JMSCR Vol||05||Issue||02||Pages 17324-17329||February 2017

www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i2.16

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Evaluation of Genotoxic, Embryotoxic and Teratogenic Potential of Paracetamol in Humans and Mice

Author
Dr Nandini Vaz Fernandes
Asst. Professor**,** Department of Zoology, Parvatibai Chowgule college of Arts and science"
Gogol, Margao Goa, India – 403602
Email: *nvf001@chowgules.ac.in / nandini_chgrl@yahoo.com, Tel: +91 9922023171*

ABSTRACT
Paracetamol being an over the counter drug is widely used as an analgesic. Though it was considered to be safe earlier, recent studies have shown that at high doses it does have a deleterious effect on humans as well as rodents. The present study was therefore undertaken to elucidate the genotoxic and teratogenic potential of paracetamol (acetaminophen) in the humans and in mice. The association of paracetamol intake during pregnancy and congenital malformations in the offspring was studied by epidemiological studies and by animal experimentation. The genotoxicity of paracetamol was tested by micronuclei test. Analysis of the data collected by proforma for the epidemiological study showed a higher frequency of occurrence of malformations in the offspring of the mothers who had consumed paracetamol during pregnancy (62.12%) as compared to those without paracetamol intake (48.48%). There was an increase in the micronuclei in the mice treated with paracetamol doses of both doses of 0.030 gms / Kg Bodyweight / day and 0.060 gms / Kg Bodyweight / day as was insignificant when compared to the control set. In the teratogenic study for both the paracetamol treated group of female mice, there was termination of pregnancies in the first trimester. It may therefore be assumed that paracetamol at the normal prescribed dose is not `genotoxic but it does have a embryotoxic potential in the mice.
Keywords: *genotoxicity, teratogenecity, Paracetamol.*

INTRODUCTION

Paracetamol is a widely used non-prescription analgesic and antipyretic drug and was considered to be a safe drug. But during the last few years, several studies indicating genotoxic effects of paracetamol has raised a question of regulatory action. This is supported by the studies of Nunes B et al, 2015, Antunes SC et al., 2013, Bergman K, et. al.[1996], Binkova B, et. al.[1990]. The genotoxicity of any chemical or drug can be evaluated by adopting various assays such as Micronuclei test, Comet assay, Dominant lethal test, Germ cell assay and cytogenetic assays. Micronuclei assay is an important component of genetic toxicology screening programs throughout the world. Teratogenicity can be evaluated by treating the pregnant female mice with selected doses of paracetamol and evaluating the fetal outcome.

Various in-vitro and in-vivo studies have shown that humans and rodents exposed to paracetamol doses above the therapeutic range have experienced hepatotoxicity. Mutagenicity of paracetamol is reported by Topinka J, et.al.,[1989]

and hepatotoxicity by Prescott LF [2000] and Ciszowski K et. al.,[2005]. Studies of Bomhard EM [2005] and Ramos As et al. 2014, also indicated an increase in the incidence of liver adenomas and carcinomas at a markedly toxic dose in mice and fishes respectively. However, at lower doses, toxic effects in rodents are minimal or absent. The genotoxicity of paracetamol, including covalent binding to DNA, induction of DNA single-strand breaks(SSBs), and inhibition of replicative and repair synthesis of DNA, has been investigated in rodents in vivo by Celik A[2006]. If paracetamol has mutagenic and Genotoxic effect, it may be necessary to evaluate its teratogenic potential too. Teratogenic studies of paracetamol are limited. Studies of Burdan F, 2003, Burdan F et. al.,.2001 indicate embryoto-xicity of paracetamol. Reliable studies on the ability of paracetamol to affect germ cell DNA are not available. Thus, there was a need to evaluate the genotoxic and teratogenic effect of paracetamol, and so the present study was undertaken.

MATERIALS AND METHODS

The association of paracetamol intake during pregnancy and congenital malformations in the offspring was studied by epidemiological studies and by animal experimentation (Teratogenicity study). The genotoxicity of paracetamol was tested by micronuclei test.

Epidemiological studies: A proforma was prepared for eliciting information for the present study. The necessary information was noted down to find out the correlation of paracetamol intake during pregnancy and congenital malformations. The data was collected by interrogating the parents of the newborns born in Goa Medical College, a major tertiary hospital in Goa. This data was then analyzed statistically using Chi-square test.

Teratogenicity test: Role of paracetamol in the induction of congenital malformation was investigated by using swiss albino mice Mus musculus as a test animal. 10-12 weeks old mice were housed in polypropylene cages and fed with standard food pellet and water ad libidum. The female mice were grouped into three groups of 5 each viz. 'A', 'B' and 'C'. Group 'A' served as control group. Females of group 'B' were administered an oral dose of paracetamol of 0.030 gms / Kg Bodyweight / day. Members of group 'C' received an oral dose of 0.060 gms of paracetamol / Kg Bodyweight / day. The females mice were crossed with healthy male mice and examined for fertilization by checking for vaginal plug. The pregnant females were then isolated and caged separately. They were then grouped into three and dosed according to the above-mentioned doses. The treated females as well as females from the control group were sacrificed on the 20th day of gestation and the uterine contents were examined for total implants, early resorptions, late resorptions and malformations.

Genotoxicity test: The genotoxicity of paracetamol was tested by micronuclei test. The females were grouped and treated with the above mentioned doses. After 24 hours of treatment the mice were sacrificed by cervical dislocation. The femur was then exposed and bone marrow was extracted in serum from each mice. The material was then smeared on the slides and air-dried for 18 hrs. The slides were then fixed in methanol and stained in 1:12 V/V of giemsa and distilled water, air-dried and screened for micronuclei in the erythrocytes of the mice. Around 2000 cells were scored for each animal and the result obtained was tabulated.

The Data was subjected to statistical analysis wherever required. Tools used were standard deviation and Chi-Square.

RESULT

Analysis of the data collected by proforma for the human epidemiological study showed higher frequency of occurrence of malformations in the offspring of the mothers who had consumed paracetamol during pregnancy. There was insignificant increase in the micronuclei in the mice treated with paracetamol. However

teratogenic study reflected embryotoxicity effect of paracetamol if consumed in early pregnancy.

Epidemiological studies: Based on the analysis of the data collected by proforma for the epidemiological study, the frequency of occurrence of malformations was found to be higher in the offspring of the mothers who had consumed paracetamol during pregnancy (62.12%) as compared to those without paracetamol intake (48.48%). The difference was found to be statistically significant (Table 1).

Teratogenicity test: The observation in the Control group and the Experimental / treated group is as follows.

Control Group (Group 'a'): The members of group 'A' served as the control group. The mean total implants, early and late deaths and live fetuses are tabulated in Table 2. The mean average weight of the fetus was 1.0046 □ 0.013.

Experimental Groups (Group 'b'and 'c'): The female mice treated with oral dose of paracetamol of 0.030 gms / Kg Bodyweight / day and 0.060 gms / Kg Bodyweight / day respectively, resulted in termination of pregnancies in the first trimester (Table 2).

Genotoxicity test: There was slight increase in the micronuclei in the mice treated with paracetamol doses of both doses of 0.030 gms / Kg Bodyweight / day and 0.060 gms / Kg Bodyweight / day as compared to the control set. The difference was found to be statistically insignificant. The results of the number and percentage of micronucleated polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) is tabulated in Table 3.

Table 1: Paracetamol Consumption During Pregnancy And Congenital Malformations

PARACETAMOL CONSUMPTION	TOTAL No. OF INDIVIDUALS	INDIVIDUALS WITH MALFORMATIONS	FREQUENCY (%)
POSITIVE	37	23	62.16
NEGATIVE	295	143	48.48

$X = 5.77$, $p < 0.05$

Table 2: Effect of Paracetamol in The Three Groups

GROUP	TOTAL IMPLANTS	EARLY DEATHS	LATE DEATHS	LIVE FETUSES	AVERAGE WT. OF FETUS
GROUP -1 (CONTROL)	10.4 ± 1.40	0	0.2	10.2 ± 1.304	1.0046 ± 0.013
GROUP -2 (0.03 gms/ Kg body wt/day)	(TERMINATION OF PREGNANCY IN ALL TREATED MICE)				
GROUP -3 (0.06 gms/ Kg body wt/day)					

Table 3: Induction of Micronuclei in Paracetamol Treated Mice

		PCE			NCE		
GROUP		MN present	MN absent	%MN	MN present	MN absent	%MN
GROUP -1	1	1	999	0.1	1	999	0.1
(CONTROL)	2	0	1000	0	0	1000	0
	3	0	1000	0	0	1000	0
	M	0.3	999.7	0.03	0.3	999.7	0.04
GROUP -2	1	2	988	0.2	1	999	0.1
(0.03 gms/	2	2	988	0.2	2	998	0.2
Kg body wt/day)	3	1	999	0.1	1	999	0.1
	M	1.7	991.7	0.17	1.3	998.7	0.13
GROUP -3	1	7	993	0.71	5	995	0.5
(0.06 gms/	2	8	992	0.81	6	994	0.6
Kg body wt/day)	3	7	993	0.71	6	994	0.6
	M	7.3	992.7	0.74	5.7	994.3	0.57

(± 0.33 for all)

DISCUSSION

Significant increase in the frequency of malformations was observed in the offspring of the mothers who had consumed paracetamol during pregnancy as compared to those without paracetamol intake. This shows a positive correlation between paracetamol intake and congenital malformations in the human population. This correlation can be attributed to the teratogenic potential of paracetamol. In the animal experiments, as there was termination of the pregnancies in all paracetamol treated mice, it may be assumed that paracetamol at the normal prescribed dose, has an embryotoxic effect in the mice. Chromosomal damage induced by paracetamol was assessed earlier [Dunn T L et. al.,1987]. His report suggests that paracetamol is capable of inducing chromosomal damage in mammalian cells. Most of the studies indicate that the use of paracetamol may contribute to an increase in the total burden of genotoxic damage in man. Paracetamol was also found to have carcinogenic effect in only some strains of mice. Studies of Antunes SC et al (2013), indicates that toxicity caused by acetaminophen is usually mediated by reactive oxygen species and can result in multiple effects, ranging from protein denaturation to lipid peroxidation and DNA damage

Further studies have also revealed that paracetamol induced DNA single strand breaks in mice treated invivo. Increase in the micronuclei in the mice treated with paracetamol doses in the present study was found to be statistically insignificant. The micronucleus test is an important component of genetic toxicology screening programs throughout the world. This test is being used to detect the cytogenetic effect of various chemicals and physical agents. Micronuclei are known to arise from acentric fragments and lagging whole chromosomes. Thus the micronucleus endpoint is capable of detecting clastogenic agents, which cause chromosomal breaks as well as aneugens. The major ultimate reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) of paracetamol at high doses was found to be the component resulting in genotoxicity [Dybing E, et. al., 1984]. It induced

decreased DNA repair synthesis in isolated mouse liver cells in monolayer cultures, at a concentration where cytotoxicity was also evident. The genotoxicity of paracetamol including covalent binding to DNA, induction of DNA single strand breaks and inhibition of replicative and repair synthesis of DNA has been investigated in the rodents in vivo [Dunn TL et. al., 1987]. The DNA damage was measured as SSBs by alkaline elution. The available data points to three possible mechanisms of paracetamol induced genotoxicity.

1) Inhibition of ribonucleotide reductase.
2) Increase in the cytosolic and intranuclear calcium levels.
3) DNA damage caused by N-acetyl-p-benzoquinone imine (NAPQI), a major ultimate reactive metabolite of paracetamol. All mechanisms involve dose thresholds. Various studies on genotoxic effect of paracetamol obtained inconclusive results. Some invivo studies revealed that paracetamol induced SCEs and chromosomal aberrations but not micronuclei [Hongslo J K et. al., 1994 and Giri AK et. al., 1992]. But studies conducted by MatterB and Schmid W [1971] revealed increase in the micronuclei frequency in the buccal mucosa after intramuscular administration of paracetamol.

Therefore it can be concluded that at high doses paracetamol results in increased incidence of liver adenomas and carcinomas and also contributes to an increase in the total burden of genotoxic damage in man and mice. Paracetamol is also found to have carcinogenic effect in some strains of mice and induces DNA single strand breaks in mice treated in-vivo. However at normal prescribed doses it is not genotoxic, but does have teratogenic potential as indicated through increased number of malformations in humans. In mice it has embryotoxic potential too, as indicated by pregnancy loses when administered in the early pregnancy.

ACKNOWLEDGEMENT

I express my gratitude to all participants who consented to take part in this study

REFERENCES

1. Nunes BI, Verde MF, Soares AM. Biochemical effects of the pharmaceutical drug paracetamol on Anguilla anguilla. *Environ Sci Pollut Res Int.* 2015 :22(15):11574-84. doi: 10.1007/s11356-015-4329-6.
2. Antunes SC, Freitas R, Figueira E, Gonç-alves F, Nunes B. Biochemical effects of Acetaminophen in aquatic species: edible clams Venerupis decussatus and Venerupis philippinarum. *Environ Sci Pollut Res.* 2013:20:6658–6666
3. Bergman K, Muller L, Teigen W S. The genotoxicity and carcinogenicity of paracetamol; A regulatory review. *Mutat. Res*. 1996: 349 (2):263-88.
4. Binkova B, Topinka J, Sram J R. The effect of paracetamol on oxidative damage in human peripheral lymphocytes. *Mutat. Res*. 1990:244: 227-231.
5. Dunn T L, Gardiner R A, Seymour G J, Lavin M F. Genotoxicity of analgesic compounds assessed by an invitro micronuclear assay. *Mutat. Res*. 1987: 189(3);299-306.
6. Topinka J, Sram RJ, Sirinjan G, Kocisova J, Binkova B. Mutagenicity studies of paracetamol in human volunteers; Unsch-eduled DNA synthesis and micronucleus test. *Mutat. Res*. 1989:327:171-179.
7. Prescott LF. Paracetamol, alcohol and the liver.*Br J Clin Pharmacol*.2000:49(4):291-301.
8. Ciszowski K, Gomółka E, Jenner B..The influence of the dose, time since ingestion and concentration of the xenobiotic on the clinical state and severity of liver damage with patients intoxicated with paracetamol. *Przegl Lek.* 2005:62(6):456-61.

9. Bomhard EM, Herbold BA. Genotoxic activities of aniline and its metabolites and their relationship to the carcinogenicity of aniline in the spleen of rats. *Crit Rev Toxicol*. .2005:35(10):783-835.
10. Ramos AS, Correia AT, Antunes S, Gonçalves F, Nunes B. Effect of acetaminophen exposure in Oncorhynchus mykiss gills and liver: detoxification mechanisms, oxidative defence system and peroxidative damage. Environ *Toxicol Pharmacol*. 2014:37:1221–1228
11. Celik A.. The assessment of genotoxicity of carbamazepine using cytokinesis-block (CB) micronucleus assay in cultured human blood lymphocytes. Drug Chem Toxicol. 2006: 29(2):227-36.
12. Burdan F. Intrauterine growth retardation and lack of teratogenic effects of prenatal exposure to the combination of paracetamol and caffeine in Wistar rats. *Reprod Toxicol*.. 2003;17(1):51-8
13. Burdan F, Siezieniewska Z, Kiś G, Blicharski T. Embryofetotoxicity of acetaminophen (paracetamol) in experimental in vivo model. *Ann Univ Mariae Curie Sklodowska* 2001:56:89-9
14. Dunn T L, Gardiner R A, Seymour G J, Lavin M F.. Genotoxicity of analgesic compounds assessed by an invitro micron-uclear assay. Mutat. Res 1987:189(3);299-306.
15. Dybing E, Holme J A, Gordon W P, Soderland E J, Nelson D S, Dahlin D C. 1984. Genotoxicity studies with paraceta-mol. *Mutat. Res.* 138(1): 21-32.
16. Hongslo J K, Smith C V, Brunburg, Soder-rland E J. Genotoxicity of paracetamol in mice and rats. Mutagenesis 1994:9 (2):93-100
17. Giri A K, Sivam S S, Khan K A.. Sister chromatid exchange and chromosomal aberrations induced by paracetamol invivo in the bone marrow cells of mice. *Mutat. Res.* 1992: 298(2):139-140
18. Matter B, Schmid W.. Trenimon induced chromosomal damage in bone marrow cells of six mammalian species evaluated by micronucleus test. *Mutat. Res* 1971:12:411-425.