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RESEARCH ARTICLE

Histopathological effect on different rat tissues induced by the trihalomethane- chloroform administered in drinking water

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Abstract

The experiment was designed to investigate the histopathological changes in rat's tissues due to the effect of the trihalomethane (THM) - chloroform when administered in drinking water. A group of male Wistar rat was exposed to trihalomethane-chloroform (TCM) at concentration 750 ppm in their drinking water and dose 16.17 mg/kg for a month. The growth rate and food intake were not affected by treatment, but TCM reduced significantly water consumption. This study demonstrate that, the THM-chloroform administered in the drinking water induced toxicity to the liver, hyperplasia in the urinary bladder and metaplasia in the small intestine of rats, but no effect on the kidney demonstrated in the current study.

INTRODUCTION

Chloroform is the major trihalomethane (THM) found in drinking water, these trihalomethanes (THMs) are halogen-substituted single-carbon compounds with the general formula CHX_3, where X represents a halogen, which may be fluorine, chlorine, bromine, or iodine, or combinations. The THMs most commonly present in drinking-water are chloroform ($CHCl_3$), bromodichloromethane or dichlorobro-momethane ($CHBrCl_2$) (BDCM), dibromochloromethane or chlorodibro-momethane ($CHClBr_2$) (DBCM), and bromoform ($CHBr_3$), The considering information relevant to the derivation of drinking-water guidelines for THMs is restricted to these compounds [1]. THM measurement assesses these four common THMs, with chloroform usually constituting the largest proportion. As well as being the most common THM, chloroform is also the principal DBPs in chlorinated drinking water[2] and [3]. Chloroform and the other THMs were ubiquitous in chlorinated drinking water [4]. National Cancer Institute published results linking chloroform to cancer in laboratory animals [5]. In 1979, the US. EPA issued a regulation to control THMs at 100 µg /l (ppb) in drinking water, and in 1998, the stage 1 disinfectants (D)/DBP rule was promulgated, which lowered permissible levels of THMs to 80 µg /1 and regulated five of the haloacetic acids (HAAs), bromate and chlorite for the first time [6]. As chloroform is the THM present in greatest concentration in drinking water, and the THM for which there are most scientific data available, a guideline developed based on data for this compound should be applicable as a guideline for the THMs identified in this document (chloroform, bromodichloromethane, dibromochloromethane and bromoform). Although not complete, available epidemiological data are consistent with the hypothesis that ingestion of chlorinated drinking water, if not THMs specifically, may be associated with cancers of the bladder and colon [7]. Additionally, epidemiological data available since 1993 have associated adverse reproductive outcomes with exposure to THMs, although neither clear evidence of a threshold,

nor a dose–response pattern of increasing risk with increasing concentration of total THMs, has been found [8]. Canadian drinking water guideline was drafted for total THMs (based on chloroform), significant effort has been made to characterize the mechanism of carcinogenicity and to understand the variability in effects from different routes and vehicles of administration. The current weight of evidence suggests that chloroform is a threshold carcinogen in rodents. There is strong evidence that the carcinogenic activity of chloroform in both rats and mice is mediated by a non-genotoxic mechanism of action that is secondary to cytotoxicity and cellular proliferation. There is strong evidence that the tumorigenicity of chloroform depends on the rate of its delivery to the target organ, and this suggests that detoxification mechanisms must be saturated before the full carcinogenic potential of chloroform is realized [9]. The weight of available evidence also indicates that chloroform has little, if any, capability of inducing gene mutation or other types of direct damage to DNA [10]. Two key studies were considered in the risk assessment for chloroform: the [11] study in dogs and the [12] Larson et al. (1994b) study in mice. The target organ in both studies was the liver. Although the [11] study was conducted in a relatively higher mammalian species (dog) and was of a reasonably long duration (7.5 years), it is an older study, used gavage dosing with a toothpaste base in a capsule, and did not cover the full life span of the dog. The [12] study, on the other hand, was conducted in a relatively lower mammalian species (mouse), used either corn oil vehicle (which may have influenced the pharmacokinetics and toxicity of the test compound) by gavage or drinking water given ad libitum, and was of short duration (3 weeks), which is insufficient for proper assessment of a lifetime exposure. In the case-control epidemiological studies conducted prior to 1993, associations were found between ingestion of chlorinated drinking water and the incidences of colon cancer for those aged 60 years or more [13] and bladder cancer among non-smokers [14]. In the investigation by [15], which involved 1244 cases and 2500 control subjects who had never been exposed in high-risk occupations for bladder cancer and for which detailed information on geographic mobility, water source (non-chlorinated ground source or chlorinated surface source for 50% of their lifetime), and potential confounders was collected, there was a positive association between bladder cancer risk, level of tap water ingestion, and duration of exposure, predominantly among study subjects with long-term residence in communities with chlorinated surface water [16]. Among non-smokers, there was an association between water intake and relative risk, and the odds ratio for those over 60 with more than median surface water intake compared with lifelong groundwater consumers was 2.3. There has been an ongoing effort since 1993 to improve the design of these epidemiological studies in order to more clearly identify both the possible agents of concern in chlorinated drinking water and the associated adverse health effects. More recent analytical epidemiological investigations of bladder cancer have been conducted in Colorado [17, 18]. Data reported thus far from a study in Iowa indicate that risk of bladder cancer is not associated with estimates of past exposure to chlorination by-products, except among men who had ever smoked, for whom bladder cancer risk increased with duration of exposure after control for cigarette smoking. No increased relative risk of bladder cancer was associated with exposure to chlorinated municipal surface water supplies, to chloroform, or to other THM species in a cohort of women, but the follow-up period of 8 years was very short, resulting in few cases for study. [18] In Ontario, [18] found an increased bladder cancer risk with increasing duration of exposure and THM levels. The association was statistically significant and of higher magnitude only after 35 or more years of exposure. The authors use a concept of THM-years to express the cumulative exposure to THM, which incorporates both levels of exposure to THMs and the period of exposure and is measured in µg/L-years. The bladder cancer incidence was about 40% higher among persons exposed to greater than 1956 µg/L-years of THMs in water compared with those exposed to less than 584 µg/L-years. Although it is not possible to conclude on the basis of available data that this association is causal, observation of associations in well-conducted studies where exposures were greatest cannot be easily dismissed. In addition, it is not possible to attribute these excesses to chloroform, although it is generally the DBP of the highest concentration in drinking water[6] (IPCS, 2000). In 2002, an expert panel convened by Health Canada to identify critical endpoints for assessment of health risks related to THMs in drinking water also agreed that THMs are used in epidemiological studies as a surrogate for exposure to CBDPs more generally, and the complexity of CDBP mixtures in drinking water makes the assignment of causation to any single component or class of components extremely difficult [19], Health Canada commissioned a review of the non-bladder cancer epidemiology of THMs in drinking water [20]. The reviewed studies focused on colon, rectal, pancreatic, kidney, brain, and haematological/lymphoreticular cancer sites. There were only a few studies with significant odds ratios for colon, rectal, brain and pancreatic cancer; studies were not significant for kidney and the blood-related cancers. For colon cancer, there were two studies showing a statistically increased risk of colon cancer with exposure to chlorinated drinking water. [21] King et al. (2000a) showed a significant association only for the male cohort, whereas [22] showed one only in females, as only females were considered. The results of the [21] study suggest that there may be different risk factor profiles for the different sexes insofar as there was no significant risk for females. However, the Iowa cohort [23] indicates that this may not be the case. Results from the studies involving rectal cancer were inconclusive. Of the studies examined, the only

study showing significance was a population-based case–control study by [24]. [24 and 23] both used the Iowa population and cancer registry for their studies. Their methodologies differed, in that [24] used a case-control design, examining rectal and colon cancers for both men and women, while [23] used a cohort design, examining only women in the population, prospectively, for colon and rectal cancers.[22] found an association only for colon cancer, while [24] found one for rectal cancer.

1. Materials and methods

This experiment was conducted during June-July, 2013 at the Departments of Biochemistry and Molecular Biology, Faculty of Science and Technology, El-Neelain University to investigate the histological change of albino rat's tissues (liver, kidney, urinary bladder and intestine) due to the effect of the Trihalomethane: chloroform administered in drinking water.

2.1 Experimental animals:

Twelve Wistar albino rats confirmed free of viral antibodies bacterial and parasitic infections were obtained from the institute of veterinary research- Khartoum. All animals were weighted their weights range from 124 to 199 g and then divided into two groups of similar weight after that were kept for 2 weeks as adaptation period and fed on basal diet. All aspects of the studies were conducted in compliance with the EPA/NHEERL Animal Care Committee.

2.2 Housing

Each group of rats was kept in a single separate cage. All groups were kept under identical condition and management, in humidity and temperature controlled room with a twelve hour light /dark cycle.

2.3 Feeding program:

During the adaptation period (which was 1 week), all rats were supplied with basal diet and distilled deionized water (DDW) was offered ad libitum, during the treatment period (which was 4 weeks long), all animals were fed basal diet and were treated as follow: Group A received THM deficient (DDW) as control group, while the other group, C received (DDW) mixed with chloroform respectively. Applied dose (16.17 mg/kg) was similar to those of [24] Moore et al., (1994) study and that doses (Table.1) were calculated from the total mean of concentration and consumption as in [25].

Animals were sacrificed after the treatment period, body and liver weights, were obtained for dose calibration and consistency.

2.4 Histology Sample Processing:

Tissue samples; liver and kidney, intestine and urinary bladder, were properly fixed for 24 h in Bouin's solution in alcohol (BSA) (tissue: BSA ratio of 1:10). Dried sections were stained by [26] for normal and ubnormal histological structure.

2.5 Statistical analysis:

Data was statistically analyzed by using one-way analysis of variance and the unpaired t-test.

Table 1: Trihalomethane concentrations, water consumption, trihalomethane doses, and body weights for Wistar rats exposed to TCM in the drinking water for 4 weeks

Treatment (N)	**Deionized water (6)**	**TCM (6)**
Target concentration (g/l)	-	0.75
Water consumption (ml/kg per day)	246.43 ±26.45	130.00±13.94*
THM dose (ml/kg per day)	-	16.17
THM dose (mmol/kg per day)	-	0.14
Body weight (g)	187.11 ±8.133	181.94±8.133

*Statistically significant when compared to the deionized water, using the unpaired t-test ($P > 0.05$).
Mean ± SEM, (N) Number of rats per group.

3. Results

3.1. Normal histology of untreated group:

Figure 1 and 2 indicated normal histological feature of liver, while Figure 3 and 4 illustrated kidney, Figure 5 indicated the intestine and urinary bladder, which is lined by transitional thin epithelium that is normally no more than 2 to 3 cell layers thick of THMs untreated group are shown in (Fig. 6) and (Fig.7) respectively.

3.2. Effect of Chloroform on liver, kidney, intestine and urinary bladder: Histological investigation showed great variability among chloroform treated group and the control in intestine, liver and bladder respectively. Histopathological analysis results in intestine of chloroform treated group, Fig. (11,12,13) showed multiple areas of squamous metaplasia within the intestinal mucosa which is the transformation of the glandular epithelium into stratified squamous epithelium. Besides, the formation of mucosal polyps (finger like projections of stratified squamous epithelium) (Fig. 12, 13). In (Fig.14,15), urothelium appeared intact , inflammatory cell infiltration and vasodilation were observed in the urinary bladder of rat exposed to chloroform in drinking water for four weeks. Slide of liver (Fig. 8) Showed necrosis in hepatocytes and degeneration of cells, kidney (Fig. 9, 10) viewed normal architecture with no significant pathological changes. Our results indicated that chloroform administered in drinking water induced preneoplastic effect in the small intestine, bladder and toxicity in the liver of rat, but not in the, kidney

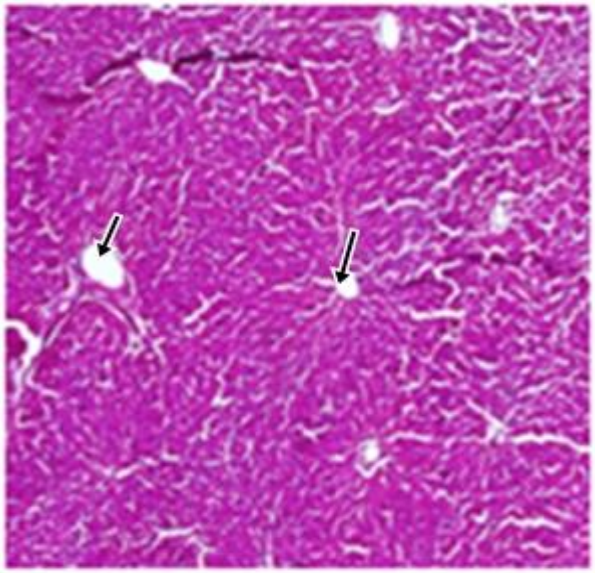
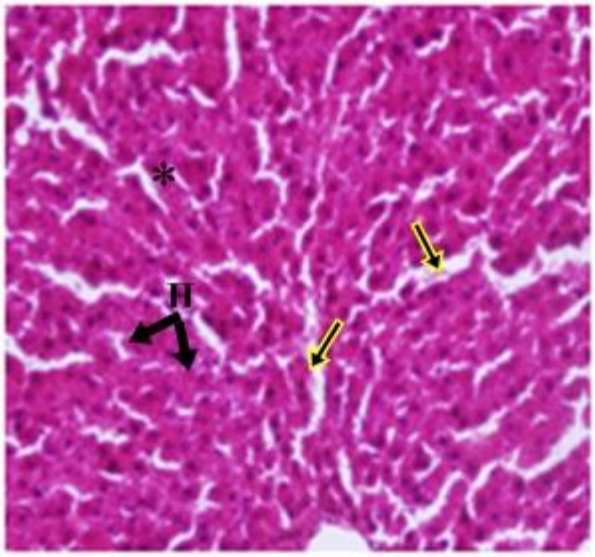
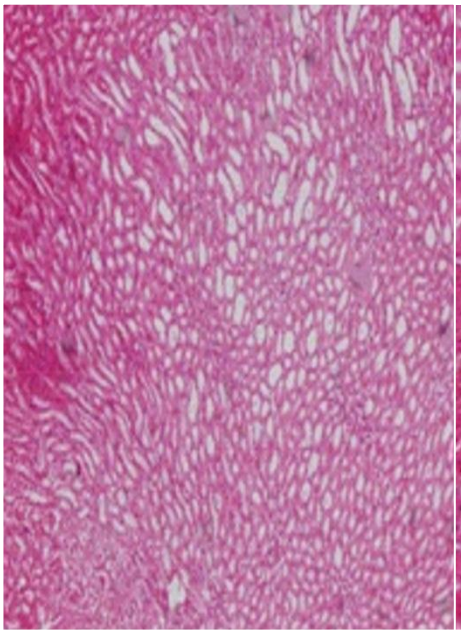


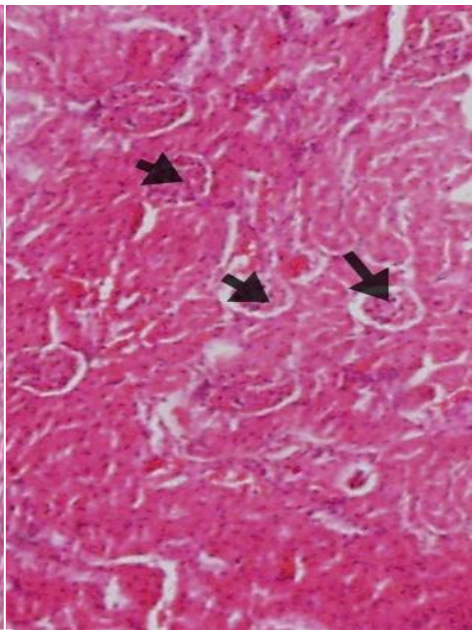
Fig. (1) Normal Hepatic Architecture of Untreated Group,Central Vein (Arrow). H&E.10x.



Fig(2) : Normal Hepatic (H)Centrally Located, Spherical Nucleus with a Clear, Dark Nucleolus (*) and Normal Sinusoids (Arrow).H&E. 40x.



Fig(3) : Normal kidney architecture. H&E.40x.



Fig(4): Normal renal tubules and melanomagrophage centre (Arrow). H&E.10x

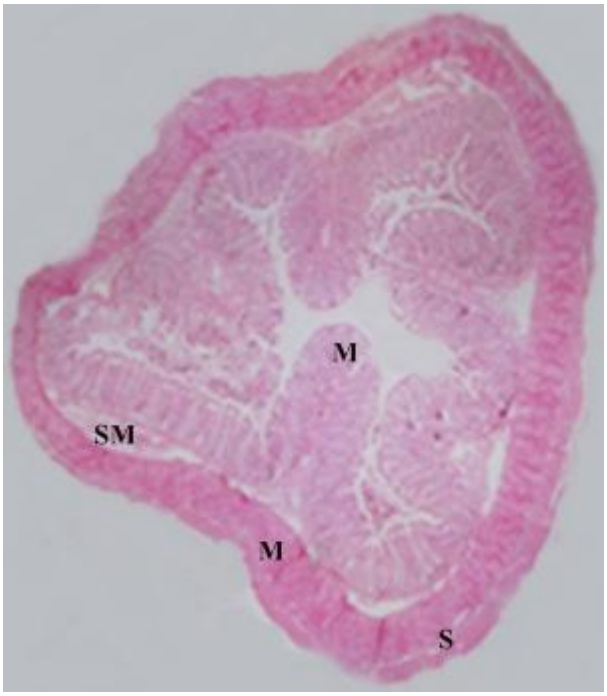
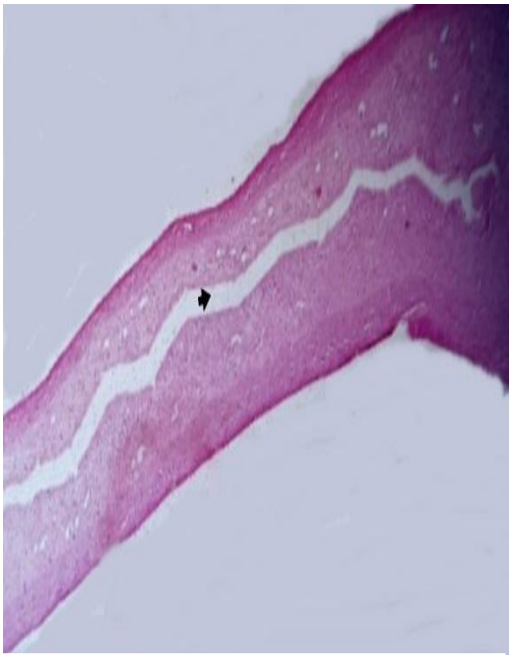


Fig (5): Whole section of Normal Rectum, Serosa (S); Musclar layer (M); Submucosa (SM); Mucosa(M). H&E 10x.



Fig(6): Showing Normal Urinary bladder, Normal epithelia (Arrow), H&E10x.

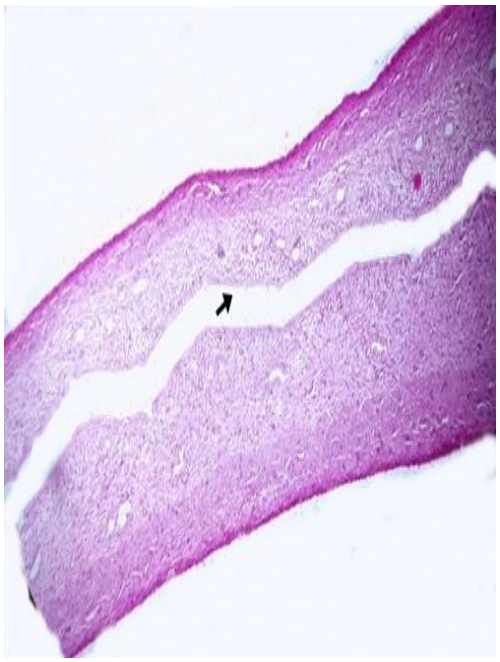
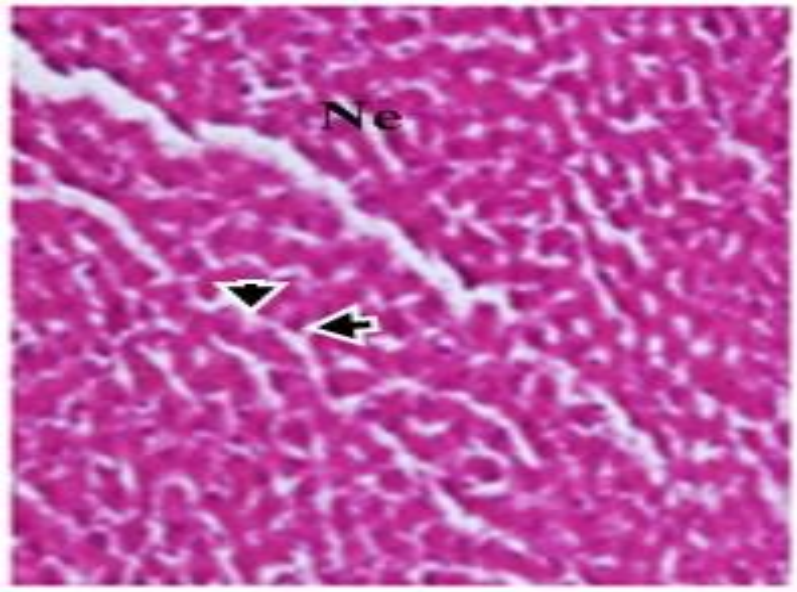
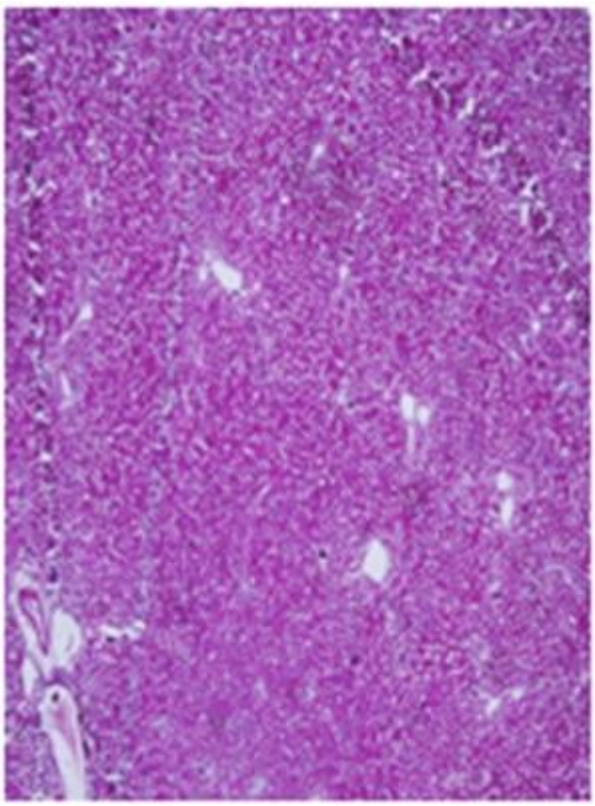


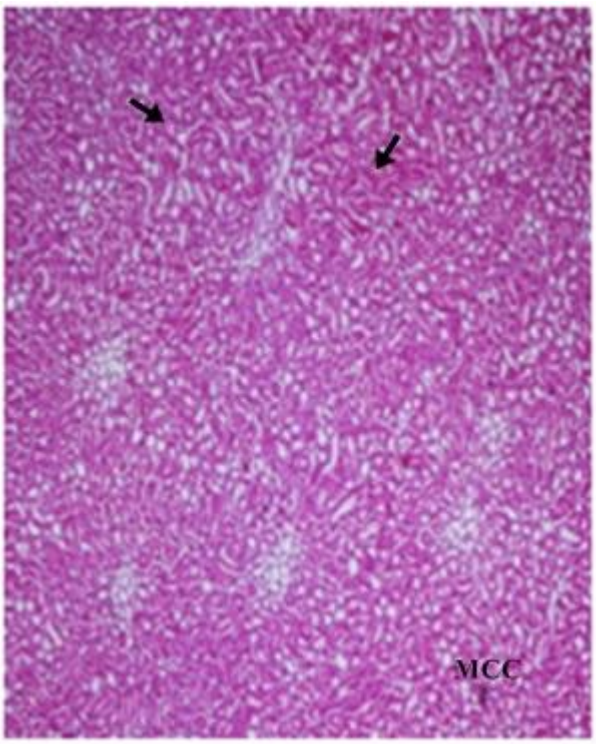
Fig (7): High power Fig(6), Normal epithelia (Arrow), H&E20x.



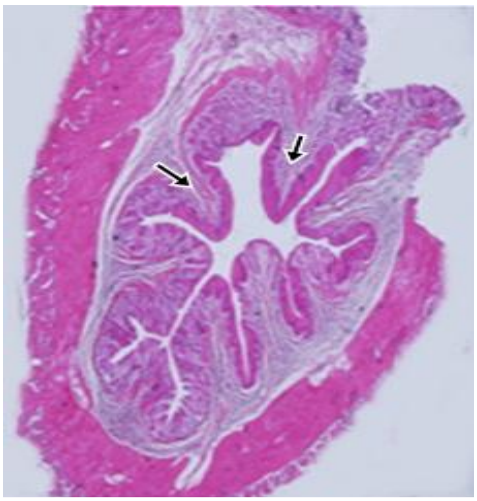
Fig(8):Showed necrosis in hepatocytes(Ne) and degeneration of cells(Arrow). H&E. 40x



Fig(9): Normal Kidney Architecture. H&E.10x.



Fig(10): Normal Renal Tubules (Arrow) and Melanomagrophagecenter(MCC) Architecture. H&E.10x.



Fig(11): Stratified Squamous Epithelia in Rectum. (Arrow) H&E. 10x

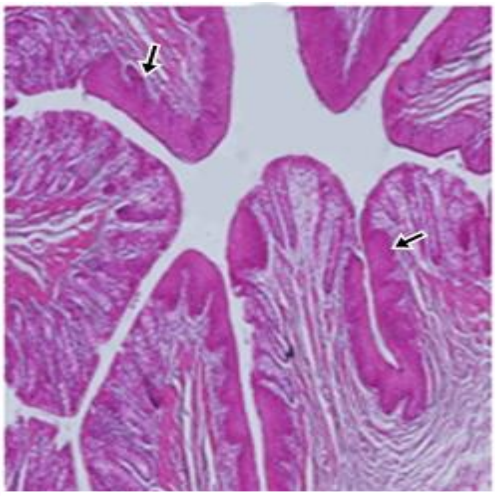
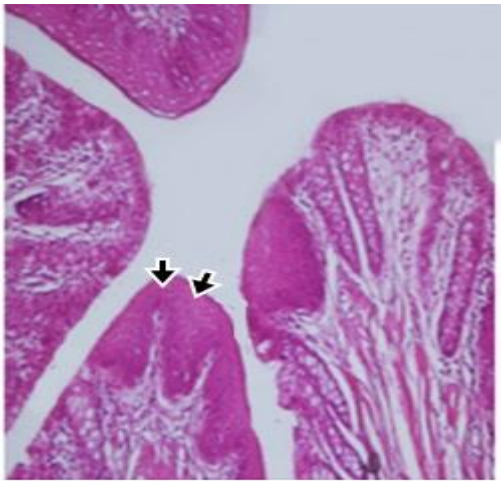
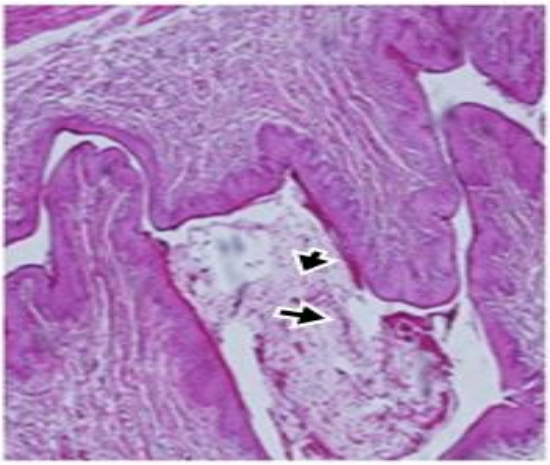


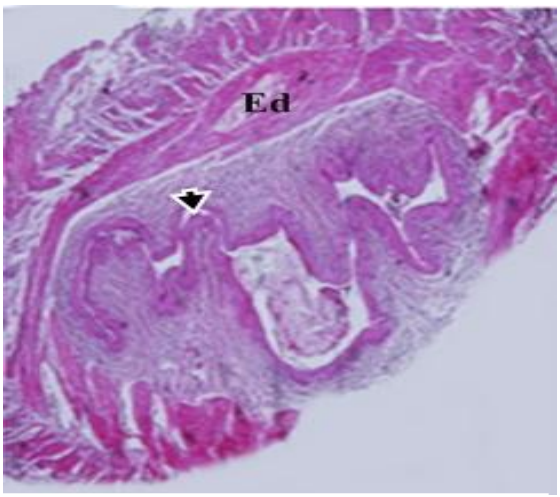
Fig (12): High power of fig (11) The Formation of Mucosal Polyps(Arrow). H&E. 40x



Fig(13): High power of fig(12)Squamous metaplasia within the Rectum mucosa. H&E.



Fig(14): High power of fig() showed thick epithelia, inflammatory cell infiltration and vasodilation(Arrow). H&E. 10x



Fig(15): Whole section of urinary bladder, urothelium appeared intact. (Arrow), Edema(Ed) H&E. 40x **Fig(1):**Normal Hepatic

4 Discussions

This study demonstrated chloroform THM toxicity on different rat tissues when administered in drinking water in concentrations of 750 mg /l and dose 16.17 mg/ kg per day.

The current study contradicted with the finding of [12] study which was conducted in lower mammalian species (mouse) and found that no treatment related changes in liver up to 329 mg/ kg per day. The variation may be due to difference in species and that rats thought to be more sensitive than mouse, also the duration of experiment was shorter, 3 weeks only, compared to the current study. [12] Considered as a key study by the [19], trihalomethane guideline report, together with [11] in the risk assessment of chloroform. The second study was conducted on higher mammalian species (dogs) and was of reasonably of long duration, thus it was used by [19] Canada health (2006) to identify the upper limit of THMs as 80µg/l in drinking water, using chloroform. [11] showed treatment related liver damage in the lowest doses and this agree with the current study finding.

The effect of chloroform in the urinary bladder agree with the epidemiological study of [17] King and Marrett, (1996) who found a significant increase in bladder cancer in Ontario, Canada after 35 or more years of exposure to THMS in drinking water. Mentioning that chloroform is the greatest chlorination by product with the highest concentration in drinking water [10].

Causing metaplasia in the small intestine which is the transformation of epithelium due to chloroform in this study, strongly supported [27] epidemiological study which showed a significant association only for the male cohort, and completely disagree with [22] study that showed one only in females, as only females were considered, but this findings are also agree with the [10] suggestion that available evidence also indicated that chloroform has a little, if any, capability of inducing gene mutation or other type of direct DNA damage.

Conclusion

The demonstration of chloroform administered in drinking water inducing toxicity to the liver, hyperplasia in the urinary bladder and metaplasia in the small intestine of rats, but did not affect the kidney. (chloroform carcinogenicity mediated by non-genotoxic mechanism of action that is secondary to cytotoxicity and cellular proliferation, and that there is strong evidence the tumorigenicity of chloroform depends on the rate of its delivery to the target organ, and this indicate that detoxification mechanisms must be saturated before the full carcinogenic potential of chloroform is realized.)

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