Global Advanced Research Journal of Educational Research and Review (ISSN: 2315-5132) Vol. 8(1) pp. 001-005, October, 2019
Available online http://garj.org/garjerr/index.htm

Full Length Research Paper

Additive Effect of Some Elements to vitamin B17 Administration in Immunosuppressed Mice Infected with Cryptosporidium

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Accepted 19 October, 2019

Cryptosporidiosis is known to trigger neoplastic gut changes in experimental animals. In this study, the efficacy of combined vitamin B17 (a newly introduced anticancer agent), and some elements such as vitamin B12, Zinc or Selenium, was tested in immunosuppressed *Cryptosporidium* infected mice. The aim of this work is to check whether the addition of either of these three elements solely to vitamin B17, could alleviate, or even hinder its deleterious effects in experimental models or not. A group of sixty (60) albino mice was used in the experiment. This group was further subdivided into ten subgroups. Subgroup1: immunocompetent infected mice. (100 Cryptosporidium oocysts four times per week for one month) Subgroup 2: immunocompetent infected mice treated with vitamin B17 (100 mg/kg b wt) 3weeks post infection, 3 times a week for 5 successive weeks. Subgroup 3: immunosuppressed infected mice. Subgroup 4: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 2 weeks post infection, 3 times a week for 5 successive weeks. Subgroup 5: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) simultaneously with the infection 3 times a week for 5 successive weeks. Subgroup 6: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 2 weeks prior to infection as a prophylactic dose, 3 times a week for 5 successive weeks. Subgroup 7: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 3 weeks post infection, 3 times a week for 5 successive weeks. Subgroup 8: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 3 weeks post infection, 3 times a week for 5 successive weeks, combined with vitamin B12 (0.57 mg/kg). Subgroup 9: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 3 weeks post infection, 3 times a week for 5 successive weeks, combined with Zinc (25 mg/kg). Subgroup 10: immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) 3 weeks post infection, 3 times a week for 5 successive weeks, combined with Selenium (50 mg/kg). Immune suppression was performed by using Dexamethasone orally in a dose of 0.25 mgm/kg/day for 4 successive days prior to inoculation with *Cryptosporidium oocysts.* Criteria of assessment included .parasitological oocysts count both in intestine and stools of infected animals. Histopathological studies included intestinal sections of all animal groups. It was found that co-administration of vitamin B17 with vitamin B12, Zinc or Selenium solely, revealed significant drop in the number of oocytes in immunosuppressed infected treated groups. As regards the histopathological findings, several degrees of inflammatory changes were seen in the infected untreated group such as villous atrophy, crypt elongation and mixed inflammatory cell infiltration in the laminapropria . These histopathological changes improved in the groups treated with B17 co- administered with vitamin B12, Zinc or Selenium solely. However, the improvement was more salient in the Zinc co-treated group.

Keywords: Vitamin B17, Cryptoporidiosis, Gut Cancer, Vitamin B12, Selenium and Zinc

INTRODUCTION

Cryptosporidiosis is known to induce inflammatory bowel changes. In this issue many studies mention that this infection could trigger neoplastic gut changes in experimental animals,.Dang et al (2017) postulated that Vitamin B17 has both a preventive and a curative role in cancer treatment by inhibiting cancer cell proliferation using the phenomenon of apoptosis. However, it is endogenously converted into a cyanide toxic radical in the intestine after its oral administration. The authors

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discovered that this side effect could be minimized by consuming citrous fruits or any acid containing food. Again, Dang et al (2017) stated that vitamin B17 interacts with other antioxidants such as vitamin A, C and E along with pancreatic enzymes to break down and eliminate harmfull cells from the body. Therefore it acts both as a detoxifying agent and an immune - potentiator.

Previously, Chang (2005) studied the effect of vitamin B17 in the treatment of asthmatic bronchitis, emphysema, leprosy, colorectal cancer and vitiligo. Later on, Chang (2006) stated that vitamin B17 is decomposed into hydrocyanic acid which is an antitumor compound and benzaldehyde, which has an analgesic action. Hence, it can be used as a double weapon both in the treatment of cancer, as well as pain alleviator. Baroni *et al* (2005), found that vitamin B17 can promote peripheral blood lymphocytes stimulated by polyhydroxy-alkanoates (PHA), secrete IL-2, IFN-γ and inhibit the secretion of TGF-B1. Therefore, it enhances the immune function. Later on, Guo (2013) added that vitamin B17 inhibits type I collagen and kidney fibroblasts (KFB) cell proliferation promoting their apoptosis.

Finally, Xu (2017) concluded that this vitamin can induce apoptosis in human pro-myelocytic leukaemia (HL60).

In this study, the addition of vitamin B12, Zinc or Selenium solely to Vitamin B17 in immune suppressed *Cryptosporidium* infected mice was tested. This co-administration was done in a trial to alleviate, or even hinder the deleterious effects of Vitamin B17 when given alone.

MATERIAL AND METHODS

Experimental animals

Male Swiss albino mice CD1 strain, weighing 18–20 g, clean from parasitic infection, were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI). Animals were maintained under standard laboratory care (controlled temperature and light environment); and were given filtered drinking water and balanced diet. Appropriate number of animals was used, to produce statistically reproducible results.

Parasite

The stool samples of infected cows were collected in sterile clean stool cups; they were repeatedly washed and sieved by using 100, 200 and 400 mesh sieves. The oocysts were suspended in phosphate-buffered saline (PBS).

Immune suppression

Immune suppression was performed by using dexamethasoneorally, in a dose of 0.25 mg/g/day for 14 successive days prior to inoculation with *Cryptosporidium* oocysts (Rehg et al., 1988).

Parasitological Examination

Stool samples were collected three weeks post infection, post treatment daily in sterile clean stool cups. The samples were stained by modified Zeihl–Neelsen (MZN) stain [Henriksen, et al 1981].

Sacrifice of mice was done after administration of drugs by intra-peritoneal anesthesia. The posterior upper part of small intestine was removed and subjected to histo-pathological examination. The duodenal contents were subjected to parasitological examination.

Drugs

Novo Dalin B17 (Amygdalin) Products"- Novodalin™ from United Kindom , given 3 week post infection (100mg/k.gm) 3 times a week for 5 successive weeks. Oravit B12 produced by Sigma Pharmaceutical Industries Egypt, given orally in a dose of 1000µg/kg/day body weight for 5 successive weeks 3 weeks post infection.

Vitazinc. Manufactured by E.I.P.I.CO. Egyptian Int. Pharmaceutical Industries CO. Tenth of Ramadan City Egypt, given orally in a dose of 25 mg/kg/day body weight for 5 successive weeks 3 weeks post infection.

Selenium ACE manufactured by Sigma Pharmaceutical Industries for Interpharma UK under license of Wassen International LTD , given orally in a dose of 50 mg/kg/day body weight for 5 successive weeks 3 weeks post infection.

Studied Groups

The experimental animals were divided into 10 subgroups (10 mice / group):

Group (1): immunocompetent infected mice (100 Cryptosporidium oocysts four times per week for one month).

Group (2): immunocompetent infected mice treated with vitamin B17 (100 mg/kg) (3weeks post infection, 3 times a week for 5 successive weeks) (Jaswal *et al*., 2018).

Group (3): immunosuppressed infected mice.

Group (4): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (2weeks post infection, 3 times a week for 5 successive weeks).

Table 1 Number of *Cryptosporidia* in intestinal contents versus oocysts count per gm stools.

Groups	Oocysts in intestinal contents (Mean±SD)x10^3	Oocysts/gm stools (Mean±SD)x10^3
Group 1	19±2.9	3000 ± 17.9
Group 2	15 ± 2.4*	2416 ± 14.2*
Group 3	22 ± 3.7	7750 ± 18.7
Group 4	8.25 ± 2.4**	1312 ± 5.4**
Group 5	17 ± 1.5	3250 ± 21.8
Group 6	18 ± 2.4**	5166 ± 15.6**
Group 7	25 ± 2.1*	2117 ± 34.2*
Group 8	9 ± 2.3*	251 ± 14.9*
Group 9	12.2 ± 2.5*	1231 ± 15.4*
Group 10	15 ± 3.7*	1378 ± 18.7*

*Statistically significant difference at P<0.05 low sig.
**Statistically significant difference at P<0.01 moderate sig.

Group (5): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (simultaneously with the infection, 3 times a week for 5 successive weeks).

Group (6): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (2weeks prior to infection as a prophylactic dose, 3 times a week for 5 successive weeks).

Group (7): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (3weeks post infection, 3 times a week for 5 successive weeks).

Group (8): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (3weeks post infection, 3 times a week for 5 successive weeks **combined with** vitamin B12 (0.57 mg/kg) (Bernard *et al*., 2018).

Group (9): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (3weeks post infection, 3 times a week for 5 successive weeks **combined with** Zinc (25 mg/kg) (Nam *et al*., 2017).

Group (10): immunosuppressed infected mice treated with vitamin B17 (100 mg/kg) (3weeks post infection, 3 times a week for 5 successive weeks **combined with** Selenium (50 mg/kg) *(*Wang *et al., 2009).*

Sacrifice of mice

Animal sacrifice was performed by intraperitoneal anesthesia. Mice received anesthetic–anticoagulant solution (500 mg/kg thiopental and 100 units/ml heparin) by intraperitoneal injection.

The excised ileo-caecal regions from the sacrificed mice were subjected to histopathological processing.

Assessment of infection

a) Stool examination:

The experimental fresh fecal pellets from each group were collected from the third day post infection, processed, stained by modified Zeihl-Neelsen stain and subjected to parasitological examination (Smith, 2008). After sacrifice, the duodenal contents were subjected to parasitological examination.

b) Histopathological examination:

The ileocecal parts are dehydrated in ascending grades of ethanol, followed by immersion in xylene, then impregnated in paraffin, and sections were cut (Tzipori,et al., 1981).

c) Statistical analysis of the data:

Mean and standard deviation (± SD) were used for parametric numerical data. A value equal to or less than 0.05 was considered significant (P ≤ 0.05).

Ethical and Regulatory Guidelines

The experimental animal studies were conducted in accordance with the international valid guidelines and they were maintained under convenient conditions at the SBSP animal house of TBRI.

RESULTS

a) Oocysts shedding monitoring: There was a significant difference in the mean number of shedded *Cryptosporidium* oocysts between immunocompetent and immunosuppressed infected treated groups with vitamin B17, 3weeks post infection (p<0.01). (Table 1)

A significant difference in the mean number of oocysts was found between immunosupressed infected groups given vitamin B17 alone, and other groups given vitamin B12, Zinc or Selenium in adjunction (p<0.01). (Table 1)

Again, there was a significant difference in the mean number of shedded oocysts between immunosuppressed infected mice treated with vitamin

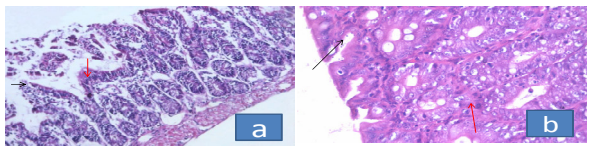

Fig. (1): picture of ileum(a) and colon(b) of group **(3)** showing (a) severe atrophy of villi with ulceration(black arrow)and surface multiform (red arrow) and (b)colonic mucosa showing mild edema and mild mixed inflammatory mononuclear cellular infiltrate in the lamina propria(red arrow) (Hx&E stain x200) and *Cryptosporidium* oocysts on the surface of the crypts(black arrow) (Hx&E stain x400)

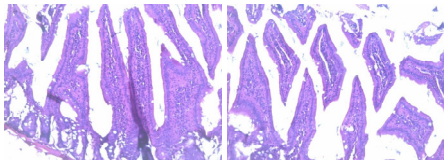

Fig. (2): pictures of ileum regions of groups **(4&7)** showing moderate inflammation in the form of broad inflamed villi (Hx&E stain x200)

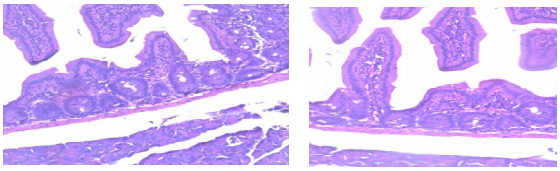

Fig. (3): pictures of ileum regions of groups **(3,5&6)** showing severe villous atrophy and broad inflamed villi (Hx&E stain x200)

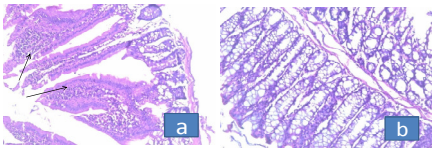

Fig. (4): picture of ileum(a) and colon(b) of group **(10)** showing broad inflamed villi (black arrows) and normal colonic mucosa with no inflammation (Hx&E stain x200)

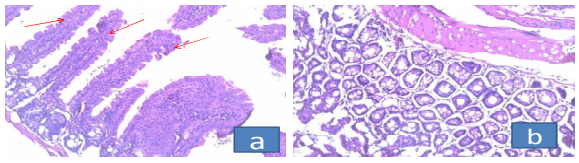

Fig. (5): picture of ileum(a) and colon(b) of group **(9)** showing only few foci of broad inflamed villi among an otherwise normal ileum(red arrows) and normal colonic mucosa with no inflammation (Hx&E stain x200)

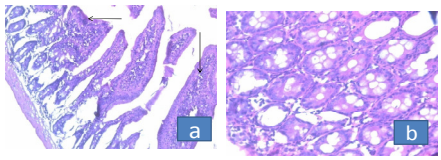

Fig. (6): picture of ileum(a) and colon(b) of group **(8)** showing broad inflamed villi (black arrows) and normal colonic mucosa (Hx&E stain x200)

B17, two weeks prior to infection, and those treated two weeks post infection ($p<0.01$).(Table 1)

Histopathological changes:\

H&E-stained intestinal sections of the immunosuppressed *Cryptosporidium*-infected control group (G3) revealed the presence of *Cryptosporidium* oocysts on the luminal surface of the epithelium lining of the crypts of colon, as small rounded organisms. Also, intestinal villi showed altered mucosal architecture, shortening, blunting and widening of the intestinal villi associated with greater inflammation in the lamina propria with lymphocytes, plasma cells and eosinophil marked intestinal oedema. In addition, there were clear depleted goblet cells, surface multiform erosions, ulcerations and sloughing of the brush border. Mucosa was damaged with villous contraction and little or absent epithelial layer (Figure 1).

The ileum was the site of the heaviest burden of intestinal cryptosporidiosis. Several degrees of inflammatory changes were seen in the groups infected with the parasites, both immunocompetent and immunosuppressed.

Intestinal sections of immunosuppressed infected groups treated with vitamin B17 alone or given simultaneously with, or prior toinfection, showed no improvement in the histopathological changes of the intestinal sections (Figure 3). While immunosuppressed infected groups treated with vitamin B17 two and three weeks post infection, showed slight improvement in the histopathological changes of the intestinal sections (Figure 2).

Again, immunosuppressed infected groups treated with vitamin B17 combined with vitamin B12 or Selenium, showed partial improvement in the histopathological changes of the intestinal sections (Figure 4 and 6).

While intestinal sections of immunosupressed infected groups treated with vitamin B17 combined with Zinc, showed slight or no pathological changes of the intestinal sections, which also regained their normal appearance with the absence of oocysts (Figure 5).

DISCUSSION

From this study, it was shown that co administration of vitamin B12, Zinc or Selenium solely with vitamin B17, could attenuate or even hinder its deleterious effects. This was shown by the drop in oocysts counts in stools in the groups given vitamin B12, Zinc or Selenium (251±4.9), (1231±15.4) and (1378±18.7) respectively, in comparison with (3000± 17.9). in the control untreated group. Although vitamin B17 is not an antiparasitic per say, yet it diminished the protozoal load due to an immunostimulatory potential.

Again, the histopathological sections revealed marked improvement with reposition of the healthy ileal mucosa, in the group given Zinc in conjunction with vitamin B17.

Previously, Ghazanfare et al (2014) reported about the anti-noxious effect of cyanocobalamin (Vit B12) in animal and human models. They noted that co-administration of Vit B12 and morphine could reduce tolerance to analgesic effect of morphine and reduces its withdrawal symptoms. It was noted the protecticve role of Zinc in preventing oxidative destruction due to free radicals release in liver cells . The author added that Zinc is also involved in stabilizing cell membrane and is essential for hepatocytes proliferation. Furthermore, the necessary role of Zinc in cell growth and multiplication and its essential role for DNA and RNA synthesis. Previously, Nawar et al (1992) studied the effect of low Zinc level in the diet of Schistosoma infected mice. They noticed stunted growth, and depressed granulomatous hypersensitivity to Schistosoma eggs in the liver of zinc deficient mice. Kapoor (2014) and Lamming et al (2014) stated that Selenium is an essential trace element to organisms from bacteria to humans. They added that it has an antioxidant action and can promote cell proliferation.

In conclusion, co –administration of vitamin B17 with vitamin B12, Zinc or Selenium solely in immune suppressed *Cryptosporidium* infected mice, could alleviate or even hinder its deleterious effects.

Further trials are being conducted to test other components which could be given in conjunction with vitamin B17 in order to minimize its toxic side effects in cancer candidates.

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