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EVALUATION THE HEMATOLOGICAL AND HISTOLOGICAL CHANGES IN WHITE RATS SPECIES ALBINO INDUCED WITH ROTAVIRUS TYPE A SUSPENSION

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ABSTRACT

Rotavirus type A one of most groups of rotavirus cause death in world. The present study establish to evaluate the hematological and histopathological changes were studied two concentration for one stool samples positive of rotavirus type A suspension are 0.2 (contain $1.2 \times 10^7 \mu\text{l}$) and 0.5 ml (contain $2.7 \times 10^9 \mu\text{l}$) treated with white rats. Hematological changes showed total white blood cells count of rats groups treated with 0.2 ml of rotavirus suspension gave significant increase ($p \leq 0.05$) compared to control group, lymphocytes percentage recorded significant increase while neutrophils recorded decrease significant with treated compared with control, neutrophils decrease significant compared to control group. Histopathological changes in small intestine (Ileum) induced by using 0.2 ml (contain $1.2 \times 10^7 \mu\text{l}$) from the positive rotavirus type A suspension indicated long and trophy of the villi. Stomach of rat has parietal cells were predominantly not present in the upper half of the muscularis was very then, consisting of an inner spiral and an outer obliquely circular layer its irregular. The tunica serosa was very thin. With sever hemorrhage.

Keywords: Rotavirus, Hematological changes, Histological changes

Introduction

Diarrhea is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms, Diarrheal disease of various causes represents a leading cause of both morbidity and mortality among infants and young children in developing countries, Rotavirus is the leading recognized cause of diarrhoea-related illness and death among infants and young children and can lead to severe and sometimes lethal dehydration (Turner *et al.*, 2013). Each year, rotavirus is associated with 25 million clinic visits, two million hospitalizations, and more than 600,000 deaths worldwide among children younger than five years of age (Midgley *et al.*, 2014).

Rotavirus is the main cause of acute viral gastroenteritis in infants and young children worldwide, and in the young animals of a large variety of species, Before the early 1970s, no virus had been confirmed as a causative agent of acute gastroenteritis, only bacterial or parasitic etiologic agents could be detected in 10–30% of children with diarrhea. Rotavirus was first described as a human pathogen in 1973 by Bishop and colleagues (Bishop *et al.*, 1973). Rotavirus infections spread easily and usually occur in the winter and early spring between about November and April. Rotavirus infection often spread in settings where many children are together such as day care centers (Atchison *et al.*, 2010).

Group A rotavirus are the main human pathogens and they are transmitted via the fecal–oral route, with a higher prevalence in winter. They infect the mature absorptive

enterocytes of the small intestine, although the exact entry mechanism is still unknown (Sánchez-San *et al.*, 2004).

In children the main symptoms are fever, vomiting, abdominal cramps and diarrhea, lasting for 3 to 8 days (Greenberg and Estes, 2009) and the virus can be spread from 2 days before and up to 8 days after the onset of diarrhea. Oral rehydration is the most usual treatment (Sánchez-San *et al.*, 2004; Greenberg and Estes, 2009).

The most widely available method for confirmation of rotavirus infection is detection of rotavirus antigen in stool by enzyme immunoassay (EIASA). Several commercial test kits are available that detect an antigen common to human rotaviruses. These kits are simple to use, inexpensive, and very sensitive, other techniques are also in use such as electron microscopy, reverse transcription polymerase chain reaction, nucleic acid hybridization, sequence analysis, and culture (Sánchez-San *et al.*, 2004; Greenberg and Estes, 2009).

The presence of rotavirus antigen in the intestinal tissues by ELISA and chromatographic immunoassay, thus confirming rotavirus infection in infants under investigation; mainly confined to the small intestinal mucosa, earlier reports were controversial for the susceptibility of different small intestinal sections to rotavirus infection, Rotavirus infection alters the function of the small intestinal epithelium, resulting in diarrhea. The diarrhea was generally considered to be malabsorptive, secondary to enterocyte destruction (Sánchez-San *et al.*, 2004; Greenberg and Estes, 2009).

The aims of the current study the pathogenesis of rotavirus type A causing diarrhea in children under three years in Babylon province and assessed this virus by histologically and physiologically parameters in rats.

Objectives of this study included the following :

1. Evaluation of physiological changes in rats infected with suspension of positive sample of Rota virus type A VP9 genes is diagnose by RT- PCR and conventional PCR.
2. Evaluation histological changes in rats infected with suspension of positive sample of Rota virus type A VP9 gene diagnose by RT-PCR and conventional PCR.

Material and Methods

Experimental study : A total of 12 males rat species albino/rat have aged six month and weight 250-300 g divided into three groups, the first group consist of four rat injected orally with 0.2ml of (rotavirus suspension G9) for one positive sample. The two group consist of four rat injected orally with 0.5ml of (rotavirus suspension G9) for one positive sample.. the three group consist of four rat divided into two groups two rat injected orally with 0.2 of rotavirus suspension G9 and other as was received 0.5 ml of sterile phosphate buffer saline (PBS) according to methods of (Abbas *et al.*, 2014; Bhrigu *et al.*, 2011). After 2-5 days clinical signs were recorded in infected animals were observed Experimental rat were sacrificed after anesthetization by chloroform and open abdomen cavity by medical scissors, tissue from small intestine, stomach, were collected from the experimentally infected rat and placed in formalin 10% for hematological and histopathological examination in later.

Histopathological examination:

Preparation of Histological Sections (Lee, 1968)

The procedure includes the following steps:

1. **Fixation:** a complex series of chemical events that is coming up by reaction between the fixative (as neutral buffered formalin 10%, in order to keep everything as their in vivo relation to each other, and that can be accomplished by keeping tissue in neutral buffered formalin 10% for several days.
2. **Tissue processing:** samples were processes to embed them in a solid medium firm enough to support the tissue tissues and give it sufficient rigidity to enable thin section to be cut, and that was made in three stages:
 - a) **Dehydration :** To remove fixative and water from the tissues and replace them with dehydration fluid, the tissue samples were washed in water for three hours to remove the formalin residue, then the sample were passed through ascending grades of increasing ethanol concentration (70%, 80%, 90%, 96% and 100%) for about 1-2 hour for each concentration.
 - b) **Cleaning :** In order to replace the dehydration fluid with a fluid that is totally miscible with both the dehydration fluid and the embedding medium, tissue sample, were macerated in xylene for 3 hours.
 - c) **Embedding :** For providing sufficient external support during sectioning, tissue samples were macerated in a container contain liquid paraffin in 56-58 °C and leave it in room temperature to be solid rectangular blocks of

paraffin which released from container to be kept in freezer.

- d) **Sectioning:** By using of rotary microtome, blocks were cutting into sections each of 5µm thickness and the ribbons, were mounted on clean glass slide protracted with him film of Mayer's albumin fixative. The slides were put on a hot plate (50°C) over night to day.

3-8-1-2- Staining of histological sections (Wood and Ellis, 1994)

It was accomplished as follows:

1. Slides were deparafinized by putting it in over (55-70°C) for 5 hours, and then it was macerated in xylene for 1 hour.
2. Slides were dried from xylene and the rehydrated by entering it in a series of ethanol concentration (100%, 95%, 70%) for 3minutes in each concentration.
3. Slides were rinsed in distilled water for (5) minutes.
4. Slides were stained in Hematoxylin stain for (6) minutes.
5. Then slides were rinsed well in running tap water for (20) minutes.
6. Slides were decolorized in acid alcohol few second, then rinsed well in tap water for (15) minutes.
7. Slides were immersed in lithium carbonate for (3) seconds and then rinsed well in tap water for (5) minutes.
8. Then slides were counterstained in Eosin stain for (15) seconds.
9. Slides were dehydrated by putting it in a graded series of ethanol concentration (95%, 95%, 100% and 100%), for (3)minutes in each concentration.
10. Slides then were cleaned in xylene for (5) minutes.

Finally mounting the slides were done by using of sticky material D.P.X. and cover slipping with cover slide and dried to be ready for microscopic examination

Hematological study

The hematological study of the blood of rats treated with 0.2ml and 0.5ml concentration of (rotavirus suspension G9).After anesthetizing the rat, we take one ml of blood and analyzed by by hematology analyzer automated .

Results

Hematological study

The hematological study of the blood of rats treated with 0.2ml concentration and 0.5ml concentration of rotavirus type A (G9) suspension was examined by analyzer automated system in the Laboratory of Al-Qasim General Hospital. The results of indicate that packed cell volume for rats groups treated with 0.2ml concentration of rotavirus suspension refer to significant decrease ($p \leq 0.05$) in treated group (34.5 ± 0.20) compared to control group $50\% \pm 2.0$).

The results for total white blood cells count of rats groups treated with 0.2 ml (contain $1.2 \times 10^7 \mu\text{l}$) of rotavirus suspension gave significant increase ($p \leq 0.05$) 12.218 ± 0.02 compared to control group 4.534 ± 0.01 . Lymphocytes percentage recorded significant increase 88.846 ± 0.066 while neutrophils recorded decrease significant with treated

10.28±0.42 compared with control 21.24±0.4 . results for total white blood cells count of rats groups treated with 0.5 ml (contain 2.7 ×10⁹ μl) of rotavirus suspension gave significant increase 14.616±0.22 compared with control group 4.534±0.01, lymphocytes percentage recorded significant increase 98.3±0.47 compared with control group 67.7±0.8, neutrophils decrease significant 12.9±0.51 compared to control group 21.24±0.4 . These results reported in Table (1).

Table 1 : Statistical analysis for patients with different concentration of Rotavirus type A suspension and control groups.

	RBCs (10 ¹² /L)	Hb (g/L)	WBC (10 ⁹ /L)	Lymphocytes %	Neutrophils %	Monocytes %	Eosinophils %
Mean ± SEM							
Control	10.276±0.05	11.078±0.03	4.534±0.01	67.7±0.8	21.24±0.4	5.58±0.2	3.46±0.1
0.2	8.332±0.18	8.32±0.08	12.218±0.02	88.846±0.066	10.28±0.42	9.68±0.21	8.2±0.15
0.5	7.264±0.09	7.066±0.03	14.616±0.22	98.3±0.47	12.9±0.51	11.18±0.57	9.7±0.1
LSD	1.021	1.201	2.014	12.52	1.85	1.96	0.961

*The number represent mean ± standard err.

* The differences number revealed to Significant differences

Histopathological study

Results of the current study showed histological changes in digestive tract which treated with 0.2 ml, 0.5 ml concentration of rotavirus type A suspension. Small intestine of rat group treated these concentration of Rotavirus type A suspension showed changes in structure apparent in figures (1,2). While stomach changes showed in figures (4,5)

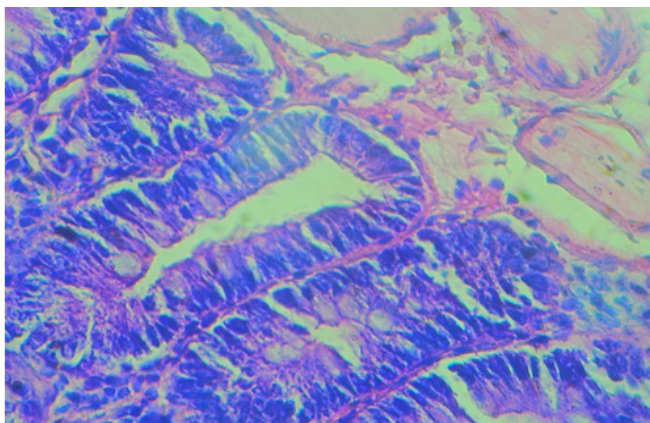


Figure (1) long villi and atrophy and hemorrhage with with 0.2 ml concentration of Rotavirus type A suspension .

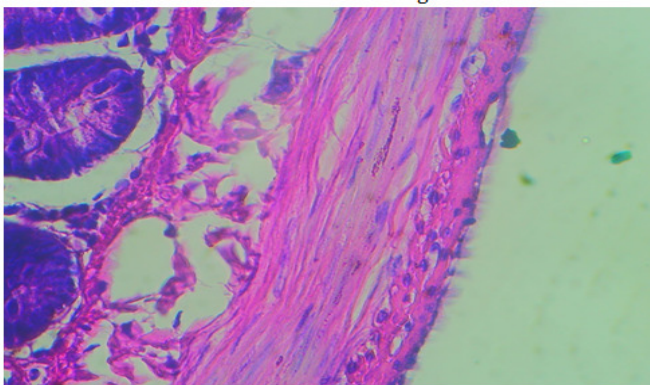


Figure (2) Rats treated with 0.5 ml concentration of rotavirus type A suspension show sever atrophy of the villi and dilation of the intestinal lumen and the short villi are flattened and sever hemorrhage.

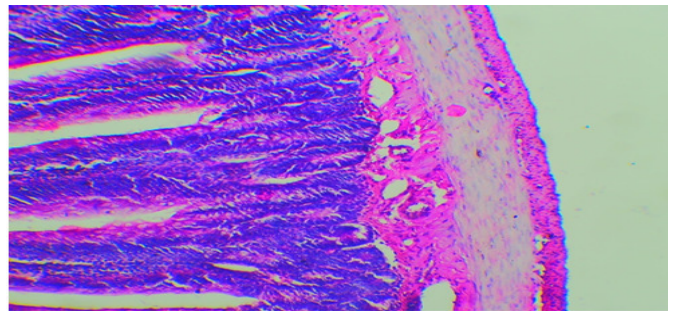


Figure (3) Small intestine of control rats group : normal long villi and no atrophy and no hemorrhage .

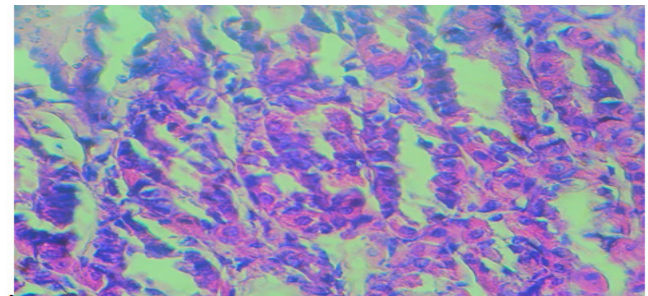


Figure (4) stomach of rats treated with 0.2 ml concentration of rotavirus type A suspension The chief cells, showing intense basophilia, occupied the lower third region of the gland; the parietal cells were predominantly not present in the upper half of the muscularis was then, consisting of an inner spiral and an outer obliquely circular layer. Ganglion cells not present between these muscle layers. The tunica serosa was very thin. With mild hemorrhage .

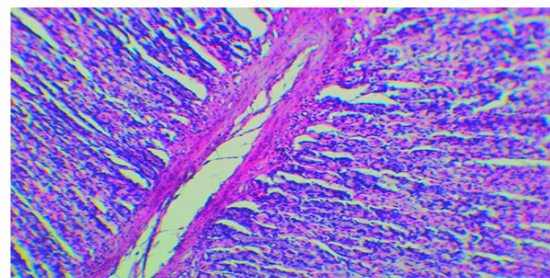


Figure (5) Stomach of rat treated with 0.5 ml concentration of rotavirus type A suspension The parietal cells were predominantly not present in the upper half of the muscularis was very then, consisting of an inner spiral and an outer obliquely circular layer its irrigreral. Ganglion cells not present between these muscle layers. The tunica serosa was very thin. With sever hemorrhage .

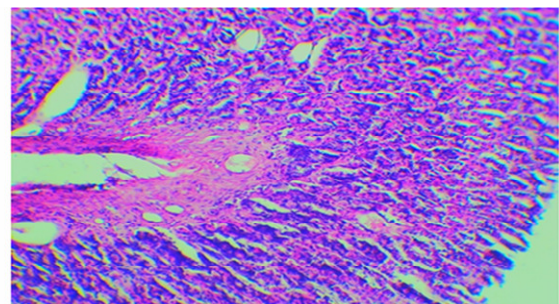


Figure (6) stomach of control rats group : The mucosa of the glandular (right) part was lined by simple columnar epithelium . The lamina propria was occupied by simple tubular, gastric glands. The cells of these glands were discernible in 3 distinct zones. The chief cells, showing intense basophilia, occupied the lower third region of the gland; the parietal cells were predominantly present in the upper half of the muscularis was thick, consisting of an inner spiral and an outer obliquely circular layer. Ganglion cells were present between these muscle layers. The tunica serosa was thin. The non glandular (left) part of the stomach was lined by keratinized, stratified

Discussion

Group A Rotaviruses are the most common causative agents of acute gastroenteritis in children under 2 years of age and are also associated with diarrhea in the young of avian (chicken, turkey, and pigeon) and many mammalian (simian, porcine, bovine, ovine, caprine, equine, canine, feline, and murine) species (Estes, 2001)

The present study establish to evaluate the hematological and histological changes in groups rats treated with two concentration of rotavirus type A suspension are 0.2, 0.5 ml compared with control rats group. Statistically analysis for hematological changes in rats treated with 0.2,0.5 ml Of rotavirus suspension revealed to significant decrease in packed cells volume and white blood cells count while recorded significant increase in lymphocytes percentage in value ($p \leq 0.05$). This result accepted with results many last study such as (Abbas *et al.*, 2014 ; Maria *et al.*, 2016).

These changes in count and percentage of blood due to the virus attacked blood form ing organs and cause failure in bone marrow and spleen (Payne *et al.*, 2009; Abbas *et al.*, 2014; Maria *et al.*, 2016).

Due to many studies in world mentioned that enteric viruses cause huge changes in histological image of digestive tract of lab animals therefore the current study also establish to evaluate histological changes in small intestine and stomach of rats induced with two certain concentration of Rota virus type A suspension (G9) diagnosed by RT-PCR and conventional PCR included 0.2, 0.5 ml. Histopathological changes were studied for one stool samples positive of Rotavirus type A in rats according to methods used (Bhriju *et al.*, 2011).

The Histopathological changes in small intestine induced by using 0.2 ml, 0.5 from the positive rotavirus type A virus suspension indicated to long villi and atrophy and hemorrhage with with 0.2 concentration, sever atrophy of the villi and dilation of the intestinal lumen and the short villi are flattened and sever hemorrhage with 0.5 concentration.

While stomach of rats has stomach of rats treated with 0.2 m l concentration of rotavirus type A suspension showed the chief cells, showing intense basophilia, occupied the lower third region of the gland; the parietal cells were predominantly not present in the upper half of the muscularis while

On other hand stomach of rat treated with 0.5 m l concentration of rotavirus type A suspension indicate to the parietal cells were predominantly not present in the upper half of the muscularis was very then, consisting of an inner spiral and an outer obliquely circular layer its iirrigeral. Ganglion cells not present between these muscle layers. The tunica serosa was very thin with sever hemorrhage.

Rotavirus infection of this study in rats was evaluated by clinical findings, histopathological changes in the small intestine, growth rate. Rotavirus infection of 2-5-day-old rats resulted in diarrhea that lasted from 1 to 10 days post inoculation. Histopathological changes in the small-intestine mucosa of 2-5-day-old. RV suspension -inoculated rats but not of PBS-inoculated rats was limited to extensive enterocyte vacuolation in the ileum. RV-inoculated rats. (Abdulazeez *et al.*,2018). The histopathological changes in

intestine at 2 days post infection showed congested diluted blood vessels with inflammatory cells particularly Lymphocytes, neutrophils and mononuclear cells and edema in sub mucosa in addition to necrosis of crypt and hypertrophy of goblet cells.

Earlier reports were controversial for the susceptibility of small intestinal sections to rotavirus infection, Rotavirus infection alters the function of the small intestinal epithelium, resulting in diarrhea. , diarrhea was generally considered to be malabsorptive, secondary to enterocyte destruction (Vanamayya and Mohanty, 1993; Abdulazeez *et al.*, 2018) in animal models, rotaviruses have also been documented to spread beyond the intestine oral infection. In the mouse model, sites of spread include the lamina propria, Peyer's patches, mesenteric lymph nodes, lung, liver, kidney, and bile duct (Kapikian *et al.*, 2001). Group A rotaviruses have been shown to induce biliary atresia in mice, but this system requires intraperitoneal not oral inoculation of virus (Conner and Ramig, 1997).

Therefore the current study demonstrated that rotavirus type A induced pathological lesion in the examined organ of treated group of rat , thus, this virus was highly virulent and have ability to produce toxic nonstructural protein and overcome of the host defense mechanism. The results match the observation who investigated the functional NSP4 enterotoxin peptide secreted from rotavirus-infected cells where they found that the NSP4, a secreted fragment of NSP4, or appropriate NSP4 peptides have an activity similar to that of toxin and stimulate diarrhea when injected into mice (Czech-Schmidt *et al.*, 2001; Nottle *et al.*, 2016). The enterocytes lining the small intestine are generally divided into two types: enterocytes and crypt cells. Villus enterocytes are mature, non-proliferating cells covering the villi digestive and absorptive functions. The absorptive enterocytes synthesize a number of disaccharidases, peptidases, and other enzymes that are expressed on the apical surface, where they carry out their digestive functions. Absorption across the enterocyte barrier occurs both by passive solutes along electrochemical or osmotic gradients and by active transport (Zhang *et al.*, 2000; Loo *et al.*, 2002). The crypt epithelium lines the crypts and is the progenitor of the villus enterocytes. Crypt cells lack the well-defined microvilli and absorptive functions of the enterocyte and actively secrete Cl^- ions into the intestinal lumen. In the normal animal, the combined activity of the enterocytes and crypt cells results in a constant bidirectional flux of electrolytes and water across the epithelium. On the villi, the balance is toward absorption, and in the crypts, the balance favors secretion (Moon, 1994).

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