J. Health Biol Sci. 2022;10(1):1-6 doi: 10.12662/2317-3206jhbs.v10i1.4461.p1-6.2022

ORIGINAL ARTICLE

Mutagenic Evaluation of Fluoxetine and Fluoxetine-Galactomannan Complex Through the Analysis of Chromosomal Aberrations in Human Peripheral Leukocytes and *Salmonella typhimurium*/Microssome Assay

Avaliação Mutagênica de Fluoxetina e Complexo Fluoxetina-Galactomanana Através da Análise de Aberrações Cromossômicas em Leucócitos Periféricos Humanos e em *Salmonella typhimurium* / Ensaio Microssômico

Bruno Coêlho Cavalcanti[1,2] ,Cecília Rocha da Silva[1,3] , João Batista de Andrade Neto[1,3,5] , Maria Aparecida Alexandre Josino[3] , Lívia Gurgel do Amaral Valente Sá[1,3,5] , Antônio Adailson de Sousa Silva[1] , Francisco Stefânio Barreto[1] , José Roberto de Oliveira Ferreira[6] , Hemerson Iury Ferreira Magalhães[7] , Ícaro Gusmão Pinto Vieira[4] , Débora Hellen Almeida Brito[4] , Nágila Maria Pontes Silva Ricardo[4] , Fátima Daiana Dias Barroso[1,3,5] ,Érica Rayanne Mota da Costa[3,5] , Hélio Vitoriano Nobre Júnior[1,3] , Manoel Odorico de Moraes[1,2]

1. Drug Research and Development Center, Federal University of Ceará, Fortaleza, CE, Brazil. 2. Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, CE, Brazil. 3. School of Pharmacy, Laboratory of Bioprospection in Antimicrobial Molecules, Federal University of Ceara, Fortaleza, CE, Brazil. 4. Department of Organic and Inorganic Chemistry, Federal University of Ceara, Fortaleza, CE, Brazil. 5. Christus University Center (UNICHRISTUS), Fortaleza, CE, Brazil. 6. School of Medical Sciences, State University of Health Sciences of Alagoas, AL, Brazil. 7. Department of Pharmaceutical Sciences, Federal University of Paraiba, João Pessoa, PB, Brazil.

Abstract

Objectives: The purpose of this study was to evaluate the mutagenic potential of fluoxetine and fluoxetine-galactomannan. Methods: Chromosomal aberration test and *Salmonella typhimurium*/microsome mutagenicity assay. Results: The results showed that fluoxetine (250 µg/mL) can cause chromosomal breaks of treated leukocytes and increase the frequency of reversion of the tester strains of *S. typhimurium* / microsome assay only at the highest concentration (5 mg/mL), while fluoxetine encapsulated in galactomannan did not cause these changes (leukocytes and *S. typhimuriums* strains). Conclusion: In summary, fluoxetine showed a mutagenic effect detectable only at high concentrations in both eukaryotic and prokaryotic models. Furthermore, the fluoxetine/galactomannan complex, in this first moment, prevented the mutagenicity attributed to fluoxetine, emphasizing that the present encapsulation process can be an alternative in preventing these effects *in vitro*.

Keywords: Fluoxetine; Galactomannan; Mutagenicity; Chromosomal Aberrations; *Salmonella typhimurium* /Microsome Assay.

Resumo

Objetivos: avaliar o potencial mutagênico da fluoxetina e da fluoxetina-galactomanana. Métodos: Teste de aberração cromossômica e ensaio de mutagenicidade de *Salmonella typhimurium* /microssoma. Resultados: a fluoxetina (250 µg/mL) pode causar quebras cromossômicas de leucócitos tratados e aumentar a frequência de reversão das cepas testadoras de *S. typhimurium* /microssoma apenas na concentração mais alta (5 mg/mL), enquanto a fluoxetina encapsulada em galactomanano não causou essas alterações (leucócitos e cepas de *S. typhimurium*). Conclusão: a fluoxetina mostrou um efeito mutagênico detectável apenas em altas concentrações em modelos eucarióticos e procarióticos. Além disso, o complexo fluoxetina/galactomanan, neste primeiro momento, evitou a mutagenicidade atribuída à fluoxetina, ressaltando que o presente processo de encapsulamento pode ser uma alternativa na prevenção desses efeitos *in vitro*.

Palavras-chave: Fluoxetina; Galactomanana; Mutagenicidade; Aberrações cromossômicas; Ensaio de *Salmonella typhimurium* /microssoma.

INTRODUCTION

Nanoparticles (NPs) generally have different physical-chemical and electronic properties, including higher specific surface area and surface reactivity. This is mainly due to the small size of the microparticles obtained during the production process[1]. The properties of NPs can also be related to another enhanced reactivity capacity, and consequently greater ability to penetrate tissues and cell membranes[2].

The use of microparticles today can be a great ally to the quality of life, but it can also raise concerns of society, as inevitable perceived risks cannot be ignored[3]. Thus, for NPs to be considered safe, they need to be tested to verify their possible toxic effects.

For NPs applications to be beneficial, they need to be quickly

Correspondence: : Érica Rayanne Mota da Costa. Laboratory of Bioprospection in Antimicrobial Molecules, Federal University of Ceara, Fortaleza, CE, Brazil. e-mail: ericaraymc@gmail.com
Conflict of interest: The authors declare that there is no conflict of interest.
Received: 2022 Jun 15; Revised: 2022 Sep 29; Accepted: 2022 Nov 11

internalized by cells[2,4]. However, NPs can also cause changes in the thickness of cell membranes, induce the formation of pores in the membrane and thus impair their integrity, and generate oxidative stress, resulting in nanotoxicity[5]. Therefore, during the processes of evaluation of NPs, it is necessary to observe the interaction with biomembranes (for example, with the cell membrane) to evaluate the ability to reach the target cells. Understanding the underlying mechanism of NPs-biomembrane interactions is important to improve the positive effects of NPs and thus avoid possible nanotoxicity.

According to[6] and the International Standardization Organization (expressed in ISO 10993), the *in vitro* cytotoxicity assay is the first test that should be used to assess the biocompatibility of any material for use in biomedical devices. Only after non-toxicity is proven can other tests be performed to complete the findings. Genotoxicity and mutagenicity assessment is also a useful component, where safety can be assessed when using certain substances, such as pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, and cosmetic ingredients, all of which are relevant in the context of international regulations aimed at protecting human and animal health[7].

Fluoxetine (FLX) is one of the selective serotonin reuptake inhibitors and galactomannan (Galact) is a natural polymer with several characteristics that can be advantageous for possible biopharmaceutical applications, among them its high capacity for gelling and the absence of toxic effects[8]

It is important to evaluate the genotoxicity potential and mutagenicity of compounds consumed by humans, particularly drugs. The toxicity of fluoxetine to various cell types and tissues is clearly described in the literature[14–17.18] reported that cytotoxicity and genotoxicity are associated with ROS production.

The main goal of the present study was to analyze the mutagenic potentials of FLX and FLX-Galact on human leukocytes (chromosomal aberration assay) and bacteria (*Salmonella typhimurium*/microsome assay).

MATERIALS AND METHODS

Chemicals

Fluoxetine (FLX) was purchased from Sigma Chemical (St. Louis, MO). Fetal bovine serum, phytohemagglutinin, RPMI 1640 medium, trypsin-EDTA, glutamine, penicillin, and streptomycin were purchased from Gibco® (Invitrogen, USA). Cyclophosphamine was supplied by Asta Medica. The S9 fraction, prepared from the livers of Sprague–Dawley rats pretreated with the polychlorinated biphenyl mixture Aroclor 1254, was purchased from Moltox Inc. (Boone, NC, USA). Colchicine, L-histidine, biotin, aflatoxin B1,4-nitroquinoline-oxide (4-NQO), and methyl methanesulfonate (MMS), were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Biomembranes

Encapsulation of fluoxetin

The formulation was prepared from a solution (1%) of the natural polymer galactomannan and stirred for 24 h at room temperature. Then fluoxetine and Tween 80 (0.1% per bioactive substance) were added and stirred for 4 h before spray drying in a Büchi B-290 spray dryer. The inlet and outlet temperatures were 120 and 90 °C, respectively. The feed flow was 10 mL/min and the aspirator flow was 35m^3/h [8] as described in [23].

The final proportion of polymer: bioactive was 90:10 w/w. The operational yield was 37%. The sample was stored with protection from humidity and light [8,9].

Isolation of peripheral blood leukocytes (PBLs)

The present study was approved by our university's research ethics committee (Process No. 161/2014). Heparinized blood was collected from healthy non-smoking donors who had not taken any medication in the 15 days before sampling and who had no history of exposure to potentially genotoxic substances. PBLs were isolated by the standard method of density-gradient centrifugation over Histopaque-1077. Cells were washed and resuspended in RPMI 1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin, at 37 °C under 5% CO2. Phytohemagglutinin (2.5%) was added at the beginning of the culture. After 24 hours, cells were treated with the test substances.

Chromosomal aberrations (CAs) test

After the end of treatment (24 hours) with FLX and FLX-Galact at concentrations of 100 and 250 µg/mL, determined from tests carried out previously with microparticles of galactomannan/fluoxetine against methicillin-resistant *S. aureus*[23], cells were washed with ice-cold PBS and re-cultivated in a complete RPMI medium for 48 h. Colchicine (0.0016%) was added 2 hours before fixation (72 h). Chromosomes were prepared according to standard procedures 10. Hypotonic treatment with KCl (0.75 M, 37 °C) was applied for 15 min. The cells were fixed with methanol and acetic acid (3:1), and the fixative solution was changed twice. Air-dried slides were stained with Giemsa (5%, pH 6.8) for 7 min and scored for CAs according to[11]. MMS (4 x 10-5 M), reference substance that has mutagenic character[22], and cyclophosphamide (50 µg/mL) were used as positive controls. Only well-spread metaphases were examined. One hundred and fifty metaphases per culture were analyzed for the presence of CAs. The mitotic index was determined for 2,000 cells and expressed as the number of mitoses per 100 cells (%)[12].

S. typhimurium /microsome mutagenicity assay

S. typhimurium TA98, TA97a, TA100, and TA102 were kindly provided by B. M. Ames (University of California, Berkeley,

CA, USA). Mutagenicity was assayed by the pre-incubation procedure. The S9 metabolic activation mixture (S9 mix) was prepared according to 13. Briefly, 100 µL of test bacterial cultures (1-2 x 10 9 cells/mL) were incubated at 37°C with FLX and FLX-Galact, at concentrations of 2500 and 5000 µg/plate, dissolved in water in the presence or absence of S9 mix for 20 min, without shaking. Subsequently, 2 mL of soft agar (0.6% agar, 0.5% NaCl, 50 M histidine, 50 M biotin, pH 7.4, 42 °C) was added to the test tube and the content was poured immediately onto a plate of minimal agar (1.5% agar, Vogel-Bonner E medium, containing 2% glucose). Aflatoxin B1 (1 µg/plate) was used as a positive control for all strains (in the presence of metabolic activation with S9 mix), 4-nitroquinoline-oxide (4-NQO, 0.5 µg/plate) for TA97a, TA98, and TA102, and sodium azide (1 µg/plate) for TA100 (absence of S9 mix). Plates were incubated in the dark at 37 °C for 48 h before counting the revertant colonies.

Statistical analysis

All experiments were performed independently three times. All statistical analyses were carried out using the GraphPad program (Intuitive Software for Science, San Diego, CA). For the chromosomal aberration test, data are presented as means ± SD and were compared by analysis of variance (ANOVA) followed by Tukey's test.

Salmonella/microsome mutagenicity data were analyzed using the Salmonel software. A compound was considered positive for mutagenicity only when: (a) the number of *revertants* was at least twice the spontaneous yield (MI ≥ 2; MI = mutagenic index: number of induced colonies in the sample/number of spontaneous colonies in the negative control samples); (b) a significant response was obtained in the analysis of variance ($p \leq 0.05$); and (c) a reproducible positive dose response ($p \leq 0.01$) was present, as evaluated by the Salmonel software.

RESULTS

Encapsulation of fluoxetin

Analysis of genetic changes at the chromosomal level induced by FLX and FLX-Galact on cultured PBLs

Table 1 shows chromosomal and numerical aberrations in cultured PBLs in the presence or absence of the S9 fraction of FLX and FLX-Galact. A slight increase in chromosome and chromatid ruptures were observed only in cultures treated with 250 µg/mL FLX in the absence of S9 mix. Moreover, numeric chromosomal changes like polyploidy and endoreduplication were not observed in any experimental cultures (FLX and FLX-Galact). Regarding cytotoxicity, FLX only at a concentration of 250 µg/mL caused a slight reduction ($p < 0.05$) in the mitotic index (cell proliferation), which was indicative of weak toxicity. This was not observed in FLX-Galact at both concentrations (100 and 250 µg/mL) in the presence or absence of the S9 fraction.

Table 1. Mitotic index, frequency of chromosomal aberrations, and numeric changes in cultured human PBLs after FLX and FLX-Galat exposure with and without metabolic activation (S9 fraction).

Compounds	S9 mix	Treatments	Mitotic index (%)[c]	Number of aberrations[d]			Aberant cells (%) [e]
				R	P	E	
Vehicle[a]	-	0.1%	6,39 ± 0,55	0	0	0	0
MMS[b]	-	4 x 10-5 M	2,84 ± 0,20*	29	0	0	9,66 ± 0,81*
Cyclophosphamide[b]	-	50 µg/mL	5,91 ± 0,55	3	0	0	1,33 ± 0,15
FLX	-	100 µg/mL	5,73 ± 0,21	1	0	0	0,44 ± 0,11
	-	250 µg/mL	5,22 ± 0,15*	5	0	0	2,21 ± 0,27*
FLX-Galat	-	100 µg/mL	6,27 ± 0,20	0	0	0	0
	-	250 µg/mL	5,95 ± 0,10	3	0	0	1,33 ± 0,15
Vehicle[a]	+	0.1%	6,83 ± 0,27	1	0	0	0,44 ± 0,11
MMS[b]	+	4 x 10^{-5} M	2,77 ± 0,56*	22	0	0	7,32 ± 0,56*
Cyclophosphamide[b]	+	50 µg/mL	3,96 ± 0,22*	18	0	0	5,99 ± 1,15*,#
FLX	+	100 µg/mL	5,78 ± 0,17	0	0	0	0
	+	250 µg/mL	5,62 ± 0,20	3	0	0	1,33 ± 0,15
FLX-Galat	+	100 µg/mL	6,41 ± 0,10	1	0	0	0,44 ± 0,11
	+	250 µg/mL	6,17 ± 0,25	0	0	0	0

[a]DMSO; [b]Positive controls; [c]determined for 2,000 cells (means ± SD); [d]number of aberrations per 150 metaphases analysed: R, ruptures (chromosome and chromatid); P, polyploid cells; E, endoreduplication); [e]percentage of cells with at least one aberration; *$p < 0.05$ compared to vehicle group by ANOVA followed by Tukey test. Data are presented as means ± SD for three independent experiments in triplicate; #$p < 0.05$ compared to experiments conducted in the absence of S9 mix by ANOVA followed by Tukey test. Data are presented as means ± SD for three independent experiments in triplicate.

Reversion analysis of mutations induced by FLX and FLX-Galact assessed by Salmonella/microsome mutagenicity assay

FLX and FLX-Galact were first tested for TA100 strain toxicity at concentrations of 1000-5000 µg/plate in the Salmonella/microsome assay. The range-finding results indicated no cytotoxicity at concentrations up to 5000 µg\mL in FLX. However, in the Salmonella/microsome assay, only FLX at 5000 µg\mL induced a significant increase ($p<0.05$) in the rate of reversion in all strains tested in the presence or not of exogenous metabolic activation (Tables 2 and 3). No mutagenic effect was observed for bacterial cultures treated with FLX-Galact at concentrations of 2500 and 5000 µg\mL. Highlighting the absence of mutagenic effect in bacterial systems in the presence or not of the metabolizing S9 fraction (Tables 2 and 3).

Table 2. Induction of his+ revertants in TA98 and 97a S.typhimurium frameshift strains by FLX and FLX-Galat with and without metabolic activation (S9 fraction).

		S. typhimurium							
		TA98				**TA97a**			
		-S9		**+S9**		**-S9**		**+S9**	
Compounds	**Treatments**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**
Vehicle[a]	0.1%	27,81 ± 8	-	31,15 ± 2	-	245,02 ± 48	-	208,37 ± 20	-
PC[b]		293,50 ± 75*	10,85	512,68 ± 154*	16,51	836,57 ± 139*	3,46	689,28 ± 118*	3,31
FLX	2500 µg/plate	12,64 ± 3	0,44	21,75 ± 7	0,67	230,28 ± 77	0,93	263,10 ± 51	1,26
	5000 µg/ plate	59,47 ± 11*	2,18	85,24 ± 15*	2,74	499,46 ± 111*	2,05	427,55 ± 95*	2,05
FLX-Galat	2500 µg/ plate	17,38 ± 5	0,62	32,91 ± 2	1,03	234,72 ± 37	0,95	266,24 ± 73	1,27
	5000 µg/ plate	35,02 ± 5	1,29	49.0 3 ± 9	1,58	252,15 ±5 5	1,02	294,16 ± 88	

aWater;
bPositive control: (-S9) 4-nitroquinoline 1-oxide (0.5 µg/plate); (+S9) aflatoxin B1 (1 µg/plate)
cNumber of revertants/plate presented as means ± SD for three independent experiments in triplicate
dMI: mutagenic index (number of his+ induced colonies in the sample/number of spontaneous his+ colonies in the negative control)
*$p < 0.05$ compared to vehicle group.

Table 3. Induction of his+ revertants in TA100 and 102 S.typhimurium frameshift strains by FLX and FLX-Galat with and without metabolic activation (S9 fraction).

		S. typhimurium							
		TA100				**TA102**			
		-S9		**+S9**		**-S9**		**+S9**	
Compounds	**Treatments**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**	**Rev/plate[c]**	**MI[d]**
Vehicle[a]	0.1%	103,48 ± 17	-	118,34 ± 32	-	347,15 ± 40	-	428,95 ± 25	-
PC[b]		571,52 ± 72*	5,54	374,50 ± 47*	3,16	2922,81 ± 218*	8,42	1131,90 ± 213*	2,64
FLX	2500 µg/plate	123,43 ± 28	1,19	139,57 ± 55	1,17	354,96 ± 63	1,02	370,26 ± 24	0,86
	5000 µg/ plate	384,60 ± 51*	3,72	475,14 ± 12*	4,02	704,48 ± 47*	2,03	952,84 ± 20*	2,22
FLX-Galat	2500 µg/ plate	111,07 ± 20	1,07	124,79 ± 31	1,05	329,61 ± 58	0,94	443,29 ± 41	1,03
	5000 µg/ plate	157,39 ± 15	1,52	203,51 ± 18	1,72	387,58 ± 37	1,11	498,35 ± 18	1,16

aWater;
bPositive control: (-S9) 4-nitroquinoline 1-oxide (0.5 µg/plate); (+S9) aflatoxin B1 (1 µg/plate)
cNumber of revertants/plate presented as means ± SD for three independent experiments in triplicate
dMI: mutagenic index (number of his+ induced colonies in the sample/number of spontaneous his+ colonies in the negative control)
*$p < 0.05$ compared to vehicle group.

DISCUSSION

There are several techniques available to measure mutagenicity, such as the Salmonella mutagenicity assay and tests for structural chromosome aberrations, micronuclei, and sister chromatid exchanges. Chromosome aberration analysis measures the frequency of breakages or exchange of chromosomal material including breaks in the chromosome or chromatid, rearrangements, translocations, inversions, or anaphase bridges. An increase in the frequency of chromosome aberrations is indicative of clastogenicity, which increases the risk for genetic ill health and cancer[19].

Fluoxetine is now the first choice antidepressant used to treat children and adolescents as monotherapy for unipolar depression[18]. There are a growing number of people suffering from anxiety and depression, therefore increasing the need for fluoxetine. So, verifying the absence of effects such as cytotoxicity or mutagenicity is of great importance.

Our results showed that fluoxetine caused chromosomal structural changes in PBLs only at the highest concentration (250 µg/mL), and provoke an increase in the number of colony formation in the Salmonella/microsome assay. Furthermore, encapsulating fluoxetine in galactomannan did not cause these changes, indicating encapsulation with the polymer decreases the clastogenic potential of fluoxetine at the highest concentration. Galactomannan, since it is a natural polymer, has several characteristics that can be advantageous for biopharmaceutical applications, one of which is its nontoxicity[8]. The assessment of genotoxic hazards to humans currently follows a stepwise approach, beginning with a basic battery of *in vitro* tests followed in some cases by in vivo testing[7]. Our data are preliminary, but galactomannan encapsulation can be an alternative to minimize mutagenic effects.

Cytotoxc effects of high concentrations of fluoxetine (2000 µg/mL) were found in A. cepa L.[14]. Root tip cells, probably because this type of drug affects carrier cells, resulting in cell death or inhibition of cell division. It was observed that fluoxetine promoted sister chromatid exchanges (SCE) and induced dose-dependent sperm abnormalities[15]. They also found that the highest dose tested increased SCE about two-fold and the level of control of sperm abnormalities about four-fold.

It was also shown that fluoxetine at concentrations of 0.2 and 1.0 mg/mL was not genotoxic by the comet assay in cultured Chinese hamster ovary (CHO) cells 21. However, they found fluoxetine to be genotoxic at a concentration of 5.0 mg/mL. These latter results were similar to our finding that only the highest concentration of fluoxetine (5000 µg\mL) showed mutagenic potential.

In summary, fluoxetine showed a mutagenic effect detectable only at high concentrations in both eukaryotic and prokaryotic models. Furthermore, the fluoxetine/galactomannan complex, in this first moment, prevented the mutagenicity attributed to fluoxetine, emphasizing that the present encapsulation process can be an alternative in preventing these effects *in vitro*.

ACKNOWLEDGEMENTS

This research was supported by grants and fellowships from National Council for Scientific and Technological Development, Higher Education Personnel Improvement Coordination / Brazil, and The Ceará Foundation for Support to Scientific and Technological Development /Ceará.

REFERENCES

1. Hochella MF Jr, Lower SK, Maurice PA, Penn RL, Sahai N, Sparks DL, et al. Nanominerals, mineral nanoparticles, and Earth systems. Science. 2008 Mar; 319(5870): 1631-1635. doi: 10.1126/science.1141134.

2. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005 Jul;113(7): 823-839. doi: 10.1289/ehp.7339.

3. Kumari M, Kumari SI, Kamal SS, Grover P. Genotoxicity assessment of cerium oxide nanoparticles in female Wistar rats after acute oral exposure. Mutat Res Genet Toxicol Environ Mutagen. 2014 Dec; 775-776: 7-19. doi: 10.1016/j.mrgentox.2014.09.009.

4. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev. 2004 Sep; 56(11):1649-1659. doi: 10.1016/j.addr.2004.02.014.

5. He XC, Lin M, Li F, Sha BY, Xu F, Qu ZG, et al. Advances in studies of nanoparticle-biomembrane interactions. Nanomedicine. 2015 jan. 10(1): 121-141. doi:10.2217/nnm.14.167.

6. Rogero SO, Lugao A, Ichikawa IT, Cruz AS. Teste *in vitro* de citotoxicidade: Estudo comparativo entre duas metodologias. Materials Research. 2003 jun. 6(3):317-320. doi: 10.1590/S1516-14392003000300003.

7. Corvi R, Madia F. *In vitro* genotoxicity testing-Can the performance be enhanced? Food Chem Toxicol. 2017 Aug; 106(Pt B): 600-608. doi: 10.1016/j.fct.2016.08.024.

8. De Souza CAG, Siqueira SMC, De Amorim AFV, Morais SM, Gonçalves T, Gomes RN, et al. Encapsulação do ácido L-ascórbico no biopolímero natural galactomanana por spray-drying: Preparação, caracterização e atividade antioxidante. Quim Nova. 2015 Aug; 38(7): 877-883. doi:10.5935/0100-4042.20150104.

9. Rodriguez-Canto W, Chel-Guerrero L, Fernandez VVA, Aguilar-Vega M. Delonix regia galactomannan hydrolysates: Rheological behavior and physicochemical characterization. Carbohydr Polym. 2019 Feb; 206: 573-582. doi:10.1016/j.carbpol.2018.11.028.

10. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res. 1960 Sep; 20(3): 613-616. doi:10.1016/0014-4827(60)90138-5.

11. Savage JR. Classification and relationships of induced chromosomal structual changes. J Med Genet. 1976 Apr; 12(2):103-122. doi:10.1136/jmg.13.2.103.

12. Arni P, Hertner T. Chromosomal aberrations *in vitro* induced by aneugens. Mutat Res. 1997 Sep; 379(1): 83-93. doi:10.1016/S0027-5107(97)00111-5.

13. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983 May; 113(3-4): 173-215. doi:10.1016/0165-1161(83)90010-9.

14. Düsman E, Almeida IV, Mariucci RG, Mantovani MS, Vicentini VEP. Cytotoxicity and mutagenicity of fluoxetine hydrochloride (Prozac), with or without vitamins A and C, in plant and animal model systems. Genet Mol Res. 2014 Jan; 13(1): 578-589. doi:10.4238/2014.January.28.3.

15. Alzahrani HAS. Sister chromatid exchanges and sperm abnormalities produced by antidepressant drug fluoxetine in mouse treated in vivo. Eur Rev Med Pharmacol Sci 2012 Dec; 16(15): 2154-2161. PMID: 23280034.

16. Lacaze E, Pédelucq J, Fortier M, Brousseau P, Auffret M, Budzinski H, et al. Genotoxic and immunotoxic potential effects of selected psychotropic drugs and antibiotics on blue mussel (Mytilus edulis) hemocytes. Environ Pollut. 2015 Jul; 202:177-186. doi:10.1016/j.envpol.2015.03.025.

17. Po WW, Thein W, Khin PP, Khing TM, Han KWW, Park CH, et al. Fluoxetine simultaneously induces both apoptosis and autophagy in human gastric adenocarcinoma cells. Biomol Ther (Seoul). 2020 Mar; 28(2): 202-210. doi:10.4062/biomolther.2019.103.

18. Elmorsy E, Al-Ghafari A, Almutairi FM, Aggour AM, Carter WG. Antidepressants are cytotoxic to rat primary blood brain barrier endothelial cells at high therapeutic concentrations. Toxicol *In vitro*. 2017 Oct; 44:154-163. doi:10.1016/j.tiv.2017.07.011.

19. Swierenga SHH, Heddle JA, Sigal EA, Gilman JPW, Brillinger RL, Douglas GR, et al. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. Mutat Res. 1991 Feb; 246(2): 301-322. doi:10.1016/0027-5107(91)90050-X.

20. Rosetti M, Frasnelli M, Tesei A, Zoli W, Conti M. Cytotoxicity of Different Selective Serotonin Reuptake Inhibitors (SSRIs) Against Cancer Cellstle. J Exp Ther Oncol. 2006; 6(1):23-9.

21. Lemos NG, Mantovani MS, Vicentini VEP. Avaliação do efeito genotóxico do Prozac (fluoxetina), sem e com adição de vitaminas A e C, através do teste do cometa em cultura de células CHO-K1. Semina: Semina: Ciênc. Biol. Saúde. 2005 Dec; 26(2): 95-100.

22. Ventura D, Grégio D'Arce LP, Lindino CA, Sebastien NY. Análise mutagênica e genotóxica do hormônio 17α-metiltestosterona por meio do sistema-teste Allium cepa. Acta Iguazu. 2019. Dec; 8(5): 52–59.

23.JOSINO, Maria AA, et al. Development and *in vitro* evaluation of microparticles of fluoxetine in galactomannan against biofilms of *S. aureus* methicilin resistant. Carbohydrate Polymers. 2021 Jan; 252: 117-184.

How to cite this article/Como citar este artigo:
Cavalcanti BC, Silva CR, Andrade JB Neto, Josino MAA, Sá LGAV, Silva AAS, et al. Mutagenic Evaluation of Fluoxetine and Fluoxetine-Galactomannan Complex Through the Analysis of Chromosomal Aberrations in Human Peripheral Leukocytes and *Salmonella typhimurium*/Microssome Assay J Health Biol Sci. 2022; 10(1):1-6.