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The Efficacy of Some Detergents on Some Intestinal Parasites and Their Histopathological Effects

Hiro M. Obaid
Medical Laboratory Techniques Department, College of Technology/ Kirkuk, Northern Technical University, Iraq.

Abstract

Detergents or surfactant are chemical substances that used for cleaning purposes. The chemical compositions of the detergents are varying greatly according to application demands and commercial competitive. The aim of the present study was to, investigate the effect of common substances, used by people in our area for cleaning vegetables and fruits. On killing some parasitic stages and also studying their histopathological effects on mice intestine. Four types of ordinary commercial detergents were used (Altunsa, Bonux, Ariel , ABC) at concentrations of 1.5, 3, 4.5 g\l against *E. hitolytica*, *E. coli*, *G. lamblia* cysts and *H. nana* eggs. The parasitic stages were incubated with the detergents used for 2, 5, 15, 30 minutes. In order to detect the efficacy of the detergents the incubated stages were administered to laboratory mice. Histological sections of mice intestinal parts were done to find out the histopathological effect of the detergents. The detergents varied in their actions on tested parasitic stages, the most effective was Ariel and ABC type followed by Bonux. The lowest efficacy was for Altunsa type. Incubating the parasitic stages with the detergent for 2 minutes had no impact with some detergents, while 5 minutes was enough for killing the stages with all detergents. The histopathological examination of intestinal parts had not revealed any dimorphty or changes comparing to the control group except in that leaved for more than 5 minutes. The conclusion is that some detergents can be used for cleaning vegetables, killing and removing parasitic stages. Detergents do not cause histopathological effects if its residues removed thoroughly and not leaved on vegetables for long period of time.

Keywords: Detergents, Parasitic stages, Histopathological effect.

تأثير بعض المنظفات على اكياس وبيوض بعض الطفيليات المعوية وتأثيراتها النسجية المرضية

هيرو محمد عبيد
قسم تقنيات التحليلات المرضية، الكلية التقنية \كركوك، الجامعة التقنية الشمالية، كركوك ، العراق.

الخلاصة

الخلفية المعرفية والهدف: ألمنظفات أو المزيلات هي مواد كيمياوية تستعمل لأغراض التنظيف. يختلف المكون الكيمياوي لها باختلاف الغرض منه والمنافسة التجارية. الغرض من الدراسة الحالية كان للبحث عن تأثير بعض المنظفات المستخدمة من قبل السكان المحليين لتنظيف الخضار و الفواكه، لأجل قتل بعض أطوار الطفيليات و دراسة التأثير النسيجي المرضي لهذه المواد على أمعاء الفئران المختبرية.

وقمنا بااستخدام اربعة أنواع من المنظفات التجارية الاعتيادية (التون سا، بونكس، اريال، أي بي سي) بالتراكيز 1.5، 3، 4.5 غرام /لتر، لبيان تأثيرها على الطور المتكيس لأمبيا النسيج و أمبيا القولون و الجارديا و

Email: dr.obaidhm13@Gmail.com.

بيوض الدودة القزمة. حضنت الاطوار الطفيلية مع المنظفات لمدة 2، 5، 15، 30 دقيقة. ولدراسة تأثير المنظفات على الاطوار الطفيلية بعد فترة الحضن، تم تغذية هذه الاطوار الطفيلية للفئران المختبرية، اخذ مقاطع لأمعاء الفئران لأجل الدراسة النسيجية المرضية للمنظفات.

وتم التوصل الى النتائج حيث اختلف تأثير المنظفات على الاطوار الطفيلية. المنظف الاكثر فعالية كان اريال و أي بي سي و تبعها البونكس، و الاقل كان التون سا. الحضن لمدة 2 دقيقة كان غير فعال مع بعض المنظفات لقتل الاطوار الطفيلية بينما الحضن لمدة 5 دقائق كان ذو تأثير فعال لكل المنظفات. لم يظهر فحص التأثير النسيجي المرضي للمنظفات على مقاطع الامعاء اية اختلافات بالمقارنة مع مجموعة السيطرة، باستثناء التي تم حضنه لمدة أكثر من 5 دقائق.

واستنتجنا بعض المنظفات و خاصة الحاوية على كميات أكبر من المواد الفعالة، من الممكن استخدامها لتنظيف الخضار و قتل و ازالة الاطوار الطفيلية منها. المنظفات لا تسبب اية تأثيرات نسيجية مرضية اذا ازيلت بقاياها جيدا أو لم يترك الخضار لفترات طويلة في هذه المنظفات.

Introduction

Detergents are chemical ingredients, most important ingredients are those named surfactants (surface active agents) these most likely is alkylbenzenesulfonates a substance that make a detergent more soluble in hard water than a soap. However the soap sometimes misnamed as a detergents but they are quietly different, a soap is restricted to types of natural products from animal or plant source, like goat's fat and wood ash but detergents include a wide range of materials in addition to natural products but mainly prepared from a diversity of petrochemicals and oleochemicals [1]. In fact soup is a kind of detergents like many other detergents as hair shampoos and clothes washing powder or dish washing liquid to shaving foam and stain removers. Detergents can be categorized according to the electrical charge on chemical surfactants they contain, to anionic detergents which are alkylbenzene-sulfonates. The alkylbenzene part is lipophilic while the sulfonate is hydrophilic [2]. Annually about 6 billion kilograms of anionic detergents are manufactured for local markets. Bile acids is an anionic detergents which include deoxycholic acid (DOC), that produced by the liver and aid in digestion and absorption of fats and oils. Other types of anionic detergents are a branched sodium dodecylbenzenesulfonate, linear sodium dodecylbenzenesulfonate, and a soap [3]. Cationic detergents analog to the anionic, consists of a hydrophobic component but its positively charged because of quaternary ammonium polar end as an alternative to the sulfonate group in the anionic type [2]. Non-ionic and zwitterionic detergents are uncharged, having hydrophilic head groups. Representative non-ionic detergents are built on polyoxyethylene or a glycoside such as Tween, Triton, and the Brij series. Two comparable detergents owning a sugar alcohol as head group are HEGA and MEGA series [4]. Zwitterionic detergents hold a net zero charge arising from the presence of equal numbers of charged chemical groups like CHAPS. Some washing detergents may contain other components like optical brighteners which make clothes gleam [5] or active chemicals called enzymes, which assist to breakdown and eliminate foodstuff and other deposits. The main enzymes are proteases (which disrupt proteins), lipases (which disrupt fats), and amylases (which break starch). Other elements comprise perfumes or chalk to help to eliminate things like burned-on cooker grease and bath-tub grime [6].

People must also not mix between detergents which is cleaning process include removal of soil, dirt and other materials, and disinfectant, the process of killing germs and microorganisms from objects and include chemicals like alcohol, chlorine, aldehydes, iodine, oxidizing agents and many other chemicals [7]. In our area the clasik question is still asked, about the suitable way for cleaning vegetables. A way that grantee microorganisms exclusion with no pathologic side effects, therefore we aimed in this study to investigate the effect of common substances used by people in our region for cleaning vegetables and fruits on killing some parasitic stages and also studying their histopathological effects on intestine.

Material and Methods

Stool samples collecting: Stool samples were collected from patient whom attended General Pediatric Hospital in Kirkuk. The specimens as in routine were proceeded for parasitological examination which included the macro and microscopic examine. After detection and diagnosing the parasite, positive samples were taken to our laboratory for isolation.

Parasites isolation: The diagnosed parasites namely *E. hitolytica* Figure-1, *E. coli* Figure- 2 and *G. lamblia* cysts Figure-3, *H. nana* eggs Figure-4. (Three positive isolates of each parasite was taken) the samples were first diluted with 10-15 ml saline, mixed thoroughly with an applicator, sieved through stainless steel mesh 75µm in to several centrifuge tubes, 10 ml saline was added to each tube then centrifuged for 3 minutes at 1000 rpm. The step was repeated until a clear supernatant was get. The supernatant was discarded while the sediment was resuspended and prepared for concentration [8].

Sedimentation technique: the prepared sediment was resuspended in 7 ml of 10% formaldhyde (1 part of 40% formalin in 3 part of saline). 3 ml of ether (or ethyl acetate) was added, the tube was closed with a stopper and shake vigorously to mix the contain, then centrifuged at 1500 rpm for 2 minutes. The tubes were rest in a stand. Four layers of ether, plug, formalin and sediment were formed, the three top layers was discarded and the sediment (purified cyst and egg) was incubated with antibiotics (penicillin 5000 Iu/ml , streptomycin 5 mg/ml and amphotericin-50 µg/ml) at 37 for 12 hrs. to kill microbial contaminants, the numbers of cyst and egg was counted using neubar slide chamber. The cysts and eggs was stored in aqueous K2Cr2O7(2.5% wt/vol) at 4-8 until used later [9].

Experimental laboratory animals: white mice of Balb/c strain were reared in clean plastic cages. They were nourished with concentrated diet prepared locally according to food ingredients. 83 mice at any age were used for detergents experiments (two mice for each concentration and each time).

Detergents preparation: four types of ordinary commercial clothes washing detergents were used (Altunsa, Bonux, Ariel, ABC) Table-1. Concentrations of 1.5, 3, 4.5 g\l (detergent\ water (each 1.5 gram is equivalent to one filled tea spoon) at room temperature) was prepared from each detergents, dissolved thoroughly, the color of each solution was differed from the other. In addition to that some substances used routinely by people for cleaning vegetables and fruits used, as vinegar 50%, potassium permanganate 10% and hydrogen peroxide (H_2O_2) 75%, all were water diluted and used as controls.

Detergents experiments: the stored parasitic stages were washed with saline until the potassium dichromate was removed completely. The infectivity of the stages were examined by orally administering them to mice. After the infectivity was confirmed, about 1000 cyst or egg was incubated in each concentration of prepared detergents solutions for 2, 5, 15, 30 minutes. After the incubation was completed the parasitic stages were washed several times to remove the detergents. Likewise parasitic stages was incubated in vinegar, potassium permanganate and H_2O_2. The washed parasitic stages were then diluted with 1ml water and administered orally to the mice. Two repetitive for each time or concentration was done. The mice feces was examined daily for parasite presence or absence by direct stool examination (examination was continued for 30 days).

Histopathological examination: for histopathological examination, vegetable like parsley and cucumber was incubated with the less and the most powerful detergents (altunsa and ariel) to the same used concentrations (1.5, 3, 4.5 g\l) and for the same times (2, 5, 15, 30 minutes). Then they washed thoroughly with water to remove the detergents as it routinely used in houses. The washed vegetables then were fed to mice daily for a month. After that the mice were killed, parts of their intestine were proceeded for histopathological examination because the intestine is the first part of the body that may be effected. A group of mice not fed the incubated vegetables were used as control. The intestine parts were cleaned, fixed, dehydrated in 60, 70, 80, 90, 100% of ethanol alcohol for 2hrs, cleared with xylene. Infiltrated in a mixture of paraplast and paraffin then embedded in paraffin wax, sectioned and rehydrated in alcoholic concentrations of 100, 90, 80, 70, 60, 50, 30% for about 1-2 min., stained with Haris hematoxylin and Eosin (H E), mounted and examined [10].

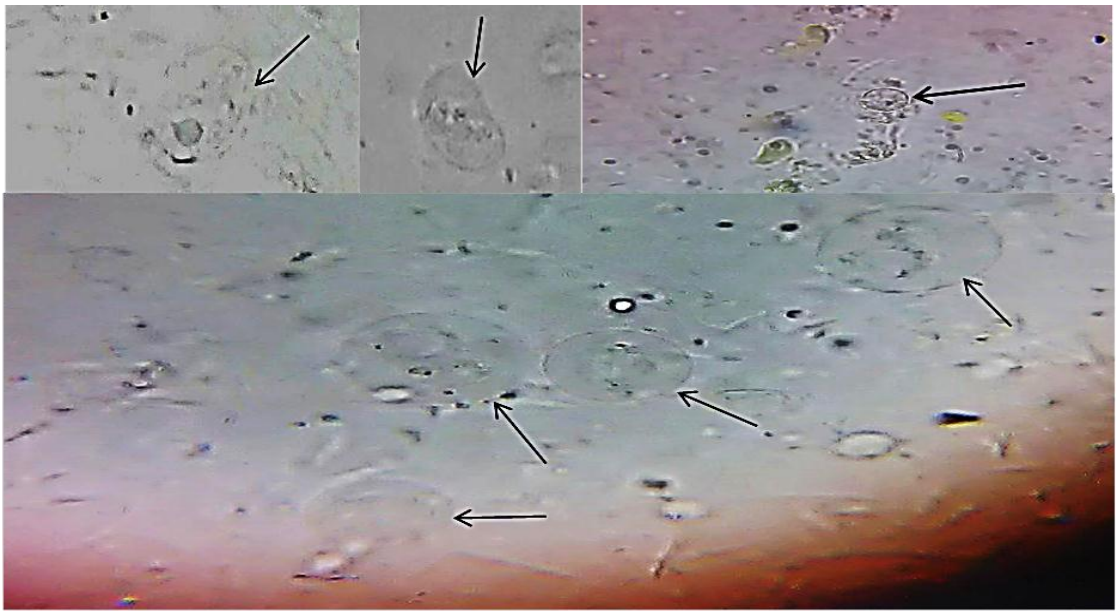

Figure 1- *E. histolytica* trophozoite and cyst. Unstained 100x. arrows refer to trophozoites and cysts.

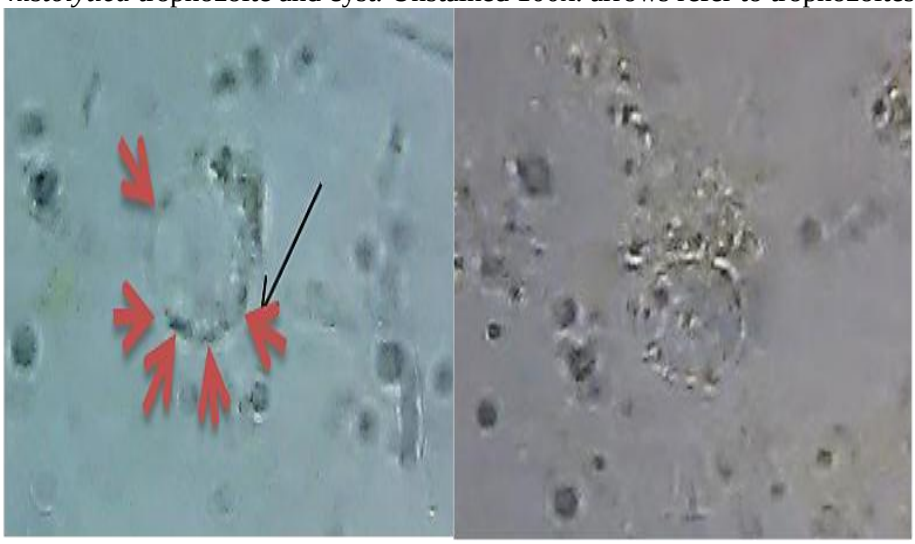

Figure 2- *E. coli* cyst. Unstained 100x small red arrows refers to nucleus.

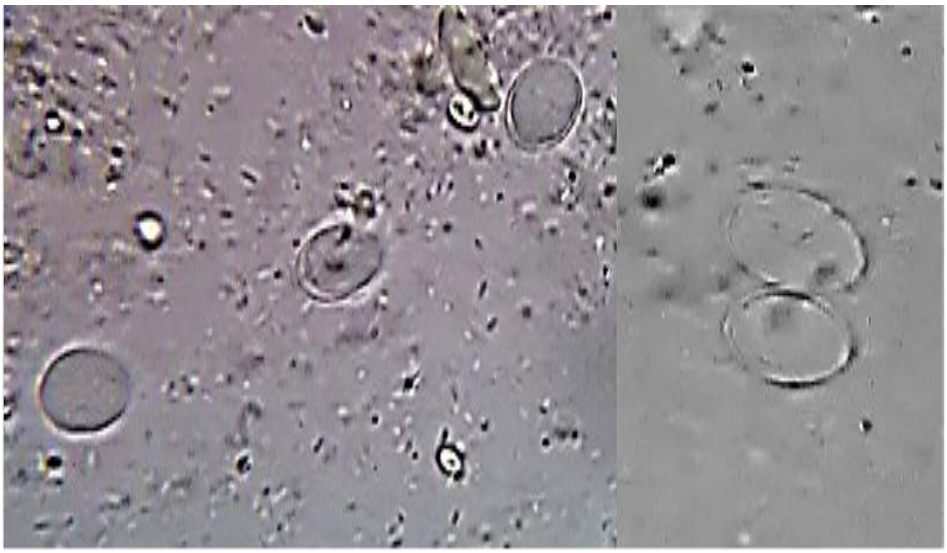

Figure 3- *G. lamblia* trophozoite and cyst. Unstained 100x

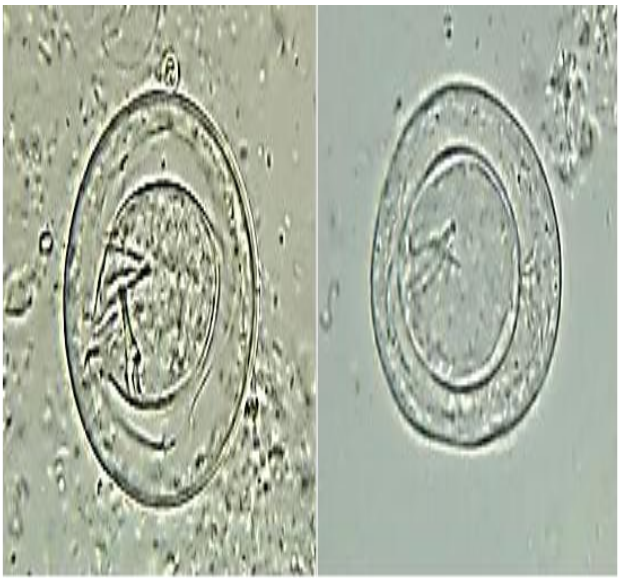

Figure 4-*H.nana* eggs. Unstained 40x.

Table 1- Used detergents and their compositions

Detergents	Compositions
Altunsa	Anionic active matter 5-15%, stpp 10%, sodium carbonate 10%,sodium silca 8%, perfume.
Ariel	Anionic active matter 5-15%, oxygen based bleach 5-30%, yuzey active matter <5%, none ionic yuzey, fosfonat polycarboxylate, zeolite, enzyme, optical brighter, perfumes
Bonux	Anionic active matter 5-15%, optical brighter, perfumes.
ABC	Anionic active matter 5-15%, oxygen based bleach 5-15%, polycarboxylate soap <965, optical brighter, perfumes.

Results

After administering the mice with the parasitic stages, all the stages were infctive to the tested mice. The cysts and trophozoites had appeared in mice feces 2-4 days post infection while the eggs of *H. nana* 15-20 days post infection. Altunsa detergent was ineffective at killing the parasitic stages, except at highest concentration (4.5 mg\l at 30 minutes) which was effective at killing *H. nana egg and E. coli cyst* only, Table-2.

Table 2-The effect of Altunsa on some parasites at different incubation times and concentrations.

Time in minutes	Conc. g\l	Parasitic stages			
		E. histilotica cyst	*H. nana egg*	*E. coli cyst*	*G. lamblia cyst*
2	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	I	I	I
5	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	I	I	I
15	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	I	I	I
30	1.5	I	I	I	I
	3	I	I	I	I
	4.5	E	I	I	E

Conc.= concentration, I=ineffective, E= effective.

As indicated in Table-3, when incubation time had increased the Ariel detergent began to be effective. The cysts and eggs were seen in large numbers in mice feces after two minute of incubation, but no parasites were detected after 5, 15, 30 minutes of incubation.

Table 3- The effect of Ariel on some parasites at different incubation times and concentrations.

Time in minutes	Conc. g\l	Parasitic stages			
		E. histilotica cyst	*H.nana egg*	*E.coli cyst*	*G.lamblia cyst*
2	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	E	E	I
5	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E
15	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E
30	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E

Conc.= concentration, I=ineffective, E= effective

Table-4, revealed that Bonux detergent was effective against the parasitic stages especially at 15, 30 minutes after incubation, while ABC detergents was the most effective comparing with other detergents, Table-5. It was effective even at 2 minutes incubation but only in concentration of 4. 5 g\l.

Table 4-The effect of Bonux on some parasites at different incubation times and concentrations.

Time in minutes	Conc. g\l	Parasitic stages			
		E. histilotica cyst	*H.nana egg*	*E.coli cyst*	*G.lamblia cyst*
2	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	I	I	I
5	1.5	I	E	I	E
	3	E	E	E	E
	4.5	E	E	E	E
15	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E
30	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E

Table 5-The effect of ABC on some parasites at different incubation times and concentrations.

Time in minutes	Conc. g\l	Parasitic stages			
		E. histilotica cyst	*H.nana egg*	*E.coli cyst*	*G.lamblia cyst*
2	1.5	I	I	I	I
	3	I	I	I	I
	4.5	I	E	E	I
5	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E
15	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E
30	1.5	E	E	E	E
	3	E	E	E	E
	4.5	E	E	E	E

Conc. =concentration, I=ineffective, E= effective

Table-6 indicated that any efficacy have not been detected when vinegar, potassium permanganate and H_2O_2 were used, at all incubation times.

Table 6-The effect of vinegar,potassium permanganate, H_2O_2 on some parasites at different incubation times and concentrations.

Substance name	Conc.	Incubation time in minutes	Parasitic stages			
			E. histilotica cyst	*H.nana egg*	*E.coli cyst*	*G.lamblia cyst*
Vinegar	50%	2	I	I	I	I
		5	I	I	I	I
		15	I	I	I	I
		30	I	I	I	I
Potassium permanganate	10%	2	I	I	I	I
		5	I	I	I	I
		15	I	I	I	I
		30	I	I	I	I
H_2O_2	75%	2	I	I	I	I
		5	I	I	I	I
		15	I	I	I	I
		30	I	I	I	I

Conc. =concentration, I=ineffective.

The histopathological effect of the detergents on mice intestine is illustrated in Figures-(5-13). No histopathological changes were noted using 2, 5 minutes incubation time with all used concentrations comparing with control group, Figure-14. The intestinal parts had maintained their typical morphology, from outside to inside they composed of serosa, muscle layer, sub mucosa and mucosa. No changes were observed in layers of mucosa (epithelial cells, lamina propria and stratum), the connective tissues covered by epithelia in mucosal folds and villi not varied from that of control group, microvilli brush border which consist of columnar cells with nuclei situated centrally or toward the cells bases had same morphology as in control. A little changes were observed when the vegetables incubated with 3, 4.5 g\l detergents for 15 or 30 minutes, even the vegetables had taken the detergents perfumes smells, the intestine had same layers but few inflammatory cell clusters were noted in the lamina propria, mild edema were detected in both lamina propria and tunica submucosa, necrosis area in entire layers were observed along with architecture changes villus blunting and atrophy and crypt hyperplasia Figures-(15-18).

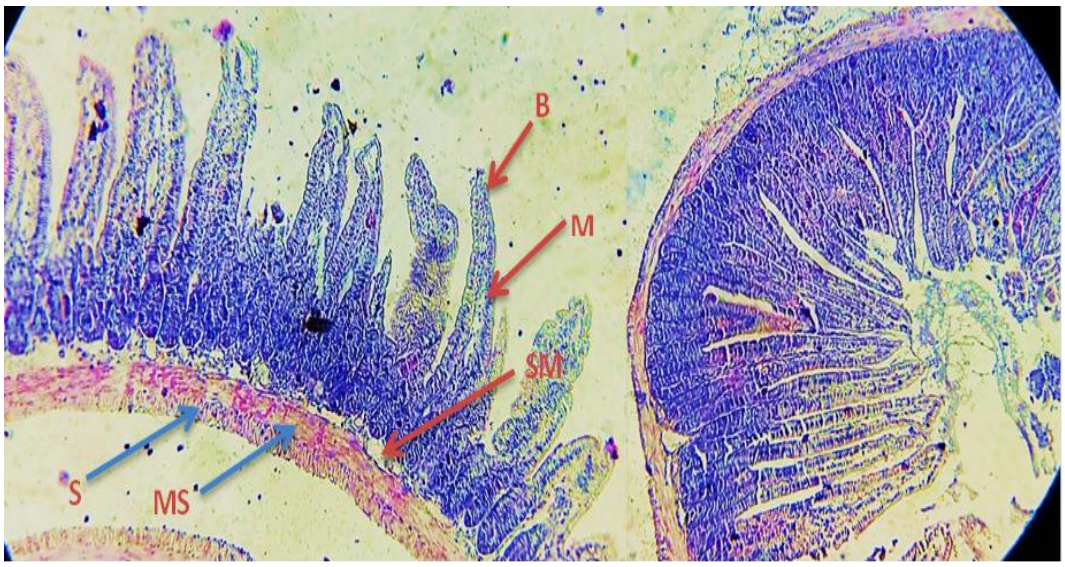

Figure 5-Intestinal section of mice treated with 1.5 g\l Altunsa for 2 minutes. No histopathological changes were observed. H E stain.
B= brush border, M= mucosa, SM= sub mucosa, MS= musculatures, S=serosa.

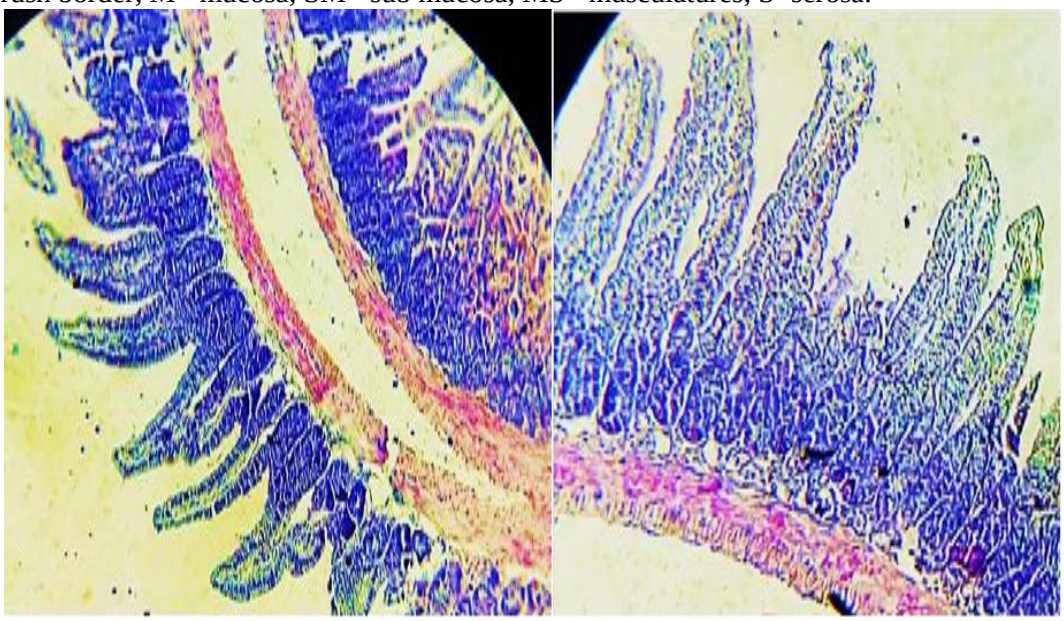

Figure 6-Intestinal section of mice treated with 1.5 g\l Altunsa for 5 minutes No histopathological changes were observed. H E stain. .

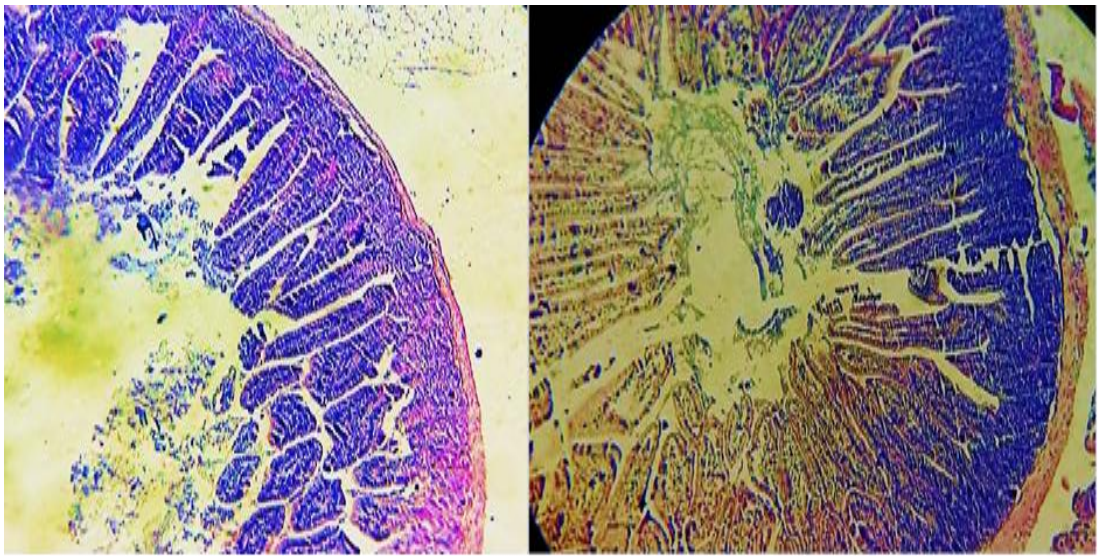

Figure7- Intestinal section of mice treated with 1.5 g\l Ariel for 2 minutes. No histopathological changes were observed. H E stain.

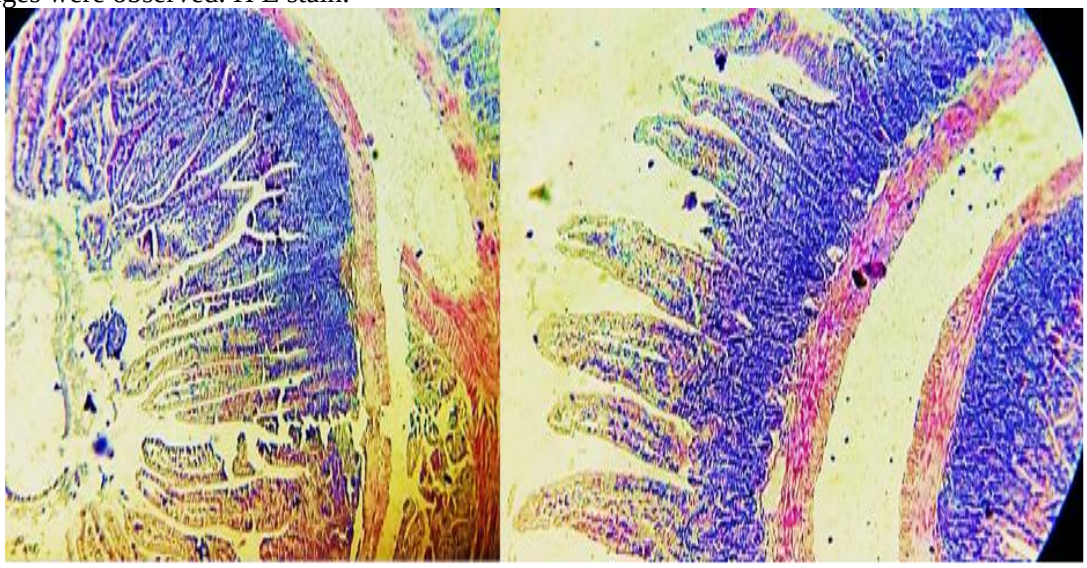

Figure 8- Intestinal section of mice treated with 1.5 g\l Ariel for 5 minutes. No histopathological changes were observed. H E stain.

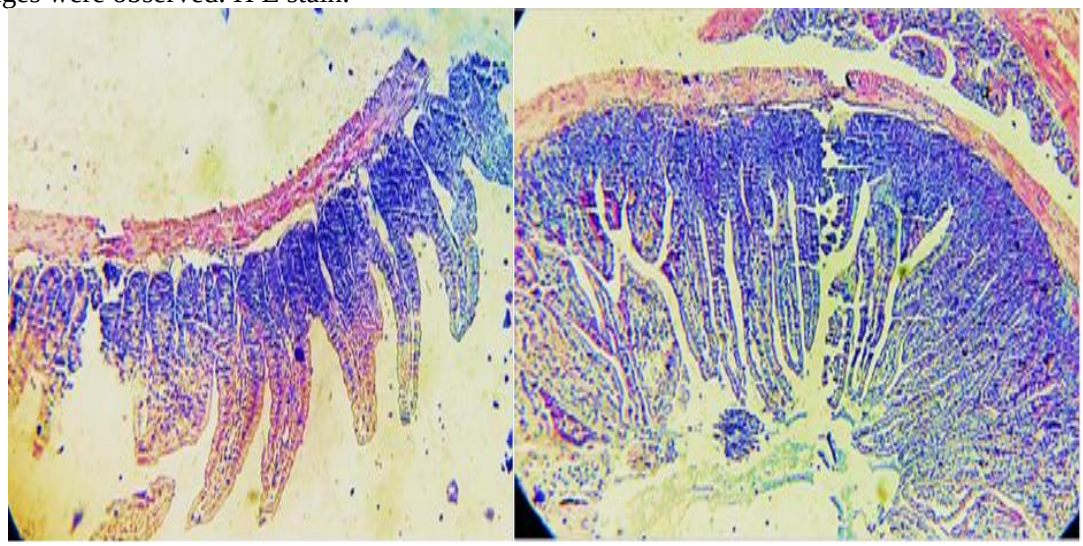

Figure 9- Intestinal section of mice treated with 3 g\l Altunsa for 2 minutes. No histopathological changes were observed. H E stain.

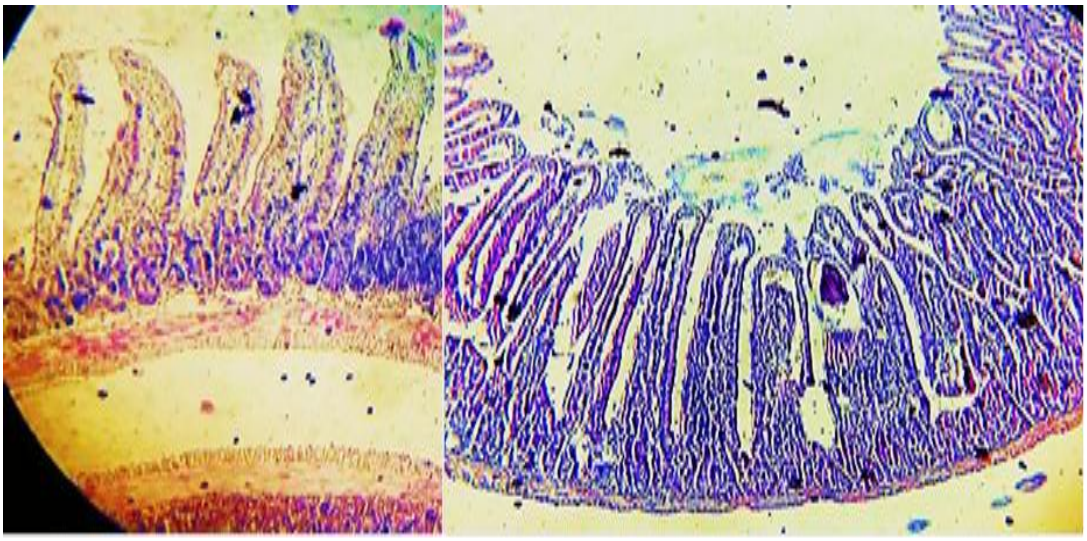

Figure 10-Intestinal section of mice treated with 3 g\l Altunsa for 2 minutes. No histopathological changes were observed. H E stain.

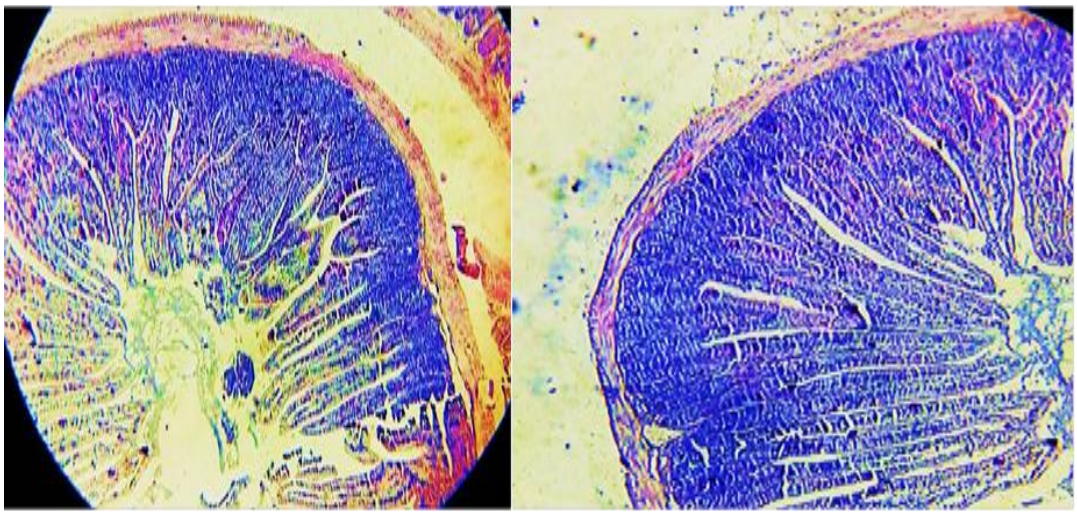

Figure11-Intestinal section of mice treated with 3 g\l Altunsa for 2 minutes. No histopathological changes were observed. H E

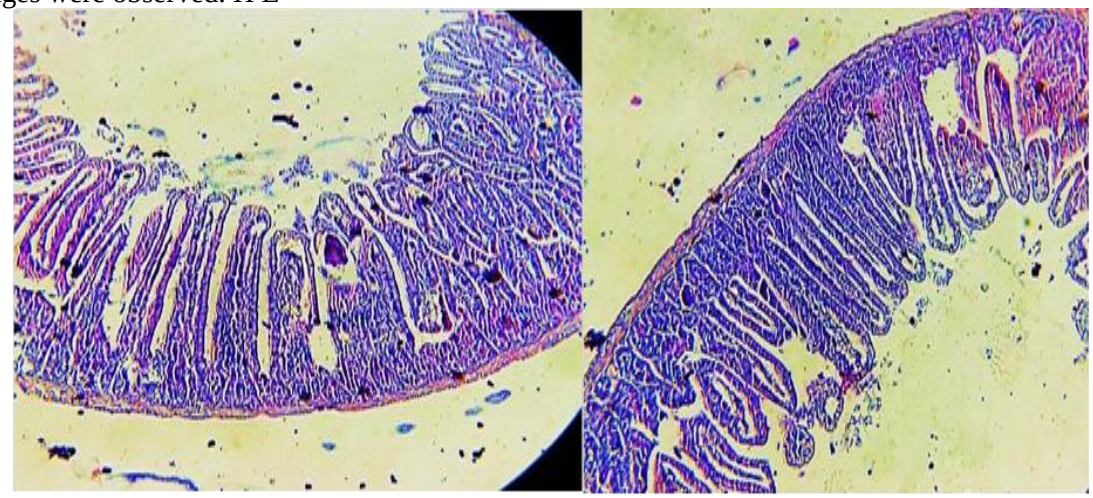

Figure 12- Intestinal section of mice treated with 3 g\l Ariel for 5 minutes. No histopathological changes were observed. H E stain.

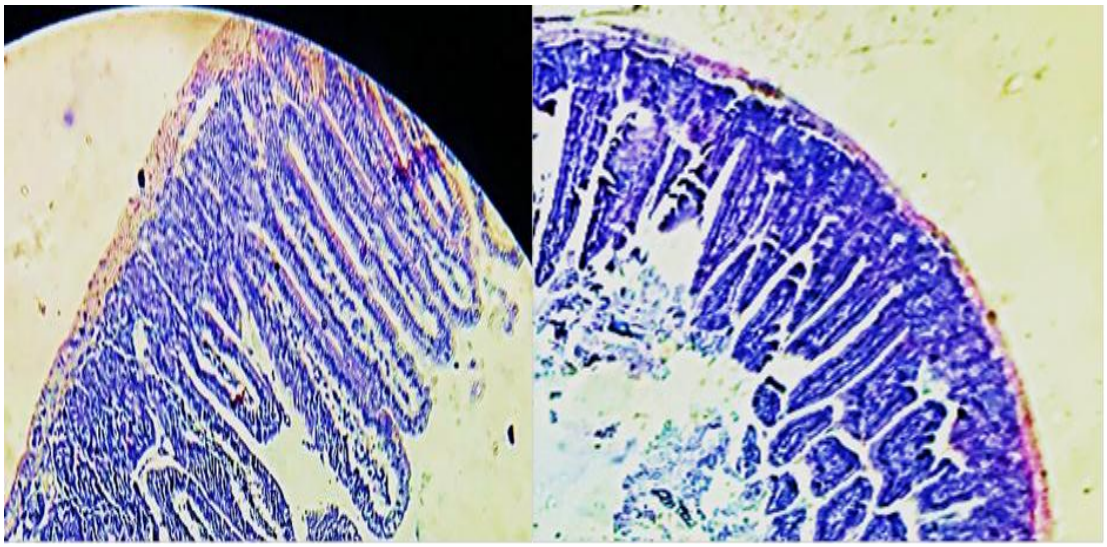

Figure 13-Intestinal section of mice treated with 3 g\l Altunsa and ariel for 30 minutes. No histopathological changes were observed. H E stain.

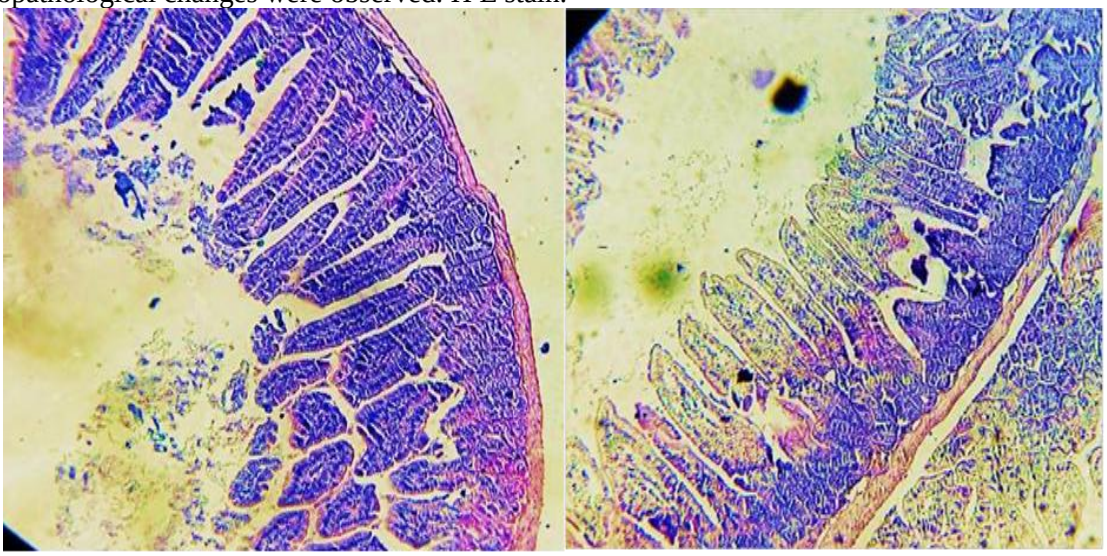

Figure 14-Intestinal section of mice untreated with detergents (control group). H E stain.

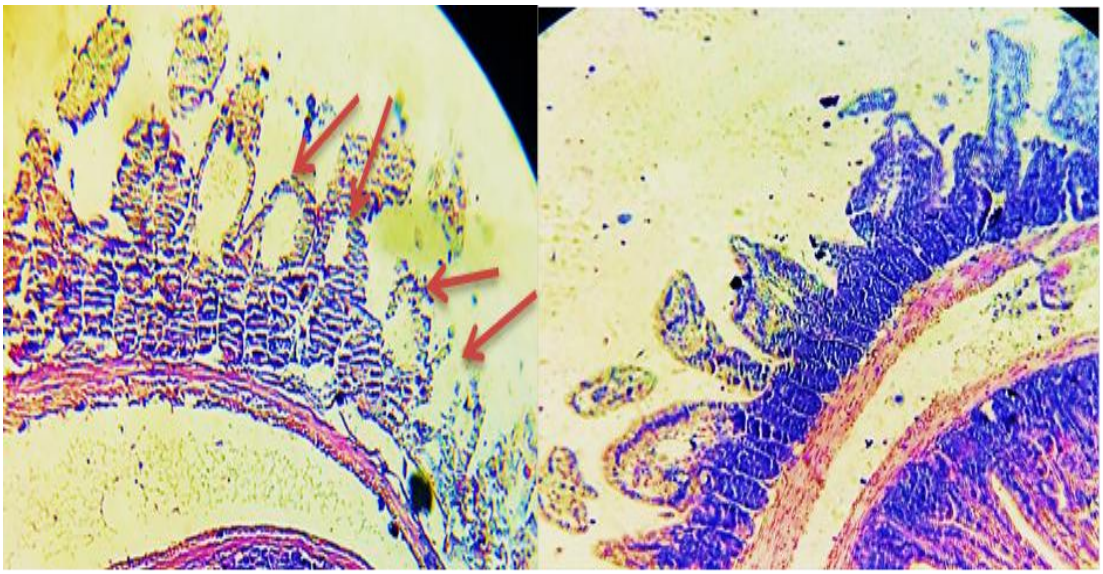

Figure 15-Intestinal section of mice treated with 3 g\l Ariel for 30 minutes. Villus atrophy and architecture changes, necrosis area with the villi were observed. H E stain.

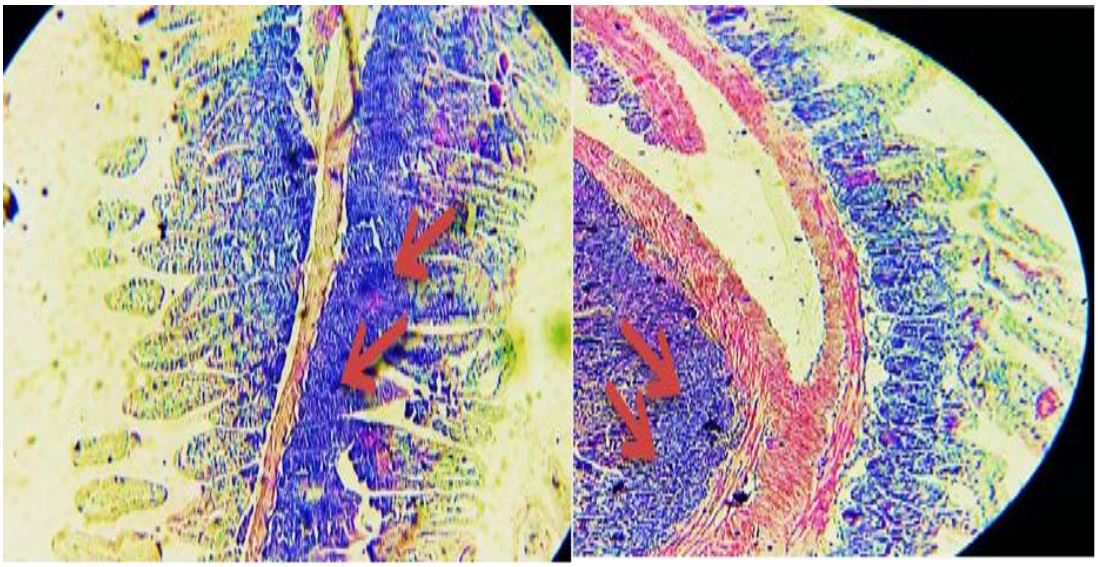

Figure 16-Intestinal section of mice treated with 3 g\l Altunsa for 30 minutes. inflammatory area and crypt hyperplasia were observed. H E stain.

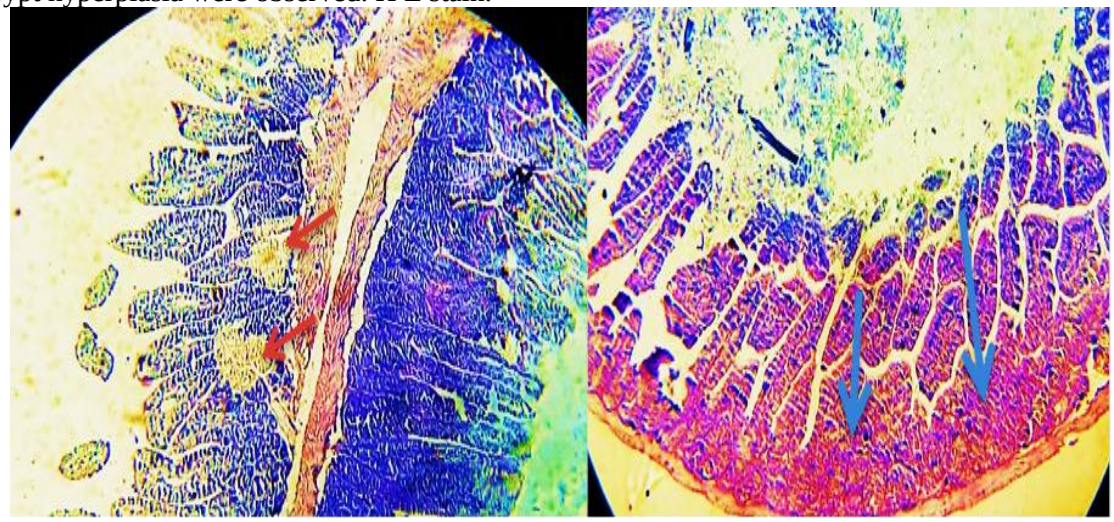

Figure 17-Intestinal section of mice treated with 4.5 g\l Altunsa for 30 minutes. inflammatory area and mucosa demorphism changes were observed. H E stain.

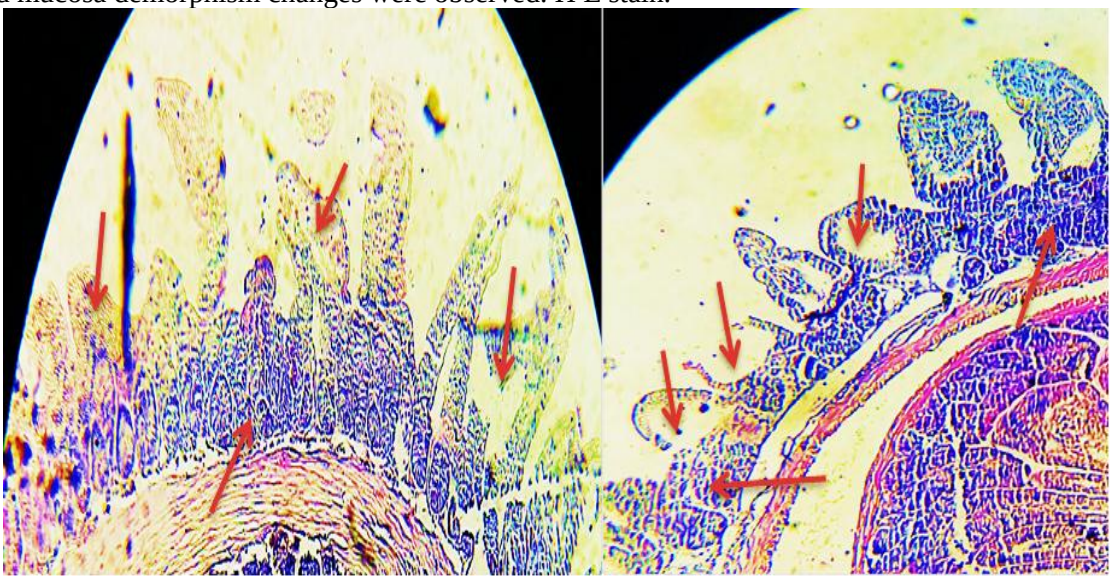

Figure 18-Intestinal section of mice treated with 4.5 g\l Ariel for 30 minutes. inflammatory cell clusters were noted in the lamina propria, mild edema were detected in both lamina propria and tunica

submucosa, necrosis area in entire layers were observed along with architecture changes villus blunting and atrophy were observed. H E stain

Discussion

Detergents are chemical constituents used for many purposes mainly cleaning. But in our community some of populations uses it for unordinary purpose like cleaning vegetables and fruits, some of those people when interviewed, were suspicion from these detergents efficacy on human body. Therefore, their opinions were controversial about using detergents for vegetables cleaning. Also the detergents ability for kill contaminant microorganisms? In the current study, partially, answers had been found for some questions asked for long time. The results of this study showed that, some detergents are capable for killing some parasitic stages more effectively than others. This may be because of the differences in the active ingredients that they contain. Some of detergents had enzymatic constitutes and bleaches that may make them more effective in microorganisms degrading than others, and may increase the permeability of cyst and eggs membranes due to lipophilic properties of these detergents. Somehow, similarly the effect of whole detergents or their chemical ingredients against parasites were studied. A concentration of 13 ppm of chlorine bleach was effective at killing *Myxobolus cerebralis triactinomyxon* parasite and 131ppm was effective 100% at room temperature [11]. The number of formed *Toxoplasma* tissue cyst was reduced when mice injected oocysts treated with chemical disinfectants [12]. A reduction in number and microscopically alteration of Cryptosporidium oocysts incubated with chemical disinfectants for 2 hours was recorded [13]. Eggs of *Echinococcus multilocularis* were not infective to intermediate host after incubation with chemical disinfectants [14, 15]. Embryonation was distinctly reduced in chemical disinfectants treated eggs of *Ascaris suum* [16]. Sodium hypochlorite was degenerated 50% of *Toxocara canis* eggs [17]. In our current study no efficacy have been detected with vinegar, potassium permanganate and H2O2 uses, other studies not agreed with us, infectivity of *Fasciola gigantica* metacercariae was reduced after 30 min incubation with sodium hydroxide (1%), potassium permanganate (>10 %) and acetic acid (>2.5 %) [18]. Exposing *Giardia* cysts to diluted vinegar (acetic acid) had not affect their viability but exposing to un diluted vinegar for 60 min had significantly reduced the viability [19].

In this investigation there were no histopathologic changes detected using low detergents concentrations although the detergents were not directly administered to animal laboratory in contrast to other studies where the detergents administered directly to experimented animals. In agreement to our result no pathological changes were detected in all tissues (intestine, kidney, liver) in doses less than LD50 dose (5000 mg/kg/d) in administered rats[20]. Histological effects of surfactant on fish intestine and liver was noted in concentrations ranged from 0.002-40.0mg/lit [21, 22]. Also pathologic effects on liver was observed in fishes treated with detergents [23]. Concentrations of 20-100 v/v detergents had effected different organs of laboratory mice [24]. This dissimilarity may be because of the differences in concentration applied in each study or due to differences in exposed time which may provide enough period for absorption, in addition to that in our research the detergents were not administered directly to experimented animals.

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