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Berberine-Induced Amelioration of the Pathological Changes in Nutrient's Homeostasis During Murine Intestinal *Eimeria papillata* Infection

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Abstract.- The current work aimed to study the ameliorative effect of berberine on the induced pathological changes in nutrient's homeostasis in mice infected with *Eimeria papillata*. Mice were randomly divided into three groups. The first group represents the control non-infected animals. Second and third groups were orally infected with 1.5×10^3 sporulated *E. papillata* oocysts. The 3rd group was treated with a daily dose (10 mg/kg) of berberine chloride solution for five successive days. All animals were sacrificed on day 5 p.i.. *E. papillata* infection induced a state of disturbance in nutrient homeostasis. Blood glucose levels and total proteins were elevated with concurrent decrease in level of carbohydrates and soluble proteins in jejunum of mice. Also, infection induced a hyperlipidemic status as shown from the increase in triglycerides, total lipids, total cholesterol, high density lipoprotein cholesterol (LDL) with the mutual decrease in high density lipoprotein cholesterol (HDL) and phospholipids. Also, *E. papillata* caused marked disturbance in blood metal ion concentrations. Both ferrous and selenium ion levels were decreased, while sodium and potassium ion concentrations were increased. Berberine treatment of infected mice with *E. papillata* showed a great enhancement in nutrient homeostatic status and also reduced blood glucose level and restored jejunal carbohydrate content. In addition, berberine exerted hypolipidemic effect on the increased fractions of carbohydrates and lipids. Finally, berberine showed a marked enhancement in the levels of altered blood metal ions by the infection. Palm pollen grains or their extracts could be used within food mixtures or water to correct the induced metabolic disturbance and growth depression associated with the intestinal coccidial infections.

Key words: Berberine, coccidiosis, impaired nutrients, *Eimeria papillata*, mice.

INTRODUCTION

Eimeriosis is a cosmopolitan serious disease infecting nearly all mammal species. Its causal agent (Sporozoites) reproduce via fast and invasive multiplication within intestinal tract causing tissue damage and induce severe local and systemic inflammatory response (Al-Quraishi *et al*., 2012; Dkhil *et al*., 2012) leading to diminished feed intake and nutrient absorption, reduced body-weight gain, dehydration, blood loss, and increased susceptibility to other diseases in different animal communities. Thus, it causes huge economic loss in the field of animal farming, milk and meat production (Bhat *et al*., 1996; Dkhil and Al-Quraishi, 2012). *Eimeria papillata* parasitize mice and provides a convenient model for studying animal coccidiosis as its intracellular development within mice jejunum is rapid resulting in fecal oocyst output on day 3 *p.i.* (Stafford and Sundermann, 1991; Schito and Barta, 1997).

The necessity appears to use traditional medicines of natural plant origin to avoid the problems with drug resistance and the adverse side effects of synthetic drug therapy. Currently, new trends have been developed to identify various dietary supplements of natural origin into feeding programs to control Eimeria infections (Abbas *et al*., 2011; Metwaly *et al*., 2012; Dkhil *et al*., 2013).

Berberine is an isoquinoline alkaloid, present in roots and stem-bark of clinically important medicinal plants (Wongbutdee, 2008; Vuddanda *et al*., 2010; Amer *et al*., 2013). Berberine based formulations, are widely used in traditional systems of medicine including, Ayurveda and Traditional Chinese Medicine. Berberine has demonstrated wide range of pharmacological activities. It has significant antimicrobial activity against a variety of organisms including bacteria, virus, fungi, protozoa,

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helminths and Chlamydia. Other physiological activities of berberine being antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, anti-diarrhoeal, cholagogue and hepatoprotective. In addition, berberine is not considered to be toxic at doses used in clinical situations, nor has been shown to be cytotoxic or mutagenic (Wongbutdee, 2008; Vuddanda *et al*., 2010).

The current study was aimed to investigate the impact of berberine administration to mice infected with *E. papillata* upon lipid, protein, carbohydrate and metal ion status.

MATERIALS AND METHODS

Preparation of E. papillata *oocysts*

A self-healing strain of *E. papillata* was kindly provided by Prof. Mehlhorn of Heinrich Heine University, Duesseldorf, Germany. Several passage processes of *E. papillata* were performed in laboratory mice (*Mus musculus*), followed by oocyst collection from faeces and sporulation process in potassium dichromate solution (2.5%). After that, the sporulated oocysts were washed several times with sterile saline and then surface-sterilized with sodium hypochlorite, and washed at least four times with sterile saline before oral inoculation as described by Schito and Barta (1997). These oocysts were used to inoculate mice by oral gavaging each mouse with 1.5×10^3 sporulated oocysts of *E. papillata* suspended in 100 µl sterile saline.

Berberine chloride treatment

Berberine chloride was purchased from Sigma Company (St Louis, MO, USA). Each mouse was orally inoculated with 10 mg/kg body weight berberine chloride. The used dose is in agreement with previous studies on other intestinal protozoan infections and toxicity measurements (Jahnke *et al*., 2006).

Animals and experimental design

Eighteen male Swiss albino mice (9-11 weeks) randomly divided into three groups (Six mice/each group). The first group received saline and served as a non-infected control group. Second and third groups were orally infected with 1.5×10^3 sporulated *E. papillata* oocysts. The 3rd group was treated with a daily dose of berberine chloride for five successive days. The experiments were approved by state authorities and followed Saudi Arabian rules for animal protection. Weight change of mice was recorded during the experimental time and at the end of the experiment.

Sample collection

Animals were cervically dislocated on day 5 *p.i.* and the blood was collected from heart into heparinized tubes. Plasma was separated and kept at -20°C until use. Parts of jejunum were weighed and homogenized immediately in ice-cold phosphate buffered saline then centrifuged at 2000 g.×15 min at 4°C to give a final yield of (10% w/v) jejunal homogenate that were kept at -20° C until use.

Blood biochemical analysis

Blood plasma was analyzed using commercial kits (Biomerieux, Marcy l'Etoil, France) for glucose (Trinder, 1969a) and total proteins (Gornall *et al*., 1949). While, jejunum homogenate was used for the determination of total carbohydrate content using phenol-sulfuric acid method (Dubois *et al.*, 1979) and for measuring soluble protein content (Lowry *et al*., 1951). In addition, plasma lipid fractions were assayed colorimetrically using commercially available kits (Biodiagnostic company, Giza, Egypt) for total lipids (Knight *et al*., 1972), total cholesterol (Trinder, 1969b), high-density lipoprotein (HDLc) cholesterol (Trinder, 1969b), phospholipids (Zilversmit and Davies, 1950), and triglycerides (T.Gs) (Fossati and Principe, 1982). Low-density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated according to Van Horn *et al.* (1988).

Plasma samples from all groups were digested with concentrated nitric acid and hydrogen peroxide as described by Bukhari *et al.* (2005). Levels of iron (Fe), potassium (K), sodium (Na), and selenium (Se) were determined using the atomic emission spectrometer with inductivity coupled plasma iCAP-6500 Duo (Thermo scientific, United Kingdom).

Histochemical studies of jejunum

Small pieces of the jejunum were quickly removed, then fixed in 10 % neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5µm thickness. Then sections were stained with periodic acid-Schiff's method for total carbohydrates demonstration (Hotchkiss, 1948), and with mercuric bromophenol blue method to demonstrate total proteins (Maize *et al*., 1953).

Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All p values are two-tailed and $P < 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Experimental coccidial infection in mice with *E. papillata* in both infected and infected treated groups was established as revealed oocyst discharging in faecal pellets beginning from day 3 *p.i*. and reached maximal level at day 5 *p.i*. (Un-published data).

Examination of carbohydrate status revealed and increase in blood glucose level from 59±3.2 to 68±4.2 mg/dl (Fig. 1). This increase was associated with depletion in the carbohydrate content within jejunum tissue from 77 to 68.3 mg/g (Figs. 2, 3). Oral administration of berberine chloride caused a significant decrease in blood glucose level in infected group to be 49±2.9 mg/dl (Fig. 1). Also, carbohydrate content in jejunum tissue was changed to be 123.8 mg/g in infected treated group (Figs. 2, 3).

Experimental infection of mice with *E. papillata* caused an increase in plasma total proteins and albumin from 4.8±0.25 and 1.87±0.21 to 5.72±0.6 g/dl and 2.67±0.21 g/dl (Fig. 4), respectively. This was associated with depletion of jejunal soluble protein content from 152.5±18.4 to 107.4±12.8 mg/g (Figs. 5, 6). Oral treatment of mice with berberine did not cause a significant change in plasma protein levels but it caused a significant increase in jejunal soluble proteins to be 166.4 mg/g in infected group (Figs. 4-6).

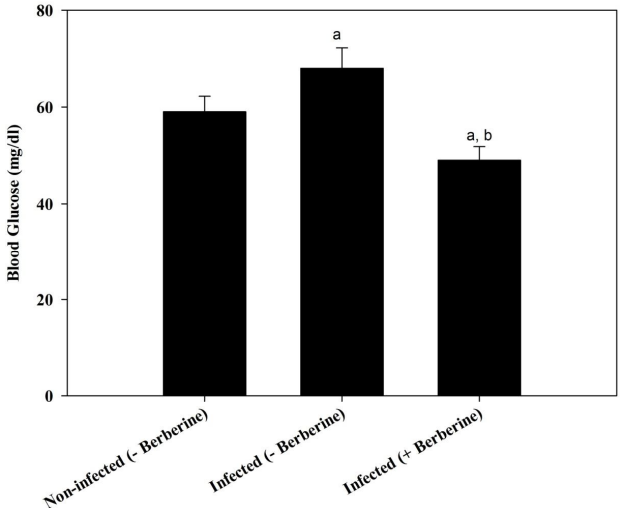

Fig. 1. Changes in blood glucose level in mice infected with *E. papillata* and after treatment with berberine. All values are means ± SD. a: Significant against non-infected control group at P≤0.05, b: Significant against infected (- Berberine) group at P≤0.05. n=6.

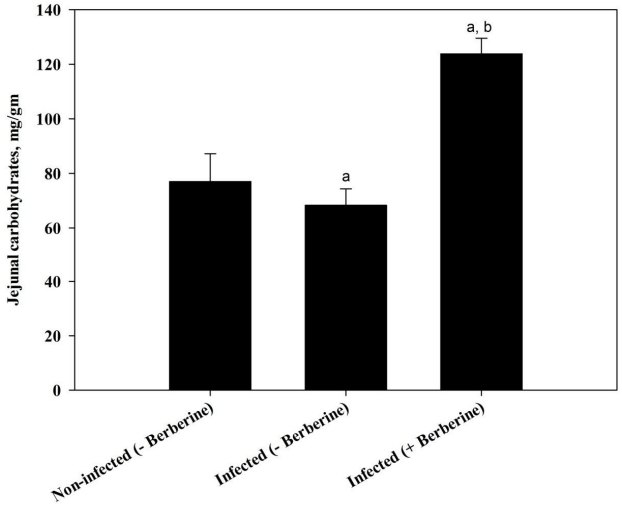

Fig 2. Changes in jejunal carbohydrate level in mice infected with *E. papillata* and after treatment with berberine. All values are means ± SD. a: Significant against non-infected control group at P≤0.05, b: Significant against infected (- Berberine) group at P≤0.05. n = 6.

There was a significant elevation in the level of total lipids, total cholesterol and triglycerides to about 77.5%, 75% and 82%, respectively (Table I). Also, infection induced a marked decrease in HDL

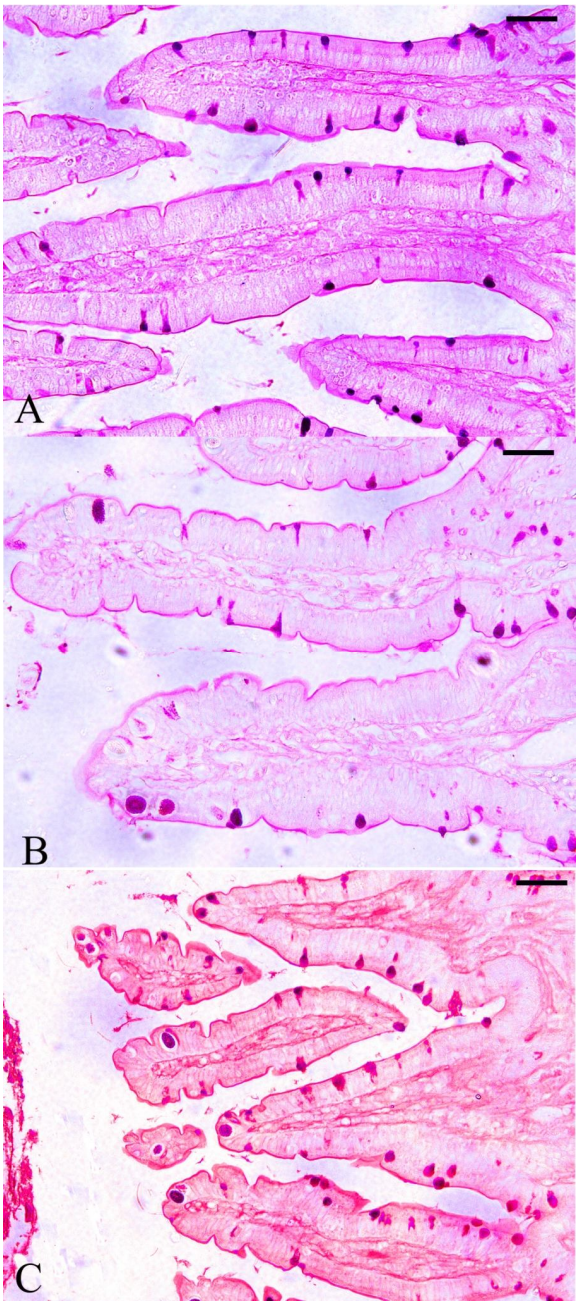

Fig. 3. Carbohydrate content in jejunum sections. A, Control non-infected mice jejunum. B, *E. papillata* infected mice jejunum. C, Berberine treated and infected mice jejunum. Jejunum tissue shows normal content in control non-infected group. Infected sections showed depletion in their carbohydrate content. After treatment with berberine, carbohydrate content showed improvement in their level. Sections were stained with periodic Schiff's method. Scale bar = 50 µm.

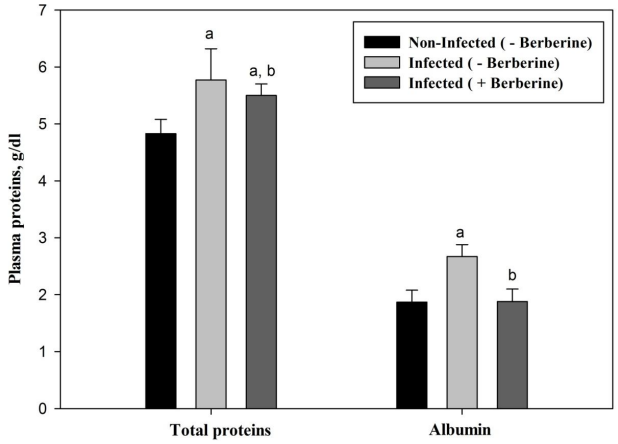

Fig. 4. Changes in plasma total protein and albumin levels in mice infected with *E. papillata* and after treatment with berberine. All values are means ± SD. a: Significant against non-infected control group at P≤0.05, b: Significant against infected (- Berberine) group at P≤0.05. n = 6

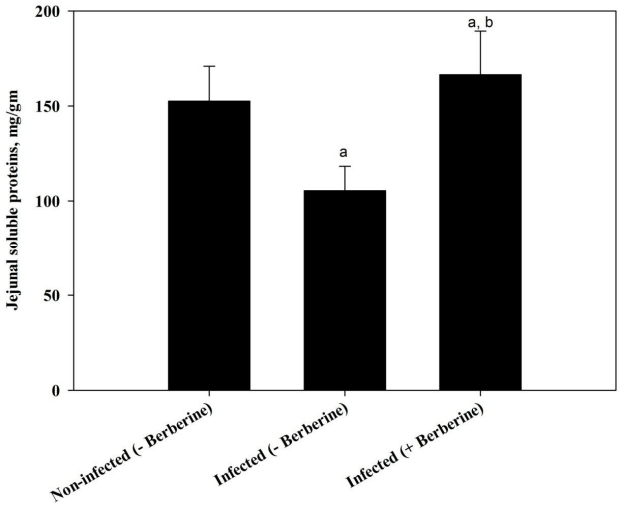

Fig 5. Changes in jejunal soluble proteins level in mice infected with *E. papillata* and after treatment with berberine. All values are means ± SD. a: Significant against non-infected control group at P≤0.05 n= 6.

cholesterol from 82.9±6.2 mg/dl to 75±4.7 and that of phospholipids from 7.2±0.3 to 4.7±0.23 mg/dl (Table I). In addition, both harmful fractions LDL cholesterol and VLDL cholesterol from 10.2 mg/dl and 12.8 mg/dl to 50.6 mg/dl and 15.6 mg/dl, respectively (Table I). Berberine showed an obvious lipid lowering activity. This can be seen from

diminishing the level of total lipids, total Cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol nearby the control value of non-infected animals. Also, HDL cholesterol and phospholipids were restored more than the control value (Table I).

Table I.- Berberine induced changes in plasma lipid fractions in mice infected with *E. papillata* on day 5 p.i.

Parameters (mg/d)	Non-infected - Berberine	Infected	
		- Berberine	+ Berberine
Total lipids	371±10.3	479.1±10.5 [a]	339.8±8.3 [a,b]
Total cholesterol	105.9±5.1	141.2±5.3 [a]	120±5 [a,b]
Triglycerides	64±8	78±4.6 [a]	44±4 [a,b]
HDL-cholesterol	82.9±7.5	75±4.8 [a]	100±5.9 [a,b]
LDL-cholesterol	10.2±0.75	50.6±3.75 [a]	11.2±0.65 [b]
VLDL-cholesterol	12.8±0.8	15.6±0.65 [a]	8.8±0.45 [a,b]
Phospholipids	7.2±0.48	4.7±0.55 [a]	7.9±0.6 [b]

All values are means ± SD. a: Significant against non-infected control group at P≤0.05, b: Significant against infected (- Berberine) group at P≤0.05 n =6

Table II.- Berberine induced changes in plasma metal ion concentrations in mice infected with *E. papillata* on day 5 p.i.

	Non-infected (-Berberine)	Infected (-Berberine)	Infected (+Berberine)
Ferrous (µg/ml)	39.9±1	30.7±0.9 [a]	35.1±1.1 [a,b]
Potassium (µg/ml)	166.5±6.5	219.8±8.8 [a]	142.9±5 [a,b]
Sodium (µg/ml)	975.3±50.5	1512.7±107.5 [a]	1033.3±16.5 [b]

All values are means ± SD. a: Significant against non-infected control group at P≤0.05, b: Significant against infected (- Berberine) group at P≤0.05 n =6

The infection caused a marked disturbance in the metal ion concentration within blood. Ferrous and Selenium levels were markedly decreased by 23 and 30% respectively. In addition, both potassium and sodium ion concentration was significantly increased from 166.5 and 975.3 µg/ml to 219.8 and 1512.7 µg/ml, respectively. Berberine could significantly increase Fe and Se levels to be 35.1

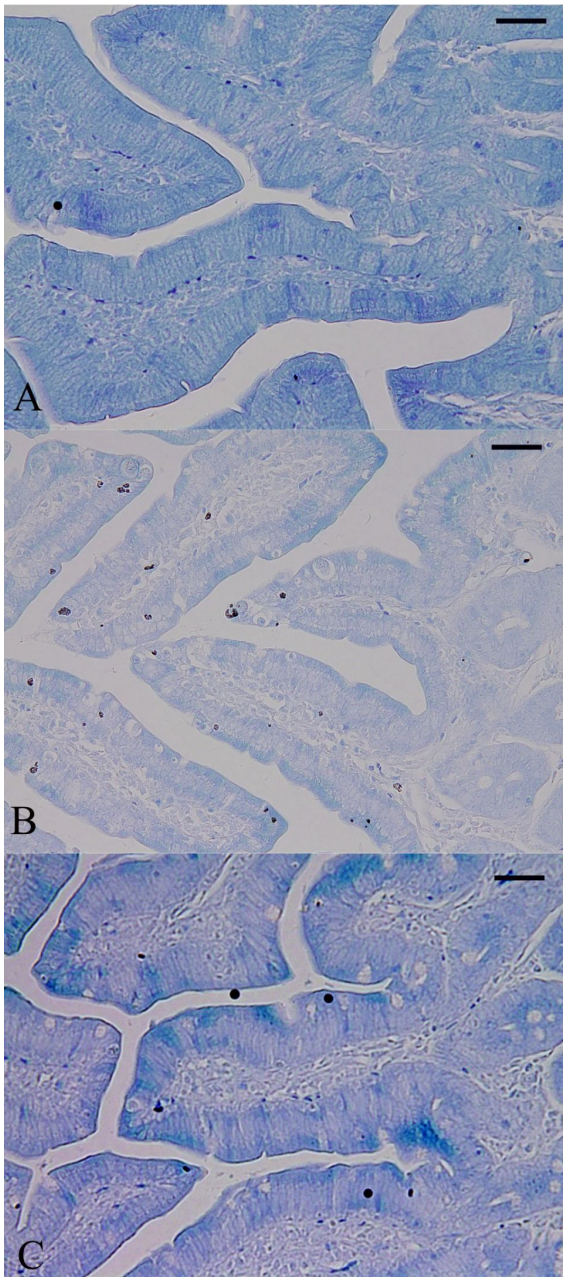

Fig. 6 Protein content in jejunum sections. A, Control non-infected mice jejunum. B, *E. papillata* infected mice jejunum. C, Berberine treated and infected mice jejunum. Jejunum tissue shows normal content in control non-infected group. Infected sections showed depletion in their protein content. After treatment with berberine, protein content showed improvement in their level. Sections were stained with mercuric bromophenol blue method. Scale bar = 50 µm.

and 1.9 µg/ml, respectively and decreased the increase in Na and K ion levels to 1033.3 and 142.9 µg/ml, respectively (Table II).

DISCUSSION

The current work was designed to explore the effect of berberine treatment on infected mice with intestinal *E. papillata* infection upon the homeostatic status of lipids, proteins, carbohydrates and metal ions.

E. papillata induced a hyperlipidemic status in infected mice as seen from the increased concentrations of plasma total lipids, total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol with concurrent decrease in HDL cholesterol and phospholipids. Upon treatment of infected mice with berberine solution, it was found that berberine has a strong hypolipidemic activity. It could significantly decrease total lipids, total cholesterol, riglyceride, LDL cholesterol and VLDL cholesterol, with mutual increase in HDL cholesterol and phospholipids.

The increased lipid fractions in blood of mice infected with *E. papillata* parasite may be due to the disturbed energy homeostatic status by such infection which leads to stress-induced secretion of pancreatic glucagon and adrenal glucocortecoides that in turn activates both gluconeogenesis processes leading to breakdown of liver carbohydrate store (Feritas *et al*., 2008; Patra *et al*., 2009; Mondal *et al*., 2011), followed by increased rate of fat mobilization and hydrolysis leading to increased level of plasma lipid fractions (Yvore and Minguy, 1972; Sharma and Fernando, 1973; Metwaly *et al*., 2013).

Berberine has a strong potential to modulate hyperlipidemic status as seen from lowering serum triglyceride, cholesterol and LDLc levels and increasing the HDLc level (Leng *et al*., 2004; Punitha *et al*., 2004). It exerts its action via different mechanisms. It can inhibit pancreatic lipase activity (Mohammad *et al*., 2012). Berberine also can acts via stabilization of hepatic LDLc receptor (LDLR) and increasing transcriptional activity of LDLR promoter and down regulation of many transcription factors related to lipogenesis processes (Kong *et al*., 2004; Ge *et al*., 2011). It also, down-regulate adipogenic enzymes and transcription factors (Choi *et al*., 2006). Centrally administered berberine can stimulate muscular and hepatic AMPK activity and fatty acid oxidation and thereby contributing to improve lipid metabolism and systemic insulin sensitivity (Kim *et al*., 2006) and inhibit cholesterol and triglyceride synthesis (Yin *et al*., 2008). Moreover, berberine downregulated the expression of genes involved in lipogenesis and upregulated those involved in energy expenditure in adipose tissue and muscle (Lee *et al*., 2006).

Intestinal *E. papillata* infection also induced a disturbance in both carbohydrate and protein status as revealed by the increased plasma glucose, total proteins and albumin levels with mutual decrease in jejunal carbohydrate and soluble protein contents. Berberine showed a hypoglycemic effect as it reduced blood glucose level and restored jejunal carbohydrate content. In spite of plasma total proteins and albumin were not increased significantly, but jejunal soluble protein content was significantly elevated.

It was found that host cell metabolism is the most affected process during the intestinal bimerian infections (Al-Quraishy *et al*., 2012; Lutz *et al*., 2011) and these parasites have a great capacity to manipulate host cells for their benefits via scavenging available nutrients and essential host cell molecules (Forst, 2006; Hermosilla *et al*., 2012).

Enhanced glycogenolysis and gluconeogenesis processes leads to breakdown of tissue carbohydrate store (Mondal *et al*., 2011; Metwaly *et al*., 2013) and hence increased blood glucose levels. Intestinal coccidial infections have been classified as protein loosing enteropathy (Kouwenhoven, 1971). Many studies proved that there is decreased amount of total proteins in the infected tissues and increased rate of protein escape into the intestinal lumen via the ruptured intestinal wall associated with reduced absorption of amino acids and decreased digestibility of protein (Bangoura and Daugscies, 2007).

Treatment with berberine was found to significantly decrease the catalytic processes of tissue protein and nucleic acid degradation (Punitha *et al*., 2004). Berberine was demonstrated to have a strong glucose lowering effects. It exerts its hypoglycemic action via either enhancing insulin

secretion and/or action (Leng *et al*., 2004) or throughout extrapancreatic mechanisms independent of insulin secretion via either activation of glycolysis process or glycogenesis and/ or inhibition of gluconeogenesis (Punitha *et al*., 2004). Also, it has a strong potential to restore glucokinase activity and inhibit lipogenesis resulting in activation of glycolysis. Berberine reduced glucose-6-phosphatase enzyme activity which probably resulted in restoration of tissue glycogen content (Lee *et al*., 2006). In addition, treatment with berberine enhanced insulin-mediated glycogen synthesis and restored insulin inhibition of triglyceride secretion (Lou *et al*., 2011) and suppressing disaccharidase activities (Liu *et al*., 2010).

Our data showed that *E. papillata* infection caused an increase in plasma levels of sodium and potassium, and a decrease in plasma selenium and ferrous ion concentrations. Again, berberine treatment reduced the increase in these metal ions and caused an increase in Se and Fe ion concentrations.

The main reason for the disturbance in blood metal ion concentration is the produced diarrhoea by such infection which accompanied by severe loss of water and electrolytes within faeces (Coudret *et al*., 1988) in addition to decreased absorbability of the damaged intestine by *E. Papillata*. Diarrhoea leads to disrupted plasma concentrations of metal ions and changes in the mineral composition of the gut (Cirak *et al*., 2004; Bangoura and Daugscies, 2007). Also, Ghanem and Abdel Raouf (2005) and Gilbert *et al*. (2011) reported that metal ions are altered in their absorption due to the suppressed appetite and intestinal mucosal destruction which leads to malabsorption and altered ion transporters.

Berberine was found to have a strong antidiarrheal activity as it could arrest diarrhea or reduce volume and frequency of diarrheal stool and their duration (Dutta *et al*., 1972; Khin *et al*., 1985). Its action occurs via depression of intestinal peristalsis and removal of inflammatory congestion of the mucosal surface of the intestine (Dutta *et al*., 1972; Khin *et al*., 1985). In addition, it is effective against the induced diarrhoea by several entero pathogenic bacteria as *Vibrio cholera, Shigella, Pseudomonas, Escherichia coli* and *Proteus*, parasites (Subbiah and Amin, 1967). It exerts its anti-secretory action via acting directly upon epithelial cells throughout blockage of potassium and calcium ion channels (Taylor *et al*., 1999; Wang *et al*., 2004). In addition, berberine is a non-selective inhibitor of transepithelial ion transport across distal colonic mucosae via inhibition of basolateral potassium channel opening which would result in attenuation of the chloride secretory response (Taylor and Baird, 1995).

Collectively, our data indicates that treatment of infected mice with berberine chloride could effectively ameliorate the induced pathological changes in lipid, protein, carbohydrate and metal ion homeostasis by *E. papillata* infection.

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