

The Impact of Vitamin B6 Supplementation on Experimental Colitis and Colonic Mucosal DNA Content in Female Rats Fed High Sucrose Diet

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Abstract: Inflammatory Bowel Disease (IBD) is a chronic replacing disorder of the gastrointestinal tract by inflammation and tissue damage. The present study aimed to evaluate the possible efficacy of vitamin B6, and its protective mechanisms on colitic female rat model fed on diet containing 30% sucrose. Rats were divided into 3 experimental groups as colitic, colitic and sucrose 30%, colitic and sucrose 30% and vitamin B6 as pyridoxal-5-phosphate (60mg/kg diet) beside a normal control group. Colitis was induced in experimental groups by intrarectal injection of 10mg 2,4,6 trinitrobenzen sulfonic acid (TNBS) dissolved in 50% ethanol. Colonic injury and inflammation were evaluated by a significant increase in colonic enzymatic activities of myeloperoxidase (MPO) and alkaline phosphatase (ALP) and colonic body weight ratio. On the other hand, colonic glutathione-S-transferase GST and glutathione peroxidase GPx activities as well as serum nitric oxide NO levels showed a significant increase demonstrating oxidative stress. Results showed that, addition of sucrose on the diet of colitic rats increased the colonic inflammation as represented by a marked increase in colonic enzymatic activities of myeloperoxidase (MPO) and alkaline phosphates (ALP) and colonic body weight ratio, whereas no significant change was observed in colon glutathione-S-transferase GST and glutathione peroxidase GPx activities in sucrose fed group when compared to colitic group. Results showed that, vitamin B6 treatment was proved to be efficient in mitigating the experimental colitis as it significantly reduced the colon/body weight ratio and myeloperoxidase (MPO) activity and restored colonic glutathione-S-transferase GST and glutathione peroxidase GPx activities and serum nitric oxide NO level. It is clear that TNBS is a potent DNA damaging agent and alters colon DNA content. Supplementation of sucrose in the diet of colitic rats induced additional DNA changes in the colon mucosal cells of rats, while vitamin B6 supplementation protects colon DNA against damage.

Key words: Vitamin B6 – Colitis- TNBS- DNA- Sucrose

INTRODUCTION

Cohn's disease and ulcerative colitis are major inflammatory bowel disease in human. The etiology of inflammatory bowel disease remains unknown. Colitis induced by various agents has been used as an experimental model to study the pathogenesis of inflammatory bowel disease (Rubio and Befrits, 2008). Colon inflammation is associated with diet and other lifestyle factors typical for the western countries, such as low intake of fruits and vegetables, sedentary life style, obesity and probably high intake of dietary energy, cooked meat and sugar (Russel *et al.*, 2007).

Inflammatory bowel disease (IBD) is a chronic replacing disorder of the gastrointestinal tract characterized by inflammation and tissue damage (Galligan, 2004). IBD may lead to serious gastrointestinal and extraintestinal complications, involving the hepatobiliary, cardiovascular and neural systems (Nicholls, 2002). One of the most widely used animal models of inflammatory bowel disease is trinitrobenzene sulfonic acid (TNBS)-induced colitis (Lee *et al.*, 2010). Chemically induced IBD models, using trinitrobenzene sulfonic acid TNBS is commonly used because of the immediate inflammation, the high reproducibility and the simplicity of the induction process. Since TNBS is a covalently reactive compound, its administration in mice results in acute necrosis of the wall of the distal colon due to oxidative damage, making it an ideal model to evaluate the effect of ROS in IBD (De Morino and Perdigon, 2010).

Vitamin B6 (pyridoxine) plays several roles in the etiology and pathogenesis of chronic inflammation and inflammatory diseases. It is water-soluble and preferentially absorbed in an acidic medium in the proximal small intestine via simple diffusion. The vitamin role in inflammation can be observed on a number of metabolic levels and in various pathologies (Zhang *et al.*, 2006).

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Vitamin B6 occurs in three forms: pyridoxal, pyridoxine, and pyridoxamine. It is part of coenzymes PLP (pyridoxal phosphate) and PMP (pyridoxamine phosphate) used in amino acid and fatty acid metabolism. It helps to convert tryptophan to niacin and to serotonin, and helps to make red blood cells. It also acts as a co-factor and antioxidant (Chen and Xiong, 2005). Recent studies are unveiling a new role of vitamin B6 as a chemopreventive agent. High levels of vitamin B6 have been reported to suppress growth of animal or human cancer cell in vitro (Valco *et al.*, 2006).

Vitamin B6 seems to be associated with some defense mechanisms especially against lipid peroxidation in tissues, this process occurs when animals are totally lacked in vitamin B6 in the diet (Bordoni *et al.*, 2006). Marginal vitamin B6 contents increased lipid peroxidation and considerably stimulated the activity of glutathione dependent enzymes. On the other hand, increased plasma and tissue lipid peroxidation has been reported in rats receiving a vitamin B6 deficient diet (Kayali and Tarhan, 2006).

Komatsu *et al.*, (2001) postulated that, colorectal cancer risk might be reduced by moderate levels of dietary vitamin B6 daily consumed by humans. Thus, it has been considered that, overdoses of vitamin B6 may have potential use in antineoplastic therapy. A positive association between colon disease and the intake of dietary sources has been found in some human studies although evidence is still insufficient (Lindecrona *et al.*, 2003).

Epidemiological studies showed significant correlations between colon inflammation and high sucrose intake. Recently, 16 of 18 studies indicated that, a high intake of sucrose is associated with increased risk of colon cancer (Giovannucci, 2001). In three weeks study in rats, sucrose increased the mutation frequency in colon, sugars has also been suggested to alter oxidative stress through glycoxidation processes. A high intake of simple sugars may also alter the colonic environment, leading to changes in pH and in the formation of fermentation products such as short chain fatty acids, which are an important energy source for the colonic epithelium (Zambell *et al.*, 2003).

Reactive oxygen species may mediate tissue injury in inflammatory bowel disease. The inflammatory response in the intestine is accompanied by significant oxidative stress both in inflammatory bowel disease and in experimental models of colitis due to the extensive release of highly reactive oxygen and nitrogen species. Oxidative stress may be one of the most important components in the pathophysiology of the inflammatory bowel disease, considering that; the use of antioxidant compounds may be useful in limiting the damage of colon disease (Raquall *et al.*, 2001). Inflammatory bowel disease are characterized by high levels of ROS that are produced by neutrophils and macrophages recruited in the inflamed tissue, as well as a decreased antioxidant capacity by plasma (Shimada *et al.*, 2005).

Evidences suggest that, reactive oxygen species (ROS), hypochlorous acid (HOCl) and oxidant derivatives are produced in excess by the inflamed mucosa and may be pathogenic in the inflammatory bowel disease. Hydroxyl and peroxides are produced compounds that cause impairment in the cell membrane stability, DNA damage and death of the cells by lipid peroxidation (D'Odorico *et al.*, 2001).

MATERIALS AND METHODS

Animals:

Thirty five female albino rats were used for the experiment. Weight matched (180-200g); female rats were kept on a constant temperature and humidity room, with a 12 hours light period. Rats were kept for one week before the onset of the experiment to acclimatize to the laboratory conditions. After the period of acclimatization, the rats were stratified by weight and assigned into four groups, according to the dietary treatment.

Group (1): rats served as control group, fed basal diet

Group (2): rats fed basal diet; colitis is induced as a single administration with TNBS

Group (3): rats fed basal diet, containing 30% sucrose; colitis is induced as a single administration with TNBS. (Sucrose is replaced with the corn starch, thereby keeping the concentration of carbohydrate and other nutrients constant).

Group (4): rats fed basal diet, containing 30% sucrose and supplemented with vitamin B6 as pyridoxal -5-phosphate (60 mg /kg diet) (Mahfouz and Kummerow, 2004). Colitis is induced as a single administration with TNBS.

Induction of Colitis:

Under these conditions, a single intra colonic dose of 10 mg of TNBS (2, 4, 6- trinitrobenzen sulphonic acid) dissolved in 0.25 ml of 50% ethanol (v:v) was administered. Induction was carried out by using rubber catheter inserted 8 cm into the anus. The catheter was left in place for 15 seconds prior to its removal to

prevent expulsion of injected solution. Four weeks after instillation, the rats in all groups were scarified, blood samples were collected and serum was separated by centrifugation at 3000 for 15 minutes r.p.m and colons were dissected.

Determination of Colon/ Body Weight Ratio:

The last 8 cm of the colon was removed, opened longitudinally and cleared of fecal materials with gentle spray of 0.9% saline and then blotted dry and weighted. The ratio of the 8 cm segment distal colon weight was calculated as an index of colonic tissue edema (Eric *et al.*, 2004). A small sample of distal colon was removed and kept in saline solution for DNA determination procedure.

The remaining distal colon specimen was homogenized in 3 ml 0.5% hexadecyltrimethyl ammonium bromide in 50mM phosphate buffer (pH 6) and kept in ice bath during homogenization to maintain maximum enzyme stability. The homogenate was then centrifuged at 4000 r.p.m for 15 minutes at 4°C; the resulting supernatant was used for the determination of enzymatic activities of myeloperoxidase (MPO), alkaline phosphatase (ALP) and glutathione-s-transferase (GST) and glutathione peroxidase (GPx).

Biochemical Analysis:

Alkaline phosphatase ALP activity was determined according to a method by Belfield and Goldberg (1971), glutathione-s-transferase (GST) activity was determined spectrophotometrically by the method of Habig *et al.* (1974), myeloperoxidase activity (MPO), an indicator of polymorphonuclear leukocyte (PMNs) accumulation was determined according to Krawisz *et al.* (1984). Glutathione peroxidase activity (GPx) was determined by the method of Paglia and Valentine (1967). Serum nitric oxide NO levels were measured as total nitrite according to method by Miranda *et al.* (2001). Total nitrite is an index of endogenous nitric oxide production.

Determination of Colon Mucosal DNA:

DNA was extracted from colon mucosal cells using QIAGEN tissue DNA kits for isolation and purification of total DNA from animal tissues. Cut up to 25mg tissue into small pieces to enable more efficient lysis and place in a 1.5 ml microcentrifuge tube, add 180 µl buffer ATL. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubates at 56° C until the tissues is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath or on rocking platform. Vortex for 15 s. Add 200 µl buffer AL to the sample, and mix thoroughly by vortexing. Then add 200µl ethanol (96-100%), and mix again thoroughly by vortexing. Distribute the mixture over two DNeasy Mini spin columns placed in 2 ml collection tubes. Centrifuge at 6000 x g for 1 minute. Add 500 µl Buffer AW1 onto the column, and centrifuge for 1 minute at 6000 x g. Discard flow-through collection tube. Place the DNeasy Mini spin columns in new 2 ml collection tubes.

Add 500 µl 80% EtOH onto the column, and centrifuge for 3 minutes at 20,000 x g to dry the DNeasy membrane. Discard flow-through and collection tube. Place the spin column in a clean 1.5 ml microcentrifuge tube, and pipet 50 µl of nuclease free water directly onto the column membrane. Incubate at room temperature for 1 minute, and then centrifuge samples for 1 minute at 6,000 x g to elute. Measure DNA concentration and purity, and analyze on an agarose gel.

Statistical Analysis:

Statistics was performed by personal computer using SPSS (Statistical Package of Social Science) version 10 for Windows. The P-values (probability value) between two groups were calculated by student "t" test, while ANOVA (Analysis of Variance) test was used to compare between more than two groups. The P-value was considered statistically significant when (p< 0.05).

Results:

TNBS Induced Chronic Colitis:

The rats that received a TNBS showed early signs of disease such as colonic hemorrhage (bloody feces) and diarrhea.

Table 1:Effect of vitamin B6 dietary supplementation on food Intake, final body weight and colon/body weight ratio

Group	G1Control	G2Colitis	G3Sucrose 30% + Colitis	G4Sucrose 30%+ Colitis + B6
Food Intake (g/week)	254±15	251±3	250±8	256±19
Final Body weight (g)	300±28	265±16	267±24	292±33
colon/body weight ratio	4.8±0.15	7.4±0.55	7.9±0.95	5.9±0.3

Values represent mean ± S.D.

Results showed that there was no significant difference in food intake between rats fed the control diet and the other groups. The average weekly dietary consumption in control rats was 254 g, and the corresponding value in the other groups ranged from 250 to 256 (Table 1).

There was a significant difference in the final average body weight in rats fed the control diet G1 when compared to the corresponding values of G2, G3 and G4. TNBS administration reduces the final average body weight, similarly the sucrose supplemented group G3 represents a significant difference compared to the control group G1, while vitamin B6 increase the value.

Colon Body Weight Ratio:

Results showed that TNBS produced damage to the rat colon. This was confirmed by a marked increase in the colon/body weight ratio compared to the control group. There was a significant increase in the colon/body weight ratio in colitic rats fed 30% sucrose containing diet, when compared to colitic rats G2. Meanwhile, the data in table (1) revealed that dietary supplementation by vitamin B6 decreased the colon/body weight ratio significantly compared to G2 and G3.

Table 2: Effect of vitamin B6 dietary supplementation on the biochemical parameters in rat colon tissues.

Group	G1 Control	G2 Colitis	G3 Sucrose 30% + Colitis	G4 Sucrose 30% + Colitis + B6	
MPO (U/g wet tissue)		3.61±0.3	13.6±0.72	14.9±0.32	7.76±0.51
ALP(U/g wet tissue)		1.36±0.17	2.84±0.27	3.21±0.13	3.0±0.2
GST(U/g wet tissue)		412.5±23.7	783±28.2	798±21.0	433±22
GPx(U/g wet tissue)		8.64±0.91	15.19±1.5	16.25±1.17	9.1±0.15
NO (nmol/ ml)		39.3±3.4	79±0.72	90.7±4.1	82.67±6.3

Values represent mean ± S.D.

Rats subjected to dietary vitamin B6 supplementation showed an overall lower impact of TNBS-induced colonic damage at the end of the experiment, when compared to TNBS group and sucrose group.

Biomarkers of Inflammation:

Colitis was indicated by a marked increase in colonic MPO and ALP activities compared to the control group. Addition of sucrose in diet increases the activities significantly when compared to colitic rats G2, on the other hand supplementation of diet with vitamin B6 resulted in a significant reduction in MPO activity when compared to either G2 or G3. Although ALP activity was not significantly altered in vitamin B6 supplemented group G4 when compared to G2 and G3, a slight reduction in its level was observed.

The Antioxidant Status of the Colon:

The oxidative status of the colon was evaluated by colon GST, GPx activities and serum NO level. Colitic group G2 was characterized by a significant increase in colonic GST and GPx activities. No significant difference observed in the GST and GPx values between colitic G2 and sucrose added group G3 rats (783±28.2 vs. 798±21.0) and (15.19±1.5 vs. 16.25±1.17) respectively. An observed normalization was detected in the vitamin B6 supplemented group G4 when compared to the G2 and G3. In addition, the nitric oxide level in TNBS group was significantly elevated compared to control group G1, vitamin B6 treatment resulted in a marked decrease in the elevated levels of NO in TNBS rats, while supplementation of diet with 30% sucrose increases it.

Discussion:

The TNBS-induced experimental colitis is probably one of the most widely used models of colonic inflammation. It is a convenient, reproducible and relatively inexpensive model which has acute phase followed by a prolonged period of progressive healing with some features of chronic inflammation (Galvez *et al.*, 2000). The technique described here differ slightly from the one originally described by Morris *et al.*,(1989) in that a lower dose of TNBS (10 mg in 50% ethanol) was used in attempt to make inflammation more amenable to the treatment. In fact, the colonic damage induced by 10 mg of TNBS was qualitatively similar to that observed with 30 mg of the compound but quantitatively lower with regard to the biochemical markers of inflammation such as MPO activity as reported previously (Sanchez *et al.*, 1996).Indeed, TNBS behaves as a contact sensitizer and so the severity of the inflammatory response is a function of the local concentration in the colonic lumen rather than of the dose: body weight ratio (Katsuyo *et al.*, 2008).

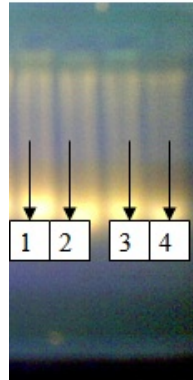


Fig 1: Agarose gel electrophoresis of DNA isolated from rat colon mucosal cells, one sample is run for each tissue, (Lan 1) G1 ctrol rats,(Lan 2) Vitamin B6 treated rats with TNBS-induced colitis (fed diets containing 30% sucrose and 60 mg/kg vitamin B6), (Lan 3) Untreated rats with TNBS-induced colitis, (Lan 4) 30% sucrose is added to the diet of colitic rats.

The animals of TNBS group represented an inflammatory response associated initially with loss of body weight and functionally with signs of diarrhea. The mechanism whereby TNBS induces colitis is still a matter of some debate. In any event, the current findings and those of other investigators (Trent *et al.*, 2005) demonstrate the utility of this model to study the initiation of changes in barrier function that may contribute to the pathogenesis of symptoms in intestinal inflammations. An increase in epithelial permeability would be expected to allow for the perpetuation of intestinal inflammation, by allowing the increased floating of microbial products and antigenic proteins into the inflamed mucosa. Likewise, it was observed that water absorption in the inflamed mucosa was markedly diminished. This effect would be expected to contribute to the diarrhea that occurs not only in this animal model but also in human intestinal inflammations (Beltran *et al.*, 2000).

TNBS-induced colitis resulted in severe ulcerative inflammation of the rat colon evidenced by the increase in colon/body weight ratio of colitic rats. Supplementation of diet with vitamin B6 resulted in a significant reduction of colon/body weight ratio indicating anti-inflammatory effect of vitamin B6 on the large bowel (Roselli *et al.*, 2009). The relation between vitamin B6 and inflammation was previously described in a study by Mousain, (2006) which indicate that vitamin B6 deficiency increased the degree of paw edema by 54% in a rat model of inflammation; it was therefore suggested that vitamin B6 deficiency might enhance inflammation.

The concurrent administration of vitamin B6 exhibited an intestinal anti-inflammatory effect, as evidenced by a significant improvement of all biochemical parameters of colonic inflammation assayed in comparison with both non-treated colitic rats and sucrose supplemented rats. Thus, both colonic myeloperoxidase (MPO) and alkaline phosphatase (ALP) activities were significantly reduced compared with untreated colitic rats. Colonic MPO activity was significantly increased as a consequence of inflammation, addition of 30% sucrose in diet increases the activities significantly when compared to colitic rats G2, and on the other hand supplementation of diet with vitamin B6 resulted in a significant reduction in MPO activity when compared to either G2 or G3. Thus, higher intake of vitamin B6 leads to higher blood levels of its active form, PLP, which is in turn associated with lower levels of inflammatory molecule (Matxain, 2006). Infiltration of neutrophils and other leukocytes is a prominent feature of TNBS experimental colitis and was demonstrated by the use of biochemical marker MPO. Myeloperoxidase (MPO) is a peroxidase enzyme most abundantly present in neutrophil granulocytes (a subtype of white blood cells). It is a lysosomal protein stored in azurophilic granules of the neutrophil. MPO has a heme pigment, which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus (Brennan *et al.*, 2003). It is considered to be a marker of tissues inflammation and has been used as a quantitative index of inflammation in several tissues, including the intestine, despite recognition that it is not specific only to neutrophils and can be released by cells such as eosinophiles, monocytes and to lesser extent, macrophages. The interaction of MPO with H₂O₂ and Cl provides a potent antimicrobial/cytotoxic system for polymorphonuclear leukocytes. MPO related cytotoxicity may be associated with the formation of toxic oxidant MPO intermediates (Hazen, 2003).

ALP activity was previously reported to be significantly correlated with colitis inflammations (Camuesco *et al.*, 2006 and Annemarie *et al.*, 2007). Oxidative stress as well as neutrophil infiltration resulted in increased activity of ALP during colonic inflammation, may due to both epithelial cells and cells of lamina propria, mainly leukocytes (Susana *et al.*, 2007). Results showed a pronounced increase in ALP activity in colitic rats when compared to control group, supplementation with sucrose increase the value, while supplementation with vitamin B6 causes a slight reduction in ALP level.

In this study, diets containing sucrose produced complex effects on the incidence of colon inflammations induced by a single dose of TNBS. Several animal studies have found a promoting effect of dietary sucrose on colon tumor developing (Hill and Caygill, 1999 and Kristiansen *et al.*, 1995). Although sucrose is hydrolyzed to the monosaccharides, fructose and glucose, shortly after ingestion, sucrose increased colon cell proliferation more than fructose and glucose in a feeding study of rats (Daly, 2003).

A study by Poulsen *et al.*, (2001) showed that dietary sucrose act as a co-initiators or promoters in the formation of colon tumours. Molck *et al.*, (2001) proved that sucrose increases the biomarkers of inflammation and act as inflammation promoter.

The oxidative status of the colon was calculated by the total glutathione-S-transferase and glutathione peroxidase. Both GST and GPx activities in colon were significantly increased following induction of colitis. This effect represents a major mechanism of protection against oxidative and chemical stress. As expected, TNBS induced colonic inflammation that associated with increased oxidative stress; this was in agreement with previous studies (Talero *et al.*, 2007 and Galvez *et al.*, 2000).

No significant difference observed in the GST and GPx values between colitic G2 and sucrose added group G3 rats. Vitamin B6 treatment effectively counteracted the oxidative stress, thus explaining the observed decrease in the GST and GPx levels and preventing the oxidative damage.

The first line of defense against oxygen radicals and hydrogen peroxide mediated injury are antioxidant enzymes, such a GPx and GST. They are the enzymes responsible for the destruction of peroxides and have a specific role in protecting tissue against oxidative damage (Hegazi *et al.*, 2005).

Glutathione-S-transferases (GST) is multifunctional enzymes, which play a key role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the more water soluble products, the glutathione conjugates are metabolized further to mercapturic acid and then excreted. These classes are comprised of both cytosolic and microsomal enzymes (Edalat *et al.*, 2004). This enzyme is found in cytoplasmic and mitochondrial fractions of cells. GPx acts on lipid hydroperoxides substrates that has released from membrane phospholipids by phospholipase. It can hydrolyse H₂O₂ at low concentration. The antioxidant enzymes catalyses the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids to water and the corresponding stable alcohol thus inhibiting the formation of free radicals and remove toxic peroxides from living cells. It plays an important role of protecting cells from potential damage by free radicals formed by peroxide decomposition (Kunikaowska and Jenner, 2003).

Addition of sucrose 30% caused a significant increase ($0 < 0.05$) in GST and GPx when compared to control group G1. It seems that no significant difference between G2 and G3, thus indicating that the increase in oxidative stress is directly related to TNBS rather than sucrose. Previous studies stated that sucrose did not induce oxidative stress in colon, liver or plasma (Dragsted *et al.*, 2002, Hansen *et al.*, 2004 and Moller *et al.*, 2003).

However, vitamin B6 normalized the values of GST and GPx and thus demonstrated the antioxidant potential of vitamin B6. These findings were in contrast with that obtained by Sathanandam, (2005). This hypothesis is also supported by the findings demonstrated by Seyithan, (2005). Pyridoxine seems to quench singlet oxygen at a rate comparable with that of vitamin C and E, two of the most highly efficient biological antioxidants (Stocker *et al.*, 2003). More recently, Kannan and Jain (2004) proposed that vitamin B6 might function as an antioxidant by scavenging oxygen radicals. They proved that, treatment with high concentrations of vitamin B6 significantly decreased the generations of oxygen radicals, lipid peroxidations and H₂O₂ induced changes in mitochondrial membrane permeability. Evidences suggest that phenolic compounds react faster with peroxy radicals rather than peroxy radicals can react with the lipids. Studies indicate that functional groups such as hydroxyl and amine can also scavenge oxygen radicals. Given the chemical structure of vitamin B6 as a phenolic compound with both hydroxyl and amine groups substituted in the pyridine ring, Hankenson, (2005) hypothesized that, alone, vitamin B6 can function as antioxidant. However, the exact chemical mechanism by which pyridoxine would scavenge oxygen radicals and inhibit lipid peroxidation has not yet been confirmed.

Ullegaddi *et al.*, (2004) reported that, B-vitamins supplementation may have antioxidant and anti-inflammatory effects. Thus, vitamin B6 seems to be involved in active oxygen resistance. Further, a study by Naoko *et al.*, (2006) reported that vitamin B6 deficient rats do not react effectively against oxidative stress and decreased antioxidant enzymes activity, and so it is suggested that vitamin B6 has antioxidant activity as a part from its role as coenzyme. Other observed effects of vitamin B6 deficiency include increased lipid peroxidation in plasma, liver, kidney and heart tissues (Cabrini *et al.*, 2005).

TNBS is a potent DNA damaging agent and carcinogenic that induces intestinal and colonic tumors in rodents. Membrane lipids are peroxidized to lipid peroxidases by various factors, including TNBS, and on further decomposition generate peroxy radicals (Mennigen *et al.*, 2009). It is clear that exposing cells and tissues to oxidant stress might tend to promote membrane DNA damage and decreased DNA repair capacity. The H₂O₂ produces a wide spectrum of DNA damage and strand breaks (Hu *et al.*, 2002).

As indicated from the results of table (2), vitamin B6 treatment resulted in a marked decrease in the elevated levels of NO in TNBS rats. NO is a highly reactive free radical. There is conflicting evidence on the concentrations of NO and its role in carcinogenesis. Studies have shown that NO can both promote and inhibit tumor progression and metastasis. In general, NO and its derivatives induce oxidative and nitrosative stress that causes DNA damage and inhibits activity of DNA repair enzymes (62).

Furthermore, this study found that the supplementation of sucrose in diet induced DNA changes in the colon mucosal cells of rats. A study by Peters and Teel, (2003) improved that, dietary sucrose act as a co-initiator or promoter in the formation of colon tumors. The neoplastic transformation is associated with the production of radicals, which necessitated the study on the levels of lipid peroxidation in tumor tissue as well as the activities of antioxidant enzymes like GST and GPx. The genotoxic and mutagenic effect of sucrose in rat colon was previously studied by Hansen *et al.*, (2005) and indicated that the genotoxicity of sucrose may not be related to oxidative DNA damage or altered DNA repair, but increased oxidative damage may still take place in other macromolecules leading to indirect effect on DNA. Interestingly, Dragsted *et al.*, (2002) concluded that a sucrose rich diet directly or indirectly increase the mutation frequency in rat colon in dose dependent manner and decreases the level of back grounded DNA. Moreover, Lindecrona *et al.*, (2003) investigated that, sucrose increased the level of DNA damage in colon indicated a possible procarcinogenic effect of sucrose.

Conclusion:

Vitamin B6 is believed to be protective against the DNA damage that can lead to cancers and is vital in DNA synthesis and repair. It converted the vitamin folate to a form that can produce thymine, a component of DNA. If vitamin B6 deficiency presents, it cannot make enough thymine and it tries to make do by substituting uracil. Uracil is not a normal component of DNA, and the normal DNA repair mechanisms of the cell become stressed. This inefficiency in the normal repair mechanisms leads to breaks in DNA strands and instability of chromosomes - possible first steps in the development of cancerous cells (Marchand *et al.*, 2002). A deficiency of vitamin B6 causes damaging of colonic DNA by causing single and double strand breaks, oxidative lesions or both (Bruce, 2001). Because of its role in the maintenance of intracellular normal DNA synthesis and methylation, vitamin B6 has been hypothesized to be associated with reduced risk of colorectal cancer (Frigola *et al.*, 2005). In conclusion: dietary vitamin B6 supplementation protects the rat colon from experimental colitis through a mechanism that involves a decrease in oxidative stress associated with inflammation. Furthermore, addition of sucrose on the diet of colitic rats increases the colonic inflammation, while no significant change was observed in oxidative enzymes activities when compared to colitic group, indicating that the increase in oxidative stress is directly related to TNBS rather than sucrose. Supplementation of sucrose in the diet of colitic rats induced DNA changes in the colon mucosal cells of rats, while vitamin B6 supplementation protects colon DNA against damage.

REFERENCES

- Annemarie, T., P. Klaas, J. Alie, B. Lisette, R. Willem, P. Markwin, G. Velders, 2007. Role of alkaline phosphatase in colitis in man and rats. *Gut*. doi., 1110-1136.
- Belfield, A. and D.M. Goldberg, 1971. Colourimetric determination of alkaline phosphatase activity, *Enzyme*, 12: 561-568.
- Beltran, B., A. Orsi, G.B. Clementi and S. Moncada, 2000. Oxidative stress and S-nitrosylation of protein in cells. *Br. J. Pharmacol.*, 129: 953-960.

- Bordoni, A., L. Cabrini, M. Marchetti, F. Danesi, D. Bocchicchio, P. Biagi and M. Maranesi, 2006. Vitamin B6 deficiency and dietary fats: Effects on lipid consumption and glutathione peroxidase activity in rat liver. *Am. Nutr. Metab.*, 50: 305-312.
- Brennan, M.L., M.S. Penn, F. Van Lente, V. Nambi, M.H. Shishehbor, R.J. Aviles, M. Goormastic, E.S. Pepoy M.L., E.S. McErlean, E.J. Topol, S.E. Nissen, 2003. Prognostic value of myeloperoxidase in patients with chest pain. *N. Engl. J. Med.*, 17: 1595-604.
- Bruce, N.A., 2001. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat. Res.*, 475: 7-20.
- Cabrini, L., D. Bochicchio, A. Bordoni, S. Sassi, M. Marchitti and M. Maranesi, 2005. Correlation between dietary unsaturated fatty acids and plasma homocysteine concentration in vitamin B6 deficient rats. *Nutr. Metab. Cardio. Dis.*, 15: 94-99.
- Camuesco, D., M. Comalada, A. Concha, A. Nieto, S. Sierra, J. Xaus, A. Zarzuelo and J. Gálvez, 2006. Intestinal anti-inflammatory activity of combined quercitrin and dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, in rats with DSS-induced colitis. *Clin. Nutr.*, 25(3): 466-76.
- Chen, H. and L. Xiong, 2005. Pyridoxine is required for post-embryonic root development and tolerance to osmotic and oxidant stresses. *Plant J.*, 44: 396-408.
- Daly, M., 2003. Sugars, insulin sensitivity, and the postprandial state. *Am. J.Clin. Nutr.*, 78: 865S-872S.
- De Moreno de LeBlanc, A. and G. Perdigon, 2010. The application of probiotic fermented milks in cancer and intestinal inflammation. *Proc. Nutr. Soc.*, 69: 421-428.
- D'Odorico, A., S. Bortolan, R. Caedin, D. Martin, A. Ferronato and G. Sturniolo, 2001. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in inflammatory bowel disease. *Scand. J. Gastroenterol.*, 36: 1289-1294.
- Dragsted, L.O., U.V. Daneshvar, H. Autrup and L. Wallin, 2002. A sucrose rich diet induces mutations in the rat colon. *Cancer Res.*, 62: 4339-4345.
- Edalat, M., B. Mannervik and L.G. Axelsson, 2004. Selective expression of detoxifying glutathione transferases in mouse colon: effect of experimental colitis and the presence of bacteria. *Histochem. Cell. Biol.*, 122: 151-159
- Eric, C., Francine Pe'toux, C. Maria and L. Annik, 2004. Beneficial effect of trimebutine and N-monodesmethyl trimebutine on trinitrobenzene sulfonic acid-induced colitis in rats. *Life Sciences*, 76: 319-329.
- Frigola, J., X. Sole and M.F. Paz, 2005. Differential DNA hypermethylation and hypomethylation signatures in colorectal cancer. *Hum. Mol. Genet.*, 14: 319-326.
- Galligan, J.J., 2004. 5-hydroxytryptamine, ulcerative colitis, and irritable bowel syndrome: molecular connections. *Gastroenterology*, 126: 1897-1899.
- Galvez, J., M. Garrido, M. Merlos, M. Torres and A. Zarzuelo, 2000. Intestinal anti-inflammatory activity of UR-12746, a novel 5-ASA conjugate, on acute and chronic experimental colitis in the rat. *Br. J. Pharmacol.*, 130: 1949-1959.
- Giovannucci, E., 2001. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J. Nutr.*, 131: 3109S-3120S.
- Habig, W., M.J. Pabst and W.J. Jakoby, 1974. Glutathione -S-transferases. The first step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130-7139.
- Hankenson, J.M., 2005. Plasma vitamin B6 and the risk of colorectal cancer in Adenoma in women. *J. Nutr. Cancer Instis.*, 97: 684-692.
- Hansen, M., M.T. Hald, H. Autrup, U. Vogel, J. Bornhold, P. Moller, A.M. Molck, R. Lindecrona, H.E. Poulsen, H. Wallin and S. Loft, 2004. Sucrose and IQ induced mutations in rat colon by independent mechanism. *Mutat. Res.*, 554: 279-286.
- Hazen, S.L., 2003. Prognostic value of myeloperoxidase in patients with chest pain. *N, Engl, J, Med.*, 349: 1595-604.
- Hegazi, R.A., K.N. Rao, A. Mayle, A.R. Sepulveda, L.E. Otterbein and S.E. Plevy, 2005. Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway. *J. Exp. Med.*, 202: 1703-1713.
- Hill, M.J. and P.J. Caygill, 1999. Sugar intake and the risk of colorectal cancer. *Eur. J.Cancer Prev.*, 6: 422-434.
- Hu, Y., J. Martin, R.L. Leu and G.P. Young, 2002. The colonic response to genotoxic carcinogenesis in the rat: regulation by dietary fiber. *Carcinogenesis*, 23: 1131-1137.

- Kannan, K. and S.K. Jain, 2004. Effect of vitamin B6 on oxygen radicals, mitochondria membrane potential and lipid peroxidation in H₂O₂ treated U937 Monocytes. *Free Radical Biology and Medicine.*, 36: 423-428.
- Katsuyo, O., S. Yasushi, K. Mitsuhsa and K. Yoichi, 2008. Abolishment of TNBS-induced visceral hypersensitivity in mast cell deficient rats. *Life Sciences*, 82: 419-423.
- Kayali, H.A. and L. Tarhan, 2006. The impact of vitamin C, B1 and B6 supplementation on antioxidant enzyme activities, membrane total sialic acid and lipid peroxidation levels in *Fusarium* species. *Process. Biochem.*, 41: 1608-1613.
- Komatsu, S., H. Watanabe, T. Oka, H. Tsuge, H. Nii and N. Kato, 2001. Vitamin B6 supplemented diets suppress Azoxymethane-induced colon tumourigenesis in mice by reducing cell proliferation. *J. Nutr.*, 131: 2204-2207.
- Krawisz, J.E., P. Sharon and W.F. Stenson, 1984. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: assessment of inflammation in the rat and hamster model. *Gastroenterology*, 87: 1344-1350.
- Kristiansen, E., O. Meyer and I. Thorup, 1995. Influence of different diets on development of DMH-induced aberrant crypt foci and colon tumor incidence in Wistar rats. *Nutr. Cancer*, 23: 151-159.
- Kunikaowska, G. and P. Jenner, 2003. Alterations in mRNA expression for Cu, Zn Superoxide dismutase and glutathione peroxidase in the basal ganglia of MPTP –treated marmosets and patients with Parkinson's disease. *Brain Res.*, 968: 206-218.
- Lee, I.A., E.A. Bae, J.H. Lee, H. Lee, Y.T. Ahn, C.S. Huh, D.H. Kim, 2010. Bifido bacterium longum HY8004 attenuates TNBS-induced colitis by inhibiting lipid peroxidation in mice. *Inflammation Research*, 59: 359-368.
- Lindecrona, R.H., L.O. Dragsted, P. Morten, V. Ulla, A. Herman and A.M. Molck, 2003. Effect of sucrose and cornstarch on 2-amino-3-methylimidazo 4, 5-f quinoline (IQ) - induced colon and liver carcinogenesis in F344 rats. *Cancer letters*, 209: 17-24.
- Mahfouz, M.M. and F.A. Kummerow, 2004. Vitamin C or vitamin B6 supplementation prevents the oxidative stress and decrease of prostacyclin generation in homocysteinemic rats. *Intr. J. Bioche. Cell Biol.*, 36: 1919-1932.
- Marchand, L., T. Donlon and J.H. Hankin, 2002. B-vitamin intake, metabolic, genes, and colorectal cancer risk (United States). *Cancer Causes Control.*, 13: 239-248.
- Matxain, J.M., 2006. Theoretical study of the antioxidant properties of pyridoxine. *J. Phys. Chem. A.*, 110: 13069:13072.
- Mennigen, R., K. Nolte, E. Rijcken, M. Utech, B. Loeffler, N. Senninger and M. Bruewer, 2009. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am. J. of Physiol. Gastrointestinal and Liver Physiology*, 296: G1140-G1149.
- Miranda, M., M.G. Espey and D.A. Wink, 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5: 62-71.
- Molck, A.M., O. Meyer and E. Kristiansen, 2001. IQ (2-amino-3-methyl- imidazo 4, 5-f quinolone) - induced aberrant crypt foci and colorectal tumour development in rats fed two different carbohydrate diets. *Eur. J. Cancer. Prev.*, 10: 501-506.
- Moller, P., M. Hansen, H. Autrup, L. Risom, U. Vogel, H. Wallin, L. Dragsted and S. Loft, 2003. Dietary low dose sucrose modulation of IQ-induced genotoxicity in the colon and liver of big blue rats. *Mutat. Res.*, 527: 91-97.
- Morris, G.P., P.L. Beck, W. Herridge, W. Depew, M.R. Szew-Czuk and J.L. Wallace, 1989. Hapten induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology*, 96: 795-803.
- Mousain, B.M., 2006. Improvement of neurobehavioral disorders in children supplemented with magnesium-vitamin B6. I. Attention deficit hyperactivity disorders. *Magnesium Research*, 19: 46-52.
- Naoko, E., N. Kazuo, O. Akira, K. Hiroaki, T. Masaki and O. Tatsuzo, 2006. Antioxidant activity of vitamin B6 delays homocysteine-induced atherosclerosis in rats. *British J. Nutr.*, 95: 1088-1093.
- Nicholls, R.J., 2002. Review article: ulcerative colitis—surgical indications and treatment. *Aliment. Pharmacol. Ther.*, 16: 25-28.
- Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J.Lab.Clin. Med.*, 70: 158-169.
- Peters, L.P. and R.W. Teel, 2003. Effect of high sucrose diet on cytochrome P450 1A and heterocyclic amine mutagenesis, *Anticancer Res.*, 23: 399-403.

Poulsen, M., M. Molck, V.B. Thorup and O. Meyer, 2001. The influence of simple sugars and starch given during pre or post – initiation on aberrant crypt foci in rat colon, *cancer lett.*, 167: 135-143.

Raquall, G., S. Fermin, G. Julio, E. Maria, D. Juan and Z. Antonio, 2001. Dietary vitamin E supplementation protects the rat large intestine from intestinal inflammation. *Int. J. Vitam. Nutr. Res.*, 71: 243-250.

Roselli, M., A. Finamore, S. Nuccitelli, P. Carnevali, P. Brigidi, B. Vitali, F. Nobili, R. Rami, I. Garaguso and E. Mengheri, 2009. Prevention of TNBS-induced colitis by different *Lactobacillus* and *Bifido* bacterium strains is associated with an expansion of $\gamma\delta$ T and regulatory T cells of intestinal intraepithelial lymphocytes. *Inflammatory Bowel Disease*, 15: 1526-1536.

Rubio, C.A. and R. Befrits, 2008. Colorectal cancer in Crohn's disease –review of a 56-year experience in Karolinska Institute University Hospital. *J. Environ. Pathol. Toxicol. Oncol.*, 27: 257-266.

Russel, W.R., A. Labat, L. Scobbie and S.H. Duncan, 2007. Availability of blueberry phenolics for microbial metabolism in the colon and the potential inflammatory implications. *Mol. Nutr. Food Res.*, 51: 726-731.

Sanchez de Madina, F., J. Galvez, J.A. Romero and A. Zarzuelo, 1996. Effect of quercitrin on acute and chronic experimental colitis in the rat. *J. Pharmacol. Exp. Ther.*, 278: 771-779.

Sathanandam, S., 2005. Protective effect of vitamin B6 in chromium-induced oxidative stress in liver. *J. Appl. Toxicol.*, 25: 440-443.

Scott, D.H., M.A. Hull, E.J. Cartwright, W.K. Lam, A. Tisbury, R. Poulson and A.F. Markham, 2001. Lack of inducible nitric synthase promotes intestinal tumorigenesis in the *Apc* (Min/+) mouse. *Gastroenterology*, 121: 889-899.

Seyithan, T., 2005. Oxidant/antioxidant status in liver tissue of vitamin B6 deficient rats. *Clin Nutr.*, 24: 385-9.

Shimada, D., A. Fukuda, H. Kawaguchi, N. Kato, H. Yoshida and O. Tatsuzo, 2005. Effect of high dose of pyridoxine on mammary tumorigenesis. *Nutrition and Cancer*, 53: 202-207.

Stocker, P., J.F. Lesgards, N. Vidal, F. Chalier and M. Prost, 2003. ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochem. Biophys. Acta.*, 1621: 1-8.

Susana, S., V. Isabel, M. Antonio, S. Marina, A. Catalina, 2007. PARP inhibition reduces acute colonic inflammation in rats. *European Journal of Pharmacology*, 563: 216-223.

Talero, E., S. Sánchez-Fidalgo, J. Calvo, V. Motilva, 2007. Chronic administration of galanin attenuates the TNBS-induced colitis in rats. *Regulatory Peptides*, 141: 96-104.

Trent, M.W., V. Thiruma, I. Arumugam, M.L. Shiels, P.A. Newman, R.C. Ross, D.P. Reid, S.M. Fairlie and T. Taylor, 2005. A potent and selective inhibitor of group IIa secretory phospholipase A2 protects rats from TNBS-induced colitis. *International Immunopharmacology*, 5: 883-892.

Ullegaddi, R., H.J. Powers and S.E. Gariballa, 2004. B-group vitamin supplementation mitigates oxidative damage after acute ischemic stroke. *Clin. Sci.*, 107: 477-484.

Valco, M., C.J. Rhodes, J. Moncol and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress induced cancer. *Chem Biol. Interaction.*, 160: 1-40.

Zambell, K., M.D. Fitch and S.E. Fleming, 2003. Acetate and butyrate are the major substrates for de novo lipogenesis in rat colonic epithelial cells. *J. Nutr.*, 133: 3509-3525.

Zhang, S.M., C.M. Storen, L. Jennifer, R.C. Nancy, E.M. Joann and E.B. Julie, 2006. Folate, vitamin B6 multivitamin supplements and colorectal cancer risk in women. *Am. J. Epidemiol.*, 163: 108-115.