{x} \pm s$. *Significantly different from control for the corresponding time period.

intravenous route the LD₅₀ was 8000 mg/kg. There was no residual effect in the animals that received oral dose and those that survived in the intravenous group, as they showed no significant difference in the body weight compared to the control group. Further, dietary supplementation of 2DG, does not reduce the food intake and maintains the body weight in rats^[37].

Intravenous administration of 2DG, though showed a time-dependent decrease in mean arterial blood pressure but was not dose-dependent. The heart rate did not change up to 1000 mg/kg of intravenous 2DG. Supplementing 2DG in the diet also shows a decrease in the heart rate and blood pressure^[37]. While 2DG does not induce any vasodilation in human skeletal muscle vascular bed, but induces vasodilation in combination with insulin^[38]. No significant alterations of ventricular rate and TS segment of rhythm in the ECG have been observed,

although prolongation of QT interval and depression of T-wave have been observed^[9]. Though there was no significant change in the tidal volume following oral administration of 2DG, the respiratory frequency decreased both in rats and in mice. The decrease in the respiratory frequency was within 1 h of 2DG administration and the effect was sustained. Unlike inhaled chemicals that can cause sensory or pulmonary irritation with a significant decrease in the percent of normal breath, oral administration of 2DG did not decrease the normal pattern of respiration, showing that the chemical may not have any deleterious effect on the respiration.

There has been a renewal of interest in using 2DG either as a primary therapeutic agent in slow growing resistant tumours or as an adjunct in the radiotherapy and chemotherapy of various neoplasms. There is also the feasibility of administering a combined treatment of 2DG plus a large dose of ionising radiation without acute or late toxicity in patients with malignant glioma^[17]. Since 2DG is absorbed similar to glucose in the tumour tissue, therapeutically relevant levels can be achieved by oral administration even in the brain as it crosses the blood brain barrier^[39]. Systematic investigations of examining the various aspects of toxicity in the form of physiological and functional disturbances like the present study promote the inclusion of this simple glucose analogue in the armamentarium of cancer therapies. In comparison to the other anti-cancer agents, perhaps 2DG is the least toxic chemical. *In vitro* and *in vivo* studies have shown that 2DG does not produce any mutagenic or carcinogenic effects^[40]. Results of the present study show that 2DG is a safe compound with a LD₅₀ of more than 8000 mg/kg. The decrease in the mean arterial blood pressure following intravenous administration and the decrease in the respiratory rate following oral administration may be due to glucoprivation and can be managed symptomatically.

REFERENCES

- Laszlo J, Harlan W R, Klein R F, *et al.* (1961). The effect of 2-deoxy-D-glucose infusions on lipid and carbohydrate metabolism in man. *J Clin Invest* **40**, 171-176.
- McComb R B, Yushok W D. Metabolism of ascites tumor cells. II effects of 2-deoxy-D-glucose phosphorylation on phosphorus metabolism. (1964). *Cancer Res* **25**, 193-197.
- Mattson M P, Duan W, Guo Z (2003). Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. *J Neurochem* **84**, 417-431.
- Dreau D, Morton D S, Foster M, *et al.* (1998). Effects of 2-deoxy-D-glucose administration on immune parameters in mice. *Immunopharmacol* **39**, 201-213.
- Tariq M, Arshaduddin M, Biary N, *et al.* (2002). 2-deoxy-D-glucose attenuates harmaline induced tremors in rats. *Brain Res* **945**, 212-218.
- Ebrahim A S, Babu E, Thirunavukkarasu C, *et al.* (2001). Protective role of vitamin E, 2-deoxy-D-glucose, and taurine on perchloroethylene induced alterations in ATPases. *Drug Chem Toxicol* **24**, 429-437.
- Lan Y W, Ishii Y, Palmer K E, B *et al.* (2003). 2-deoxy-D-glucose protects retinal ganglion cells against excitotoxicity. *Neuroreport* **14**, 2369-2372.
- Duke W W, Hirschowitz B I, Sachs G (1965). Vagal stimulation of gastric secretion in man by 2-deoxy-D-glucose. *Lancet* **2**, 871-876.
- Stadler G A, Schultheiss H R, Allogower M (1972). Use of 2-DG for testing completeness of vagotomy in man. *Gastroenterol* **63**, 552-556.
- Wei J, Cohen D M, Quast M J. (2003). Effects of 2-deoxy-d-glucose on focal cerebral ischemia in hyperglycemic rats. *J Cereb. Blood Flow Metab* **23**, 556-564.
- Saydjari R, Upp JR Jr, Alexander RW, *et al.* (1989). 2-deoxy-D-glucose inhibits the antitumor effects of alpha-difluoromethylornithine on the growth of colon cancer *in vivo*. *Invest New Drugs* **7**, 131-138.
- Landau B R, Laszalo J, Stengle J, *et al.* (1958). Certain metabolic and pharmacological effects in cancer patients given infusions of 2-deoxy-D-glucose. *J. National Cancer Institute* **21**, 485-494.
- Jain V K, Holtz G W, Pohlit W, *et al.* (1977). Inhibition of unscheduled DNA synthesis and repair of potentially lethal X-ray damage by 2-deoxy-D-glucose in yeast. *Int J Rad Oncol Biol Physiol* **32**, 175-180.
- Dwarakanath B S, Jain V K. (1989). Energy linked modifications of the radiation response in a human cerebral glioma cell line. *Int J Rad Oncol Biol Physiol* **17** 1033-1040.
- Dwarakanath B S, Jain V (1991). Effects of gamma-rays and glucose analogs on the energy metabolism of a cell line derived from human cerebral glioma. *Indian J Biochem Biophys* **28**, 203-209.
- Jha B, Pohlit W (1992). Effect of 2-deoxy-D-glucose on DNA double strand break repair, cell survival and energy metabolism in euoxic Ehrlich ascites tumour cells. *Int J Rad Biol* **62**, 409-415.
- Mohanti B K, Rath G K, Anantha N, *et al.* (1996). Improving Cancer Radiotherapy with 2-Deoxy-D-Glucose: Phase I/II Clinical Trials on Human Cerebral Gliomas. *Int J Rad Oncol Biol Physiol* **35**, 103-111.
- Halicka H D, Ardel B, Li X, *et al.* (1995). 2-Deoxy-D-glucose enhances sensitivity of human histiocytic lymphoma U937 cells to apoptosis induced by tumor necrosis factor. *Cancer Res* **55**, 444-449.
- Padilla-Paz I, Donat N J, Smith H O (2003). The effect of 2DG as a chemosensitizer and radiosensitizer on cervical cancer cells. *Proc. Am Soc Clin Oncol* p461.
- Lin X, Zhang F, Bradbury CM, *et al.* (2003). 2-Deoxy-D-glucose induced cytotoxicity and radiosensitization in tumor cells is mediated via disruptions in thiol metabolism. *Cancer Res* **63**, 3413-3417.
- Maschek G, Savaraj N, Priebe W *et al.* (2004). 2-deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers *in vivo*. *Cancer Res* **64**, 31-34.
- Bell S E, Quinn D M, Kellett G L *et al.* (1998). 2-deoxy-D-glucose preferentially kills multidrug-resistant human KB carcinoma cell lines by apoptosis *Brit J Cancer* **78**, 1464-1470.
- Keenan J, Liang Y, Clynes M (2004). Two-deoxyglucose as an

- anti-metabolite in human carcinoma cell line RPMI-2650 and drug-resistant variants. *Anticancer Res* **24**, 433-440.
24. Mack P, Ahren B, Jeppsson B *et al.* (1998). Influence of 2-deoxy-D-glucose and arterial ischaemia on glucose oxidation and growth of liver cancer in the rat. *Eur J Cancer Clin Oncol* **24**, 1433-1437.
25. Hervias I, Lasheras B, Aguirre N (2000). Deoxy-D-glucose prevents and nicotinamide potentiates 3, 4-methylenedioxyamphetamine-induced serotonin neurotoxicity. *J Neurochem* **75**, 982-990
26. Thompson D A, Lilavivathana U, Campbell R G (1981). Suppression of prolactin and growth hormone responses to 2-deoxy-D-glucose-induced glucoprivation by mazindol in humans. *Metabolism* **30**, 1015-1020.
27. Schurr A, Miller J J, Payne R S, *et al.* (1999). An Increase in Lactate Output by Brain Tissue Serves to Meet the Energy Needs of Glutamate-Activated Neurons. *J Neurosci* **19**, 34-39.
28. Miller ES, Koebel DA, and Sonnenfeld G. (1993). The metabolic stressor 2-deoxy-D-glucose (2-DG) enhances LPS-stimulated cytokine production in mice. *Brain Behav Immunol* **7**, 317-325.
29. Gad S C, Weil C S. (1989). Statistics for toxicologists. In: Hayes AW (Ed), Principles and Methods of Toxicology, second edition. Raven Press, New York, pp. 463-467.
30. Vijayaraghavan R, Thomson R, Schaper M., *et al.* (1994). Computer assisted recognition and quantification of sensory irritation, airway constriction and pulmonary irritation. *Archiv Toxicol* **68**, 490-499.
31. Chen W, Gueron M. (1992). The inhibition of bovine heart hexokinase by 2-deoxy-D-glucose-6-phosphate: characterization by ³¹P NMR and metabolic implications. *Biochimie*. **74**, 867-873.
32. Nelson C A, Wang J Q, Leav I, Crane P D. (1996). The interaction among glucose transport, hexokinase, and glucose-6-phosphatase with respect to 3H-2-deoxyglucose retention in murine tumor models. *Nuclear Med Biol* **23**, 533-541.
33. Chi M M, Pusateri M E, Carter JG, *et al.* (1987). Enzymatic assays for 2-deoxyglucose and 2-deoxyglucose 6-phosphate. *Anal. Biochem.* **161**, 508-513.
34. Haga N, Naito M, Seimiya H, *et al.* (1998). 2-Deoxyglucose inhibits chemotherapeutic drug-induced apoptosis in human monocytic leukemia U937 cells with inhibition of c-Jun N-terminal kinase 1/stress-activated protein kinase activation. *Int. J. Cancer.* **76**, 86-90.
35. Jain V K, Kalia V K, Gopinath PM, *et al.* (1979). Optimization of cancer therapy. part III - Effects of combining 2-deoxy-D-glucose treatment with gamma irradiation on normal mice. *Indian J Exp Biol* **17**, 1320-1325.
36. Thompson D A, Lilavivathana U, Campbell R G (1980). Thermoregulatory and related responses to 2-DG administration in humans. *Am J Physiol* **239**, 291-295.
37. Wan R, Camandola S, Mattson M P (2003). Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *FASEB J.* **17**, 1133-1134.
38. Abbink E J, Tack C J, Smits P (2001). Vascular effects of metabolic inhibition by 2-deoxy-D-glucose in humans. *J Cardiovas Pharmacol* **37**, 94-100.
39. Breier A, Crane A M, Kennedy C, Sokoloff L (1993). The effects of pharmacologic doses of 2-deoxy-D-glucose on local cerebral blood flow in the awake, unrestrained rat. *Brain Res* **618**, 277-282.
40. Singh S P, Singh S, Jain V (1990). Effects of 5-bromo-2-deoxyuridine and 2-deoxy-D-glucose on radiation induced micronuclei in mouse bone marrow. *Int J Rad Biol* **58**, 791-797.

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