Asian
Science
Bulletin

OPEN ACCESS

Comparative Study of LD_{50} Determination of Bisphenol A in Albino Wistar Rats Using Different Method

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ABSTRACT

Background and Objective: The intake of chemical substances by man has increased dramatically, mostly as consumables such as food and other household products. Bisphenol A (BPA) can elicit mild or severe toxicity, depending the extent of exposure. Bisphenol A is commonly used in most consumer goods and products. Attention has been placed on BPA's impact as an endocrine disruptor. The toxicity studies of BPA have been debated over the years and the LD_{50} values of Bisphenol A found in literature are different. This study attempts to determine the oral and subcutaneous LD_{50} of Bisphenol A in male and female albino Wistar rats. **Materials and Methods:** The LD_{50} was estimated using Lorkes, Karbers arithmetic methods and grouping methods. Graded doses of Bisphenol A ranging from 5000-1000 mg/kg b.wt., were for oral administration and 500-100 mg/kg b.wt., was used for subcutaneous administration. All rats were examined for mortality and clinical symptoms during the experiment. **Results:** The results showed that the LD_{50} was significantly different between the male and female species using the 3 different methods, with the male rats having higher LD_{50} values. The values of the LD_{50} were higher for the oral route of administration compared to the subcutaneous route and similar clinical sign changes were observed in the various experimental groups. **Conclusion:** The LD_{50} obtained is high, this finding implies that BPA can produce effects that lead to biochemical and functional impairments in cells, tissues and organs, this will in turn alter the biomolecules and functioning of the organism which if prolonged can be fatal.

KEYWORDS

Bisphenol A, lethal dose (LD_{50}), Lorkes method, Karbers method, subcutaneous administration, oral administration

INTRODUCTION

The quantity of chemical substances that are in use has increased to an innumerable amount. Especially Bisphenol A (BPA) which is found as a food component and additive. All these chemical substances found as additives, beverages, drugs and other household consumables components, when put together, can result in toxicity if used repeatedly. The LD_{50}, the dose that kills 50% of test animals' population is used in measuring toxicity[1] and different methods have been developed and adopted for measuring the LD_{50}.

Bisphenol A, is a chemical compound found in products like plastic. It is used mostly in the industrial production of materials used for packaging and storage. Numerous reports state that BPA production has

ISSN: 1813-0070 (Print)
https://doi.org/10.3923/asb.2024.92.98

Received: 22 Oct. 2023
Accepted: 11 Feb. 2024
Published: 23 Feb. 2024

significantly increased over the years[2]. It is the major chemical used in the production of polycarbonate plastics and epoxy-phenolic resins. These plastics are in turn used as food and drink containers, while the epoxy resins are used as an interior protective lining for cans. Because of its major applications in the production of plastic containers mostly used for food and beverage packing and in coating of cans, people are exposed to it[3]. Humans are regularly exposed to minute quantities of BPA, mainly through diet and occupation[4]. Universally, the existence and extensive use of BPA-containing products results in the major factor leading to high human exposure[5]. Research evidence has also shown exposure through dermal contact with thermal papers[6].

The effect of BPA on animals has been investigated and has shown BPA elicit adverse effects on brain[7,8]. It has also been accepted that BPA has endocrine-disrupting activity, low-dose effects, dose-response relationships and developmental effects. To date, there is an argument about the toxicity of BPA. The FDA labeled BPA as a safe agent[9], but more recent data generated from research has stirred discussion on the urgency for more risk assessment studies of BPA[10].

Some of the LD_{50} of Bisphenol A found in some literature includes; 4100 mg/kg b.wt., by NTP[11]. Morrissey *et al.*[12] and Goodman *et al.*[13] reported 3300-4240 mg/kg, Chapin *et al.*[14] and Lakind *et al.*[15], reported 3,250 mg/kg.

This study aims to determine oral and subcutaneous LD_{50} of Bisphenol A on male and female albino Wistar rats using Lorkes and Karber arithemetic methods of LD_{50} analysis and also compare the outcome.

MATERIALS AND METHODS

Study area: The study was carried out at Biochemistry Department Research Laboratory, Faculty of Natural and Applied Sciences, Gregory University, Uturu, Abia State Nigeria, from August to September 2023.

Chemicals: Bisphenol A (BPA) (CAS # 80-05-7) of analytical reagent grade (Purity>90%) was procured from Sigma Aldrich.

Feed, water and housing: The pellet diet was used in this research; BPA was delivered in drinking water and Wire meched cages have been used successfully in these studies[16].

Analysis of LD_{50}: Four weeks old male and female (n = 280) of body weight 140±6 g albino Wistar rats were purchased from Veterinary Department, University of Nigeria, Nsukka, Enugu, Nigeria. The rats were fed on a pellet diet (vitafeed Ltd., Enugu, Nigeria) and water *ad libitum*, maintained under standard laboratory conditions. The rats were acclimatized for one week before experimentation. Rats were randomly divided into groups consisting of six animals in each group.

The experiment was carried out in two major phases; oral administration group and subcutaneous administration group. Each of these was divided into two categories; category 1 for male Wistar rats and category 2 for female Wistar rats. These categories were further subdivided into various test groups and control groups. The treatment groups received different graded doses of Bisphenol A ranging from 5000-1000 mg/kg b.wt., for oral administration phase and 500-50 mg/kg b.wt., for subcutaneous administration. All rats were examined for mortality and clinical manifestation during the period of experiment.

Ethical consideration: The ethical clearance was obtained from animal ethics and consideration committee of Nnamdi Azikiwe University, Awka.

Statistical analysis: The statistical analysis was used to determine the level of significance between the methods and the LD_{50} between the sexes, at $p>0.05$.

METHODS

Lorke's method[1]: This method was carried out in two phases which are phases 1 and 2 for the oral and subcutaneous administration of Bisphenol A in male and female rats respectively.

In both phases 1 and 2, the control group was administered with the vehicle in which the test substance was dissolved that is water.

Phase 1: This phase was carried out for the male and female rats. The male and female animals are divided into five groups of six animals each respectively. Each group of animals are administered different doses of Bisphenol A orally and subcutaneously. The animals are placed under observation for 24 hrs to monitor their behavior as well as if mortality will occur.

Phase 2: This phase involves the use of male and female rats, which are distributed into four groups of six animals each. The animals were administered graded doses of BPA, the dosage of BPA was dependent on the outcome of the phase 1 experiment and then observed for 24 hrs for behavior as well as mortality.

Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where:

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Karber's method[1]: This method involves the administration of different doses of a test substance to five groups, which have six animals each for the male and female rats' categories, respectively. The control group was administered with the vehicle in which the test substance was dissolved that is water. The test group (5 groups) receive different doses of BPA orally and subcutaneously, respectively. The animals in each group receive specific doses, while an increment in dose progresses from group to group. The LD_{50} was calculated using the arithmetical method of Karber, which is as follows:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

Where:

LD_{50} = Median lethal dose

LD_{100} = Least dose required to kill 100%

a = Dose difference

b = Mean mortality

n = Group population

LD_{50} by grouping: This method involves administering the test substance and observing which dose concentration was able to kill half of the population samples within 24 hrs. These were carried out in two phases.

In the first phase, the treatment groups (1 to 9) received different graded doses of Bisphenol A (G-I = 5000 mg/kg, G-II = 4000 mg/kg, G-III = 3000 mg/kg, G-IV = 2000 mg/kg, G-V = 1000 mg/kg,

G-VI = 2200 mg/kg, G-VII = 2400 mg/kg, G-VIII = 2600 mg/kg G-XIX = 2800) orally for female. While, G-I = 5000 mg/kg, G-II = 4000 mg/kg, G-III = 3000 mg/kg, G-IV = 2000 mg/kg, G-V = 1000 mg/kg, G-VI = 2600 mg/kg, G-VII = 2700 mg/kg, G-VIII = 2800 mg/kg and G-XIX = 2900 orally for males.

For subcutaneous administration, (G-I = 500 mg/kg, G-II = 400 mg/kg, G-III = 300 mg/kg, G-IV = 200 mg/kg, G-V = 100 mg/kg, G-VI = 180 mg/kg, G-VII = 160 mg/kg, G-VIII = 140 mg/kg G-XIX = 120) orally for female. While, G-I = 500 mg/kg, G-II = 400 mg/kg, G-III = 300 mg/kg, G-IV = 200 mg/kg, G-V = 100 mg/kg, G-VI = 225 mg/kg, G-VII = 250 mg/kg, G-VIII = 275 mg/kg and G-XIX = 280 orally for males.

RESULTS

Result of oral route of administration: The results in Table 1, showed the number of mortality recorded after the oral administration of Bisphenol A in the male and female Wistar rats during the phase 1 study. It showed that 5000 and 4000 mg/kg were able to cause the death of all the experiment animals whereas at 2000 mg/kg, 1 rat died in the male rats as against 2 rats in the female. The dose of 1000 did not record any death in the female. The result in Table 2 shows the number of mortality recorded after the oral administration in phase 2 of the study in both male and female rats. The result revealed that 3 rats died at the dosage of 2200 mg/kg for the female rats and at 2800 mg/kg for the male rats.

Result for subcutaneous route of administration: The result in Table 3, showed the number of mortality recorded after the subcutaneous administration of Bisphenol A in the male and female Wistar rats during the phase 1 study. It showed that 500 and 400 mg/kg were able to cause the death of all the experiment animals whereas at 100 mg/kg, 2 rats died in the female rat's category and none was recorded for the male category. The result in Table 4 showed the number of mortality recorded after the subcutaneous administration in phase 2 of the study in both male and female rats. The result revealed that 3 rats died at the dosage of 160 mg/kg for the female rats and at 250 mg/kg for the male rats.

LD_{50} results: The result in Table 5, showed the different LD_{50} obtained from the experiment using three different methods. The LD_{50} obtained from the male category is higher than that of the female rat's category for all three methods used after oral and subcutaneous administration of BPA. It was observed that the oral route of administration has higher LD_{50} values than those of subcutaneous route.

Table 1: Number of mortality following oral administration of BPA, phase 1

	Dose (mg/kg b.wt.)		Number of death	
Group	Female	Male	Female	Male
1	5000	4000	6	6
2	4000	3500	6	5
3	3000	3000	5	4
4	2000	2500	2	2
5	1000	2000	0	1
6	Control			

Table 2: Number of mortality following oral administration of BPA, phase 2

	Dose (mg/kg b.wt.)		Number of death	
Group	Female	Male	Female	Male
1	2200	2600	3	2
2	2400	2700	4	2
3	2600	2800	4	3
4	2800	2900	4	4
5	Control			

Table 3: Number of mortality following subcutaneous administration of BPA, phase 1

	Dose (mg/kg b.wt.)		Number of death	
Group	Female	Male	Female	Male
1	500	500	6	6
2	400	400	6	6
3	300	300	5	4
4	200	200	4	2
5	100	100	2	0
6	Control			

Table 4: Number of mortality following subcutaneous administration of BPA, phase 2

	Dose (mg/kg b.wt.)		Number of death	
Group	Female	Male	Female	Male
1	180	225	4	2
2	160	250	3	3
3	140	275	2	4
4	120		2	
5	Control			

Table 5: LD_{50} obtained using grouping method, Lorke's and Karber's equation for LD_{50} determination

		LD_{50} (mg/kg b.wt.)		
Route of administration	Sex	LM	KM	Dosing
Oral	Female	2146.60*	2166.67*	2160*
	Male	2828.43*	2825.00*	2800*
Subcutaneous	Female	163.10#	170.00#	160#
	Male	240.83#	235.42#	240#

#Significance in the values of LD_{50} obtained for the male and female rats after subcutaneous administration of BPA for the methods used, *Significance in the values of LD_{50} obtained for the male and female rats after oral administration of BPA for the methods used, LM: Lorkes method and KM: Karbers method

DISCUSSION

Clinical signs: All the Bisphenol A treated animals showed treatment-related clinical signs such as soft stool and frequent urination were observed. Irregular movements, unrest tremors, convulsions and muscle spasms were observed in increased dosage concentration of Bisphenol A treated rats[17].

The result of the median lethal dose (LD_{50}) of Bisphenol A obtained is shown in Table 5 above, for all three methods used on the male and female Wistar rats, for both oral and subcutaneous routes of administration. The results indicated that the LD_{50} of Bisphenol A obtained for the male rats was significantly higher than those of the female species in all cases for the oral and subcutaneous route of administration. Comparing the effect of the method on the LD_{50} of the rats, it was observed that there is no significant difference between the three methods for both the male and the female rats. The result in Table 5, shows the different LD_{50} obtained from the experiment using three different methods. The LD_{50} obtained from the male category is higher than that of the female rats category for all three (3) methods used after oral and subcutaneous administration of BPA. It was observed that oral route of administration has higher LD_{50} values than those of subcutaneous route. The maximum toxicity was observed in the dosage of group that received 4000-5000 mg/kg (Table 1) and half of the animals were dead at the dosage above 2200 and 2800 mg/kg b.wt., for both female and male respectively after oral administration (Table 2) whereas for subcutaneous phase, LD_{50} of above 160 and 250 mg/kg b.wt., was observed for female and male respectively (Table 4).

Some of the LD_{50} values of Bisphenol A found in literature are divergent. The older once-recorded LD_{50} value for BPA was 4100 mg/kg b.wt., by NTP[11]; 3300-4240 mg/kg b.wt., by Morrissey *et al.*[12] and

Goodman *et al.*[13]; 3,250 mg/kg b.wt., by Chapin *et al.*[14] and LaKind *et al.*[15]; 3228 mg/Kg by Preethi *et al.*[3]. The LD_{50} value published in Material Safety Data Sheet of Societies producing Bisphenol A as Sigma Aldrich was >2000 mg/kg of Bisphenol A by oral route. Also, Oral LD_{50} values beyond 2000 mg/kg are indicated in the rat and mouse and dermal LD_{50} values above 2000 mg/kg are evident in the rabbit[11,18,19]. These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health.

CONCLUSION

Although the LD_{50} of Bisphenol A data were divergent in the literature, the oral administration of BPA was used in this study because the primary route of environmental exposure was through dietary intake of BPA-contaminated food and water. Toxicity can occur either immediately or at a short time interval as a result of a single, multiple and prolonged administration of chemical substances. The effect that is produced is capable of causing biochemical and functional impairments in cells, tissues and organs of the organism, the toxic effect of BPA can alter an organism's biochemical molecules (biomolecules) and their functionality. The extent of toxicity will depend on the route of administration and the sex of the organism among other factors. This study established the LD_{50} of Bisphenol A in male and female Wistar rats using the oral and subcutaneous routes of administration. The route of administration and sex of the organism have tremendous effects on the LD_{50} values obtained in the experiment for Bisphenol A.

SIGNIFICANCE STATEMENT

Toxicity can occur either immediately or at a short time interval as a result of a single, multiple and prolonged administration of chemical substances. The effect that is produced is capable of causing biochemical and functional impairments in cells, tissues and organs of the organism, the toxic effect of BPA can alter organisms' biochemical molecules (biomolecules) and their functionality. The extent of toxicity will depend on the route of administration and sex of the organism among other factors. This study established the LD_{50} of Bisphenol A in male and female Wistar rats using the oral and subcutaneous routes of administration. The route of administration and sex of the organism has a tremendous effect on the LD_{50} values obtained in the experiment for Bisphenol A.

REFERENCES

1. Chinedu, E., D. Arome and F.S. Ameh, 2013. A new method for determining acute toxicity in animal models. Toxicol. Int., 20: 224-226.
2. Calafat, A.M., Z. Kuklenyik, J.A. Reidy, S.P. Caudill, J. Ekong and L.L. Needham, 2004. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ. Health Perspect., 113: 391-395.
3. Preethi, S., L.D. Esther, B. Sreedevi, K. Chandrasekhar, B. Sarojamma and K.J. Pramoda, 2014. Toxicity studies of bisphenol-A induced Sprague Dawley rats. Int. J. Pharm. Pharm. Res., 2: 11-19.
4. Vandenberg, L.N., R. Hauser, M. Marcus, N. Olea and W.V. Welshons, 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol., 24: 139-177.
5. Vandenberg, L.N., I. Chahoud, J.J. Heindel, V. Padmanabhan, F.J.R. Paumgartten and G. Schoenfelder, 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect., 118: 1055-1070.
6. Biedermann, S., P. Tschudin and K. Grob, 2010. Transfer of bisphenol A from thermal printer paper to the skin. Anal. Bioanal. Chem., 398: 571-576.
7. Inagaki, T., M. Frankfurt and V. Luine, 2012. Estrogen-induced memory enhancements are blocked by acute bisphenol a in adult female rats: Role of dendritic spines. Endocrinology, 153: 3357-3367.
8. Eilam-Stock, T., P. Serrano, M. Frankfurt and V. Luine, 2012. Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behav. Neurosci., 126: 175-185.
9. Okada, H., T. Tokunaga, X. Liu, S. Takayanagi, A. Matsushima and Y. Shimohigashi, 2007. Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-γ. Environ. Health Perspect., 116: 32-38.

10. Hugo, E.R., T.D. Brandebourg, J.G. Woo, J. Loftus, J.W. Alexander and N. Ben-Jonathan, 2008. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ. Health Perspect., 116: 1642-1647.
11. Lee, H.S., M.Y. Pyo and M.H. Yang, 2012. Set, a putative oncogene, as a biomarker for prenatal exposure to bisphenol A. Asian Pac. J. Cancer Prev., 13: 2711-2715.
12. Morrissey, R.E., J.D. George, C.J. Price, R.W. Tyl, M.C. Marr and C.A. Kimmel, 1987. The developmental toxicity of bisphenol A in rats and mice. Fundam. Appl. Toxicol., 8: 571-582.
13. Goodman, J.E., E.E. McConnell, I.G. Sipes, R.J. Witorsch and T.M. Slayton *et al*., 2006. An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit. Rev. Toxicol., 36: 387-457.
14. Chapin, R.E., J. Adams, K. Boekelheide, L.E. Gray Jr. and S.W. Hayward *et al*., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. Part B: Dev. Reprod. Toxicol., 83: 157-395.
15. LaKind, J.S., M. Goodman and D.Q. Naiman, 2012. Use of NHANES data to link chemical exposures to chronic diseases: A cautionary tale. PLoS ONE, Vol. 7. 10.1371/journal.pone.0051086.
16. Howdeshell, K.L., P.H. Peterman, B.M. Judy, J.A. Taylor and C.E. Orazio *et al*., 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect., 111: 1180-1187.
17. Cichna-Markl, M., 2012. Sample clean-up by sol-gel immunoaffinity chromatography for the determination of bisphenol A in food and urine. Methods, 56: 186-191.
18. Peretz, J., L. Vrooman, W.A. Ricke, P.A. Hunt and S. Ehrlich *et al*., 2014. Bisphenol A and reproductive health: Update of experimental and human evidence, 2007-2013. Environ. Health Perspect., 122: 775-786.
19. Demierre, A.L., R. Peter, A. Oberli and M. Bourqui-Pittet, 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol. Lett., 213: 305-308.