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Toxicopathological studies of Butylparaben in Wistar rats (*Rattus norvegicus*)

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Abstract
Butylparaben (BP) is widely used as preservatives in cosmetics, as a food additive and in the drug formulations. It has antifungal and antimicrobial properties and is active against moulds and yeasts and, to a lesser extent, bacteria. The present toxicopathological studies on Butylparaben was carried out on forty Wistar rats including 20 male and 20 female rats by dividing them into four equal groups, *viz.*, Group I, II, III and IV. Group I served as vehicle control and received only corn oil whereas Group II, III and IV served as treatment groups, orally administered with butylparaben at the dose of 2000 mg/kg (low), 4000 mg/kg (mid) and 6000 mg/kg (high), respectively. The experimental animals were closely observed daily for physical and behavioural changes, body weights (weekly) for 28 days. All the rats were subjected to haemato-biochemical profile, organ weight and pathomorphological studies on 29th day of experiment. There were no noticeable behavioural and other clinical signs in rats throughout the experimental period of 28 days. The haematological parameters revealed no significant changes in any parameter except significant (p<0.05) decrease in MCHC level in Group III and IV male rats as compared to control rats. The biochemical parameters revealed significant (p<0.05) increase in AST level in male of Group IV and female rats of Group III and IV whereas significant (p<0.05) increase in ALT level was recorded in Group IV male rats. There was significant (p<0.05) increase in ALP levelin the male and female rats of Group III and IV as compared to control group animals. Varying degree of pathomorphological changes comprised of periportal vacuolation in liver and acanthosis, hyperkeratosis and parakeratosis in fore stomach of rats of Group IV.

Keywords: Butylparaben, *Wistar rats*, toxicopathology, forestomach

Introduction
Environmental pollution is one of the greatest threats to human and animal health. Parabens (PB), a group of substances commonly employed as preservatives can potentially be termed as environment pollutants. The global widespread use of PB has resulted in their ubiquitous occurrence in the environment. There are five widely marketed parabens: Methylparaben (MP), Ethylparaben (EP), Propylparaben (PP), Butylparaben (BP) and Benzylparaben (BeP).
Butylparaben (BP) is widely used as preservatives in cosmetics such as fragrance powders, men's talcum, skin care preparations, cold creams, lotions, liquids, moisturizing skin care preparations, arthritis analgesic creams with methylsulfonylmethane, lipsticks, mascara and other eye make-up, pharyngeal antiseptic, acne cleansing products, pore cleansers, exfoliants, and cleansing cloths, perineal cleansing preparations, vaginal contraceptives and cradle cap treatment. It is used as a food additive (especially in beer) and in the drug formulations (Yang *et al.*, 2018) [13]. BP has antifungal and antimicrobial properties also (David *et al.*, 2012) [4]. BP is most active against moulds and yeasts and, to a lesser extent, bacteria. In comparison to other parabens. Indiscriminate use of parabens increases its concentrations in the surface as well as in the river water. Presence of paraben has also been detected from agricultural and forestry soil in the different areas of the Spain (Núñez*et al*., 2008) [6]. Pérez et *al*. (2012) [7] observed the presence of several parabens in agricultural soils amended with treated sewage sludge and industrial soil. Other places including indoor dust and air originate from personal care products used in households showed detectable level of Parabens to which individuals exposed by inhalation and oral ingestion (Rudel *et al*., 2003; Canosa *et al*., 2007) [9, 2].

There are very few reports available regarding parabens/ butylparaben toxicity in laboratory animals in India and in abroad.

Materials and Methods

The present study was carried out at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Gujarat, India. The experiment has been designed to study toxicopathology of Butylparaben (BP) in Wistar rats (*Rattus norvegicus*). The study was conducted on 40 healthy and adult non pregnant Wistar rats having 10 to 12 weeks of age. Research protocols as per the CPCSEA guidelines on the care and use of laboratory animals were followed and approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design

The toxic pathological studies on butylparaben toxicity were carried out on 20 male and 20 female rats. All the 40 rats were randomly divided into four different groups. Each group consisted of five male and five female rats. The groups were numbered as group I to IV. Group I served as control and received only vehicle (corn oil), while Group II, III and IV received butylparaben dissolved in corn oil at doses of 2000 mg/kg (low dose), 4000 mg/kg (mid dose) and 6000 mg/kg body weight (high dose), respectively, by oral gavage daily for 28 days. The detail of experimental design is mentioned in Table 1.

Table 1: Experimental design

Group	No. of Wistar Rats		Treatment	Dose mg/kg b.wt/day	Route
	Male	Female			
I	101-105 (5)	151-155 (5)	Vehicle (Corn oil)	4 ml/kg b. wt (Control)	Oral gavage
II	201-205 (5)	251-255 (5)	Butylparaben in corn oil	2000 mg/kg b. wt (Low)	Oral gavage
III	301-305 (5)	351-355 (5)	Butylparaben in corn oil	4000 mg/kg b. wt (Mid)	Oral gavage
IV	401-405 (5)	451-455 (5)	Butylparaben n corn oil	6000 mg/kg b. wt (High)	Oral gavage

Physical and behavioural examinations

All animals were observed regularly for any abnormal physical or behavioural symptoms. The time of onset, intensity and duration of such symptom, if any, were recorded daily up to 28 days of study.

Haematology

Rats were anesthetized by using isoflurane for blood collection. Blood was collected from all experimental groups on 29th day of experiment from retro-orbital plexus with the help of capillary tube. Blood was collected in sterilized vials containing 4.0 per cent potassium ethylene diamine tetra acetic acid (K_3EDTA) as an anticoagulant for estimation of haematological parameters and in serum clot activator vial for biochemical estimates. Blood smears were prepared for differential leukocyte count. Estimation of hematological parameters in K_3EDTA was carried out from collected blood samples by using Automated Blood Analyzer (Exigo haematology analyzer, Boule Medical AB, Sweden) by impedance method. The parameters studied were Total leukocytes count (TLC), (10^3/ µL), Differential leukocyte count (DLC), Haemoglobin (Hb), (g/dL),Total Erythrocyte count (TEC), (10^6/ µL), Packed Cell Volume (PCV) or Haematocrit HCT (%), Mean Corpuscular Volume (MCV), (fL), Mean Corpuscular Haemoglobin concentration (MCHC), (g/dL), Mean Corpuscular Haemoglobin MCH (pg), Red cell Distribution Width (RDW), (%), Red cell Distribution Width (RDW), Platelet (PLT), (10^3/µL) and Mean Platelet Volume (MPV), (fL).

Biochemical study

Estimation of biochemical parameters in serum was carried out using Fully Automated Biochemistry Analyzer (RANDOX-RX Monaco, United Kingdom). The parameters studied were Alanine Aminotransferase (ALT), (U/L), Aspartate Aminotransferase (AST), (U/L), Alkaline phosphatase (ALP), (U/L), Urea (mg/dL), Creatinine (mg/dL), Total protein (TP), (g/dL), Albumin (g/dL), Cholesterol (mg/dL), Triglycerides (mg/dL), etc.

Histopathology

On 29th, day all the rats were sacrificed by decapitation. All sacrificed animals were subjected to post mortem examination to determine the presence/absence of any appreciable gross lesions. Detailed post mortem lesions from all the animals were recorded. Tissue samples were collected and preserved in 10% neutral buffered formalin**.** Fixed tissues were trimmed, labelled and washed under running tape water for 2 hours. Dehydration was done using ascending grade (30%, 70%, 90%, and 100%) of isopropyl alcohol. The dehydrated tissues were cleared by three changes of xylene and impregnated in melted paraffin wax. The entire tissue processing was done in automatic tissue processor (Leica TP1020). The paraffin impregnated tissues were embedded using Leica EG1160 paraffin embedding station and cooled on Leica EG1150 C Cold Plate. The 4 to 5µ thick sections were cut using Leica RM2255 fully automated rotary microtome. The sections were taken on poly-L-lysine-coated (0.1% w/v in H2O) slides and stained with Mayer's Hematoxylin and Eosin (H & E). The sections were deparaffinised by xylene, and rehydrated through descending grade of isopropyl alcohol and water. The hydrated tissue sections were stained with hematoxylin, differentiated in acid alcohol and blueing was carried out by ammonia water. Then tissue sections were stained with eosine, dehydrated with absolute isopropyl alcohol, followed by xylene wash and mounted with DPX (Suvarna *et al*., 2012) [12]. The entire slide staining was done in Gemini AS Automated Slide Stainer, Thermo Scientific.

The statistical analysis of data generated on various parameters were subjected to statistical analysis using 2-way analysis of variance (ANOVA). Pairwise comparisons with control, for each sex separately, was made using Dunnett's test, (Snedecor and Cochran, 1980) [11].

Results and Discussion

The results obtained from the present study have been presented and discussed under the following headings:

Symptomatogy: Rats of Group II, III and IV did not reveal any noticeable symptoms and clinical signs in comparison to control Group I rats throughout the experimental period of 28

days. There seems to be no published report available on symptoms of oral BP toxicity in rats. However, reported rapid onset of ataxia, paralysis and depression in mice. All the male and female rats of Group I, Group II, Group III and Group IV were observed daily for mortality (if any) till day 28 of experiment. No mortality was noticed in all the four Group rats throughout the study period of 28 days. The finding of present study was in accordance with Rodrigues *et al.* (1986) [8]. They didn't observe any mortality in rats orally administered with BP @ dose rate of BP at 4000 mg/kg for 27 consecutive days.

Body weight: The body weight of male and female rats of all groups was measured at weekly interval i.e. on Day 0, Day 7, Day 14, Day 21 and Day 28 of the study and presented in Table 2 and Table 3, respectively.

Table 2: Effect of Butylparaben on weekly body weight (g), (Mean ± SD, N=5.) in male rats after daily oral administration for 28 days.

Body weight (g) of male rats				
Group	**I**	**II**	**III**	**IV**
Days	**0 mg/kg**	**2000 mg/kg**	**4000 mg/kg**	**6000 mg/kg**
0 Day	298.89±23.652	310.99±21.840	310.20±25.972	325.85±24.568
7 Day	309.23±21.612	317.94±22.027	316.81±26.312	318.16±21.864
14 Day	317.20±23.507	322.81±23.350	316.43±25.208	316.70±21.155
21 Day	326.32±27.277	326.03±21.369	312.08±23.058	309.99±21.359
28 Day	332.87±30.174	328.29±21.713	306.61±20.869	298.47±29.457

Table 3: Effect of Butylparaben on weekly body weight (g), (Mean ± SD, N=5.) in female rats after daily oral administration for 28 days.

Body weight (g) of female rats				
Groups	**I**	**II**	**III**	**IV**
Days	**0 mg/kg**	**2000 mg/kg**	**4000 mg/kg**	**6000 mg/kg**
0 Day	219.65±18.825	220.50±16.736	218.49±16.553	211.56±14.027
7 Day	222.11±15.592	222.91±13.555	219.02±14.800	218.27±10.950
14 Day	226.52±14.114	227.71±13.219	217.97±11.439	221.09±8.322
21 Day	230.15±15.767	226.02±15.113	216.90±10.806	210.11±9.062
28 Day	232.31±16.601	225.77±16.316	210.67±9.845*	200.91±6.944*

* Significant (p<0.05)

On day of 28 day of experiment, the mean body wt. of male rats was found to be 328.29±21.713 g, 306.61±20.869 g and 298.47±29.457 g respectively in Group II, III and IV rats as compared to 332.87±30.174 g in Group I rats. There was no significant change in mean body weights in male animals of all treatment groups in comparison to control group animals throughout the experimental period.

The mean body wt. of females on day 28 was 225.77±16.316 g, 210.67±9.845 g and 200.91±6.994 g respectively in Group II, III and IV rats as compared to 232.31±16.601 g in Group I control rats. There was significant (p<0.05) reduction in the body wt. of female rats belonging to Group III and Group IV rats as compared to Group I control female rats. However, there was no significant change in the body wt. of female rats of Group II as compared to Group I control rats on day 28 of experiment. The reduction in the body wt. as observed in the present study was in agreement with the report by Daston (2004) [3], in Sprague-Dawley pregnant rats orally administered with BP at 1000 mg/kg body wt. on gestation day 6-19. Inai*et al.*, (1985) [5] also reported 10 per cent reduction in body weight in mice orally administered with BP at dose rate of 1900 or 3800 mg/kg b. wt.

Haematology

Haematological parameters of all the male and female rats of control Group I, Group II (2000 mg/kg), Group III (4000 mg/kg) and Group IV (6000 mg/kg) were studied on 29th day and presented in Table 4 and 5. There was no significant changes in any of the hematological parameters in all experimental groups male and female rats except Group III and IV male rats that showed significant (p<0.05) decrease in MCHC only as compared to control group rats.

Table 4: Effect of Butylparaben on haematological parameters (Mean ± SD, N=5) in male rats after daily oral administration for 28 days.

Haematology data of male rats					
Parameter	**Unit**	**G I**	**G II**	**G III**	**G IV**
		0 mg/kg	**2000 mg/kg**	**4000 mg/kg**	**6000 mg/kg**
TLC or WBC	10^3/µL	10.72±2.009	9.52±1.103	10.40±3.126	10.56±1.155
Neutrophils	10^3/µL	4.28±1.137	3.68±0.356	3.52±0.915	4.52±0.986
Lymphocytes	10^3/µL	5.84±1.428	4.58±1.057	6.02±2.499	5.26±0.885
Monocytes	10^3/µL	0.68±0.303	0.70±0.200	0.72±0.164	0.64±0.416
Eosinophils	10^3/µL	0.12±0.045	0.14±1.055	0.14±0.055	0.14±0.089
Basophils	10^3/µL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Haemoglobin	g/dL	13.88±0.589	14.82±0.650	14.74±1.582	13.86±0.688
PCV or HCT	%	42.96±2.793	47.14±2.668	47.38±4.956	44.04±1.278
TEC or RBC	10^6/ µL	8.12±0.444	8.68±0.509	8.41±1.062	8.34±0.590
MCV	fL	52.38±1.424	55.30±1.786	54.54±1.555	52.88±2.308
MCHC	g/dL	32.48±0.835	31.40±0.663	30.82±1.026*	30.70±1.239*
MCH	pg	17.10±0.224	17.08±0.259	17.20±0.316	16.88±0.311
RDW	%	19.90±0.339	21.30±0.828	20.48±0.756	20.78±0.960
PLT	10^3/µL	781.40±96.909	795.60±103.493	833.40±138.509	816.40±142.042
MPV	L	5.74±0.297	5.98±0.653	5.38±0.460	5.42±0.597

* Significant (p<0.05)

Table 5: Effect of Butylparabenon haematological parameters (Mean ± SD, N=5) in female rats after daily oral administration for 28 days.

Haematology data of female rats					
Parameter	**Unit**	**G I**	**G II**	**G III**	**G IV**
		0 mg/kg	**2000 mg/kg**	**4000 mg/kg**	**6000 mg/kg**
TLC or WBC	10^3/µL	7.96±1.783	8.00±2.152	8.04±1.746	8.36±1.973
Neutrophils	10^3/µL	2.82±0.798	2.62±1.121	2.96±0.844	3.74±0.981
Lymphocytes	10^3/µL	4.18±0.691	4.74±1.324	4.32±0.944	3.98±1.173
Monocytes	10^3/µL	0.80±0.418	0.48±0.217	0.58±0.356	0.50±0.300
Eosinophils	10^3/µL	0.16±0.089	0.16±0.089	0.18±0.084	0.14±0.055
Basophils	10^3/µL	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Haemoglobin	g/dL	12.82±0.870	12.82±1.236*	12.36±0.940	12.96±2.873
PCV or HCT	%	40.00±3.569	39.28±3.629	39.84±2.614	40.72±8.158
TEC or RBC	10^6/ µL	7.39±0.646	7.12±0.368*	6.82±1.052	7.30±1.502
MCV	fL	54.02±0.904	55.66±2.184	55.22±1.422	56.00±1.308
MCHC	g/dL	33.16±1.043	32.64±0.744	31.46±1.222	31.76±1.062
MCH	Pg	18.02±0.268	18.14±0.817	17.48±0.701	17.90±0.738
RDW	%	19.50±0.632	19.18±0.342	19.84±0.462	19.62±0.614
PLT	10^3/µL	530.80±120.442	572.20±129.978	510.00±129.043	611.20±181.565
MPV	fL	6.32±0.531	6.48±0.482	5.92±0.164	6.06±0.134

On the basis of various haematological parameters, it has been assumed that the dose rate of BP up to 6000 mg/kg orally for 28 days or duration of the study was not sufficient to produce any significant changes in any parameter except MCHC in Group III and IV male rats. MCHC indicates the amount of hemoglobin per unit volume. MCHC correlates the hemoglobin content with the volume of the cell. In present study, there was no significant alteration in hemoglobin value in any of the Group of male as well as female rats. So significant decrease (p<0.05) in MCHC values in Group III and IV male rats as compared to vehicle control rats seems to be of no biological significance. There seems to be no published reports on haematology of BP induced toxicity in rats.

Biochemical Profile

Biochemical parameters of all the male and female rats of Group I (control Group), Group II (2000 mg/kg), Group III (4000 mg/kg) and Group IV (6000 mg/kg) after daily oral administration of BP for 28 days are presented in Table 6 and Table 7.

Table 6: Effect of Butylparaben on biochemical parameters (Mean ± SD, N=5) in male rats after daily oral administration for 28 days.

Biochemical profile of male rats					
Parameter	**Unit**	**G I**	**G II**	**G III**	**G IV**
		0 mg/kg	6 mg/kg	12 mg/kg	24 mg/kg
Alanine Aminotransferase (ALT)	U/L	22.28±4.553	43.96±17.128	36.90±6.948	99.78±26.484*
Aspartate Aminotransferase (AST)	U/L	142.98±13.227	161.44±40.290	204.38±50.569	326.06±172.317*
Alkaline Phosphatase (ALP)	U/L	127.62±29.971	190.66±47.493	215.52±68.607*	249.48±52.95*
Total Protein	g/dL	6.10±0.406	6.60±1.181	6.48±0.522	5.42±0.476
Albumin	g/dL	3.10±0.324	2.71±0.522	2.84±0.230	2.58±0.164
Urea	mg/dL	25.28±3.173	35.26±9.461	35.80±6.336	32.80±7.308
Creatinine	g/dL	0.43±0.164	0.52±0.192	0.45±0.216	0.44±0.192
Triglyceride	mg/dL	75.28±18.233	78.40±10.096	91.44±10.663	104.52±27.109
Calcium	mg/dL	10.82±1.630	10.16±1.036	9.74±1.254	9.78±2.146
Iron	µg/dL	170.30±29.986	155.42±20.296	169.28±35.611	129.34±12.038
Cholesterol	mg/dL	60.04±16.766	69.14±16.671	68.22±9.700	80.62±12.612
Glucose	mg/dL	66.00±10.050	85.00±40.386	77.20±31.412	78.80±17.341
Magnesium	mg/dL	5.15±0.265	5.94±0.329	5.80±0.394	5.74±0.568
Phosphorous	mg/dL	5.02±0.958	4.78±0.952	5.04±0.986	5.36±1.431
Creatine Kinase (CK)	U/L	407.40±177.115	424±183.067	430.20±171.632	475.40±243.894
Gamma Glutamyltransferase (GGT)	U/L	15.88±8.865	18.20±7.412	12.94±5.636	16.92±6.415

* Significant (p<0.05)

Table 7: Effect of Butylparaben on biochemical parameters (Mean ± SD, N=5) in female rats after daily oral administration for 28 days.

Biochemical profile of female rats					
Parameter	**Unit**	**G I**	**G II**	**G III**	**G IV**
		0 mg/kg	**2000 mg/kg**	**4000 mg/kg**	**6000 mg/kg**
Alanine Aminotransferase (ALT)	U/L	29.98±9.549	33.64±4.754	44.72±21.784	41.96±6672
Aspartate Aminotransferase (AST)	U/L	150.22±25.783	183.80±31.529	203.10±17.668*	198.06±33.904*
Alkaline Phosphatase (ALP)	U/L	118.94±29.691	184.10±31.449	285.52±53.224*	234.58±53.996*
Total Protein	g/dL	6.16±0.513	6.54±0.397	6.54±0.555	6.12±0.311
Albumin	g/dL	3.14±0.195	3.26±0.167	3.14±0.279	3.16±0.207
Urea	mg/dL	42.68±13.469	32.62±5.398	36.60±6.819	42.00±12.426
Creatinine	g/dL	0.90±0.158	0.70±0.200	0.70±0.122	0.74±0.134
Triglyceride	mg/dL	82.28±9.150	93.46±13.616	70.86±17.594	100.44±31.404
Calcium	mg/dL	10.76±1.569	10.04±1.376	10.00±1.592	9.78±2.242
Iron	µg/dL	275.52±47.965	252.04±54.500	230.32±23.720	238.40±70.710
Cholesterol	mg/dL	63.20±12.884	68.00±9.350	73.40±16.904	74.38±23.008
Glucose	mg/dL	73.20±10.569	62.00±12.104	82.60±22.401	86.00±13.838
Magnesium	mg/dL	5.58±0.492	5.76±0.643	5.74±0.288	5.62±0.311
Phosphorous	mg/dL	5.34±1.309	4.92±1.331	4.88±1.381	4.44±1.481
Creatine Kinase (CK)	U/L	404.60±146.004	512.80±118.491	555.20±154.960	507.20±186.152
Gamma Glutamyl transferase (GGT)	U/L	13.98±6.836	14.90±4.717	16.12±6.558	20.68±10.663

* Significant (p<0.05)

Biochemical profile in general revealed significant (p<0.05) increase in enzyme AST was observed in Group IV male rats as well as in Group III and IV of female rats as compared to vehicle control rats. There was significant (p<0.05) increase in ALT was observed in Group IV male rats as compare to control rats. Statistically significant increase (p<0.05) in other liver specific enzyme *viz*, ALP was observed in Group III and IV of male as well as female rats, while other biochemical parameters *viz*. GGT, ALB, TP, CK, glucose, triglyceride, cholesterol, BUN and creatinine along with minerals such as calcium, phosphorous, magnesium and iron did not reveal any significant changes in all the treated group as compared to control group.

Significant increase in the ALT, AST and ALP as observed in the present study is indicative of hepatocyte damage and ultimately heptatoxic potential of BP. Asnani and Verma (2009) [1] reported that oral administration of BP induced oxidative stress in mice liver, might be due to formation of covalent bond by BP in the lipid bilayer of cell with sulfhydryl groups of membrane molecules such as GSH which ultimately prevents it from scavenging the free radicals. As a result increase in the leakage of liver enzymes such as ALT and AST may be possibly due to disruption of membrane integrity leading to their elevation in plasma/serum. Increase in the ALP level as observed from the present experiment is possibly due to blockage or inflammation/injury to bile duct or hepatocytes. Shah et al. (2011) [10] also reported that oral administration of BP for 30 days in Swiss-albino mice significantly reduced the activities of enzymes of antioxidant system like GSH, GST and SOD and resulted in increased production of reactive oxygen species that induce lipid peroxidation. Thus, BP induced lipid peroxidation might be the ultimate cause for hepatocytes damage and subsequent elevation of these enzymes.

Pathomorphology

No appreciable gross lesions were observed in any organs of rats after oral administration of BP daily for 28 days.

Histolpathology was carried out on various organ *viz*, liver, spleen, heart, lung, kidney, salivary glands, stomach, intestine brain, thymus, adrenal, eyes, testes and epididymis (male), ovary and uterus with cervix (female). There were no specific drug induced pathomorphological changes observed in any organ except liver and non-glandular stomach. In the present study, liver and stomach were found to be primary target organs of BP induced toxicity.

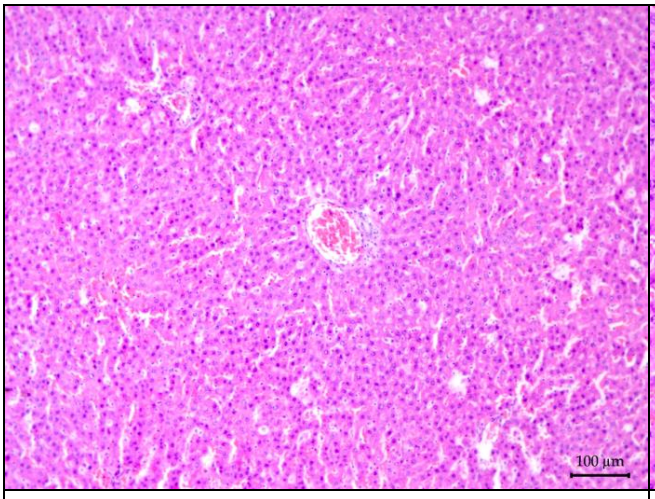

Fig 1: Group I, Male. Liver showing normal architectural detail. H&E X100

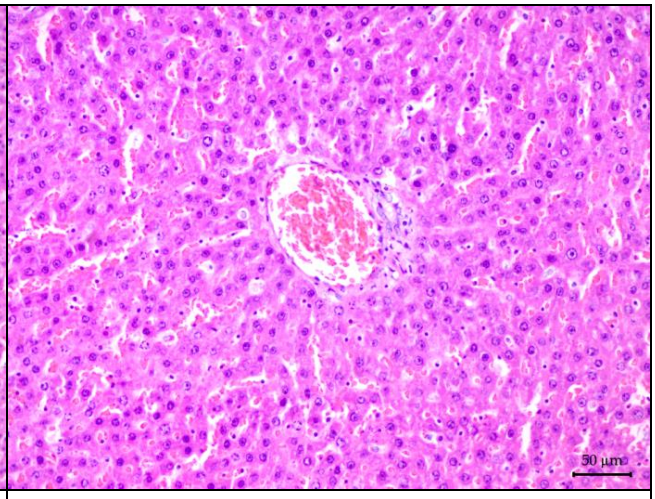

Fig 2: Group I, Male. Higher magnification of figure 1. Liver of control rat. H&E X200

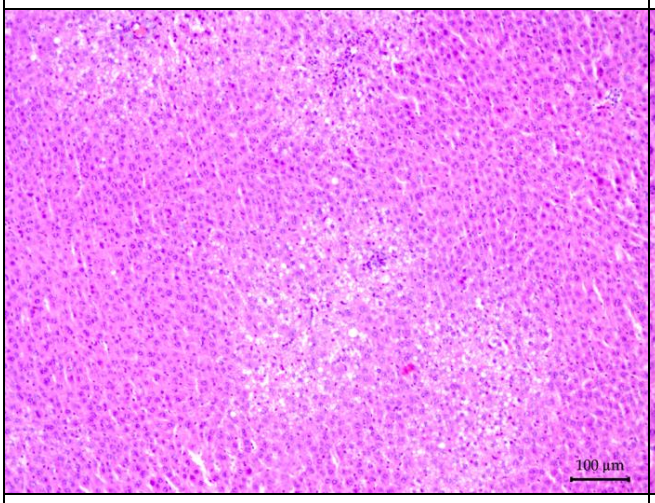

Fig 3: Group IV, Male. Liver showing diffuse moderate periportal vacuolation, characterized by presence of variable sized ill-defined clear vacuoles in cytoplasm of hepatocytes. H&E X100

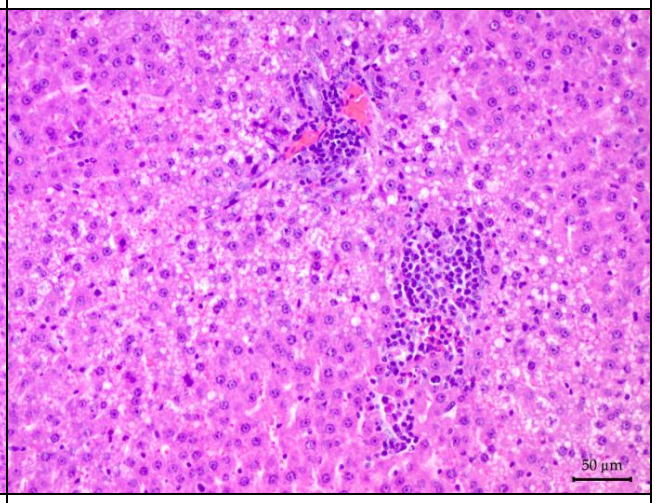

Fig 4: Group IV, Male. Liver showing diffuse moderate periportal vacuolation and minimal mononuclear cell infiltration. H&E X200

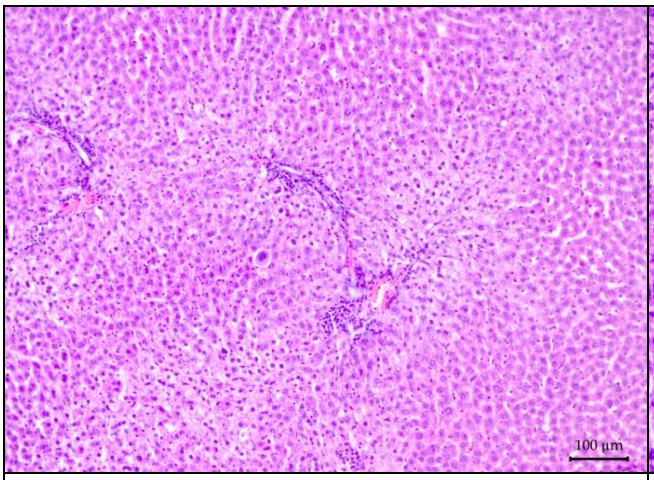

Fig 5: Group IV, Female. Liver showing diffuse moderate periportal vacuolation and one megakaryocyte (arrow) was appreciated in liver parenchyma. H&E X100

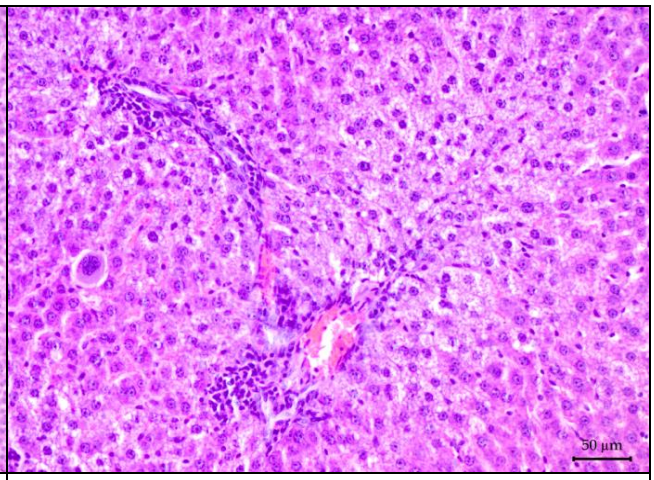

Fig 6: Group IV, Female. Higher magnification of figure 5. Liver showing diffuse moderate periportal vacuolation and one megakaryocyte was appreciated in liver parenchyma. H&E X200

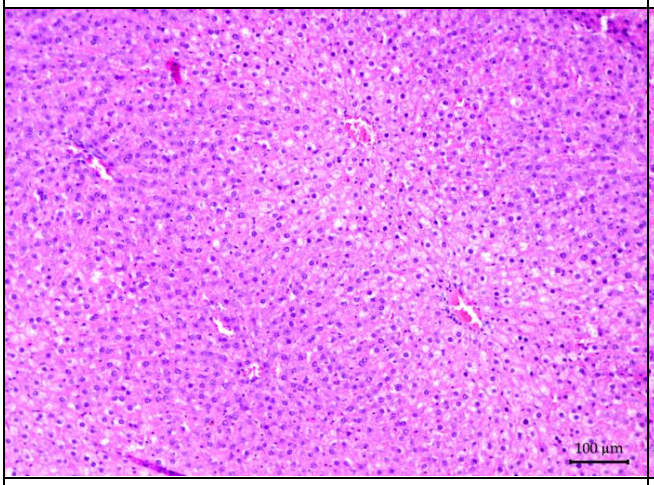

Fig 7: Group III, Male. Liver showing diffuse mild periportal vacuolation. H&E X100

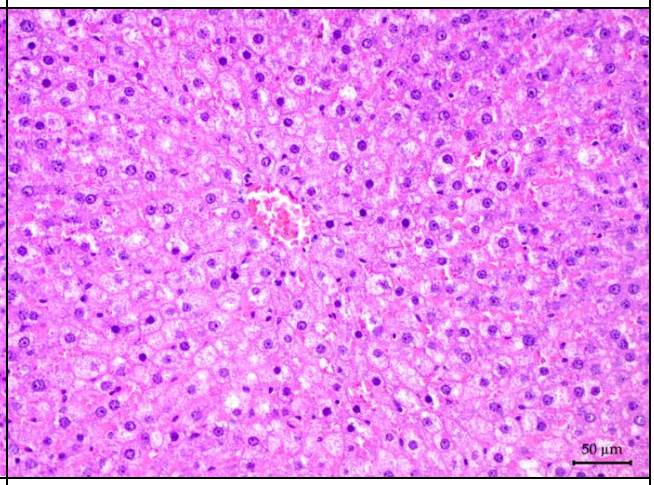

Fig 8: Group III, Male. Higher magnification of figure 7. Liver showing diffuse mild periportal vacuolation. H&E X200

Liver

The histopathological changes in liver comprised of diffuse moderate periportal vacuolation with presence of clear vacuoles in the hepatocytes with eccentric nucleus in Group IV male rats (Figure 3 and 4) as compared to normal architectural details of liver (Figure 1 and 2) in Group I control rats. Group IV female rats orally administered with BP @ 6000 mg/kg b.wt for 28 days also resulted in diffuse moderate periportal vacuolation (Figure 5 and 6). There was minimal infiltration of mononuclear cells (MNC) in the liver of 3 male and one female rats belonging to Group IV. Rarely megakaryocyte was noticed in liver parenchyma of two Group IV females. Group III male rats also showed diffuse mild periportal vacuolation (Figure 7 and 8). Liver of Group II rats showed no pathological changes. In the present study, nature of hepatocellular vacuolation was not confirmed by special stain, however microscopically they were consist with glycogen.

Stomach

No appreciable gross lesions were observed in the stomach of rats treated with various doses of BP. However, microscopic changes were observed in the non-glandular stomach of Group IV and III of male and female rats. Group I control rats showed normal architectural details in the non-glandular stomach (Figure 9). In control males and females, non-glandular stomach was lined by stratified squamous epithelium that have up to six stratum spinosum layers, few keratohyalin granules and appreciable keratin layer.

The pathomorphological changes in Group IV rats were characterized by diffuse squamous hyperplasia along with prominent keratohayline granules, prominent rete pegs and hyperkeratosis (Figure 10, 11and 12). Hyperkeratosis was evident by thickening of superficial layer without nuclear proliferation. Besides hyperkeratosis diffuse squamous hyperplasia with prominent rete pegs, two rats of Group IV showed focal extensive granulation tissue characterized by fibro vascular stroma (Figure 13 and14). In squamous cell hyperplasia, non-glandular stomach was lined by stratified squamous epithelium that have up to fourteen stratum spinosum layers. The severity of lesions was less in Group III rats which also showed the similar pathomorphological changes *viz*, squamous cell hyperplasia, hyperkeratosis, and prominent rete peg and keratohayline granules (Figure 15 and 16). Dose depended decreased in prominence of rete peg formation and keratohyalin granules were noted in mid and low dosed male and females. The pathomorphological changes observed in the non-glandular stomach of treated rats were in conformity with the findings of Rodrigues *et al.* (1986) [8]. These changes may be due to functional inability of esterase enzyme commonly found in epithelium of non-glandular stomach to hydrolyse esters with larger alkyl groups, or to the greater lipophilicity of the higher alkyl esters.

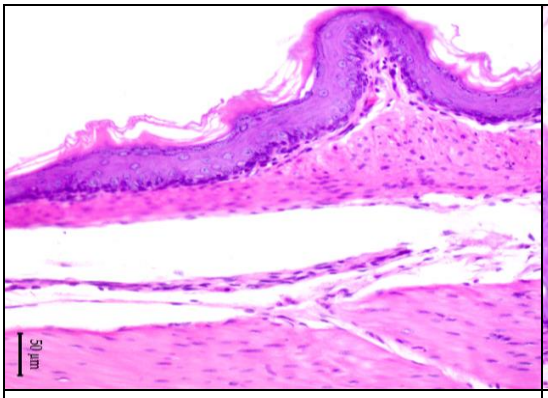

Fig 9: Group I, Male, non-glandular stomach of control rat showing normal architectural details. H&E X200

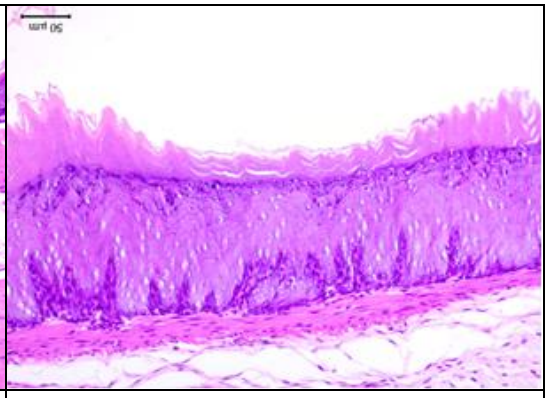

Fig 10: Group IV, Male. Non-glandular stomach showing diffuse squamous cell hyperplasia, prominent keratohyalin granules, hyperkeratosis and prominent rete peg. H&E X200

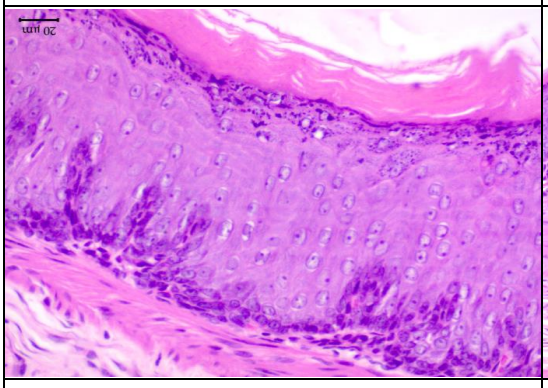

Fig 11: Group IV, Male. Higher magnification of figure 10. H&E X400

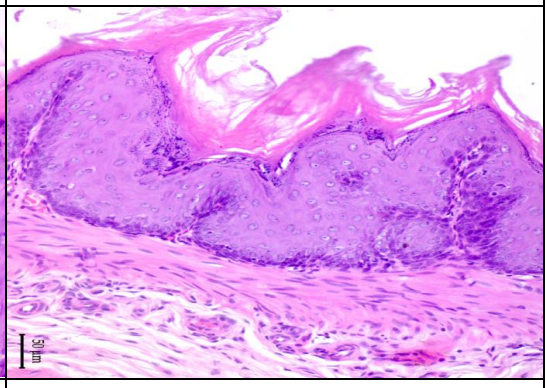

Fig 12: Group IV, female, non-glandular stomach showing diffuse squamous cell hyperplasia, minimal papillary projection, prominent keratohyalin granules, hyperkeratosis and prominent rete peg. H&E X200

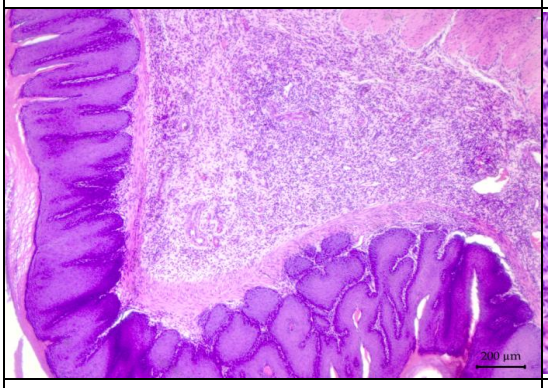

Fig 13: Group IV, Male, non-glandular stomach showing diffuse squamous cell hyperplasia, prominent keratohyalin granules, hyperkeratosis and prominent rete peg. Submucosa showing focal extensive granulation tissue. H&E X50

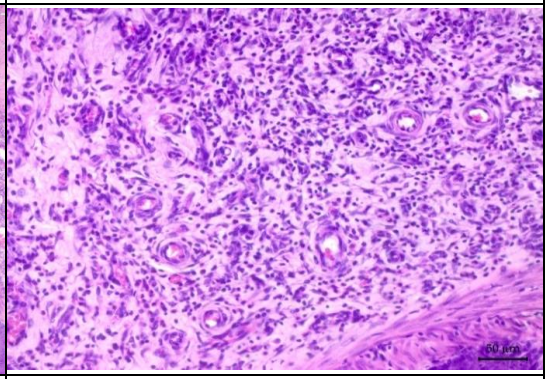

Fig 14: Group IV, Male. Higher magnification of figure 13 showing Fibrovascular stroma infiltrated with inflammatory cells. H&E X200

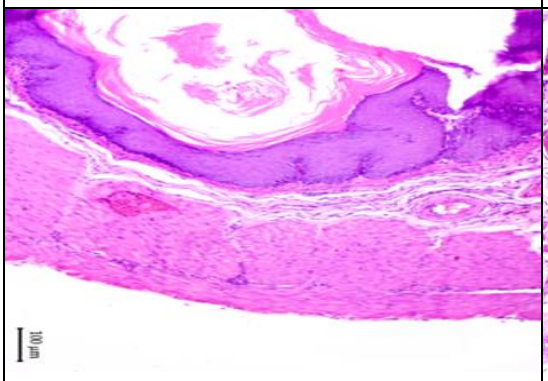

Fig 15: Group III, Male, non-glandular stomach showing diffuse squamous cell hyperplasia and keratohyalin granules. H&E X100

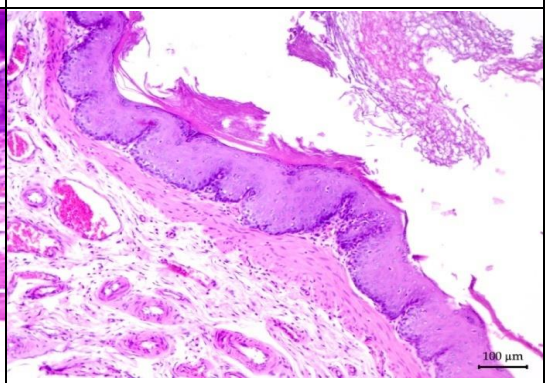

Fig 16: Group III, Male, non-glandular stomach showing diffuse squamous cell hyperplasia, prominent keratohyalin granules, hyperkeratosis and prominent rete peg. H&E X100

Conclusions

Butylparaben oral administration @ dose rate of 2000 mg/kg, 4000 mg/kg and 6000 mg/kg b. wt. did not produced any noticeable symptoms and clinical signs throughout the experimental period of 28 days. Haematological parameters revealed significant (p<0.05) decrease in MCHC in Group III and IV of male rats. Butylparaben produced alterations in biochemical profile as evident by significant (p<0.05) increase ALP in Group III and IV of male and female rats. Group IV male rats revealed significant (p<0.05) increase in ALT. Group III and IV female rats and Group IV male rats revealedsignificant (p<0.05) increase in AST. Butylparaben produced appreciable pathomorphological changes in liver, and in non-glandular stomach.

Conflict of Interest

Not available

Financial Support

Not available

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