CHAPTER III

Impact of monocrotophos (MCP) on small intestine of experimentally induced diabetic rats

PREFACE

Evidence obtained in the previous chapters (Chapter I and II) clearly demonstrated that monocrotophos (MCP) possesses the potential to induce biochemical and structural alterations in small intestine of rats after repeated oral doses. The incidence of diabetes is on the rise globally and more so in India. This demographic data necessitates the generation of evidence on the possible impact of pesticide residues in general and OPI in particular on small intestine under diabetic conditions. Hence, the hypothesis that MCP exposure may cause enhanced responsiveness of small intestine in experimentally-induced diabetic rats has been examined in this study. The main objective was to obtain evidence on the potential of MCP (repeated oral doses) to augment the intestinal dysfunctions in STZ-diabetic rat model.
1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder of multiple etiology characterized by hyperglycemia with interruptions in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. In addition to the major complications of diabetes like, cardiovascular disease, nephropathy, neuropathy, retinopathy and diabetic cataract, gastrointestinal (GI) disorders are common in diabetic patients (Folwaczny et al., 1999).

In diabetics, the entire GI tract from the oesophagus to the anorectal region is reported to be affected with common complaints including dysphasia, early satiety, reflux, constipation, abdominal pain, nausea, vomiting and diarrhea (Feldman and Schiller, 1983). When compared to esophageal or gastric dysfunctions, the small intestinal dysfunctions are more common in diabetes. A decrease in the intestinal tone has been reported in diabetic patients, which is due to an increase in cholinergic activities and decrease in adrenergic receptor activation (Anjaneyulu and Ramarao, 2002). The result is seen as rapid transit of the small intestine and is well recognized in animal studies. Further, structural and functional changes observed in the small intestine of diabetic patients are responsible for the impairments of motility, altered transit time, and compromised secretory and absorptive functions (De Freitas et al., 2008).

Structural/functional alterations in the GI tract of diabetic patients are often accompanied by increase in absorption of intestinal glucose and activities of brush-border disaccharidases (Liu et al., 2011). Small intestine of diabetics also exhibit copious changes, including hyperplasia and hypertrophy of epithelial cells (Zoubi et al., 1995), enhanced absorption of sugars and amino acids (Fedorak, 1990) and increased endogenous cholesterol synthesis (Feingold et al., 1982). Inflammatory cytokines are also reported to be increased in diabetes (Esposito et al., 2002). A number of unusual conditions have been described in different sections of the GI tract in patients with diabetic autonomic neuropathy.
(DAN) such as oesophagus (dysmotility), stomach (dysmotility, delayed emptying) and small and large intestine (dysmotility, bacterial overgrowth, delayed transit, and diarrhoea) (Horowitz et al., 2004). Several studies have demonstrated prominent morphological changes of the small intestine and oesophagus in DM (Zoubi et al., 1995; Horowitz et al., 2004).

Epidemiological and experimental studies provide sufficient evidence regarding the role of pesticides in inducing and/or aggravating the pathophysiology of DM. Hyperglycemia is widely reported as one of the adverse effects in poisoning by OPI in humans and animals (Abdollahi et al., 2003; Hagar et al., 2002; Seifert, 2001; Shoba and Prakash, 2000) and several mechanisms, including altered gluconeogenesis, are believed to mediate OPI-induced hyperglycemia (Rahimi and Abdollahi, 2007). Studies from our laboratory have established that OPI possess the propensity to disrupt glucose homeostasis in rats (Joshi and Rajini, 2012). Further, studies also showed that MCP possesses the potential to intensify the pre-existing disrupted glucose homeostasis among diabetic rats (Begum and Rajini, 2011a). Although MCP per se caused only a marginal increase in blood glucose levels, significant elevation in glucose levels was evident among diabetic rats and was accompanied by disrupted glucose homeostasis.

Data obtained in previous chapters indicated that single oral of MCP did not cause any change in the activity of intestinal disaccharidases (Chapter I) whereas, repeated doses of MCP enhanced the activities all brush border enzymes in all the regions of small intestine and caused altered redox status (Chapter II). In addition, histological changes and ultra-structural alterations in villi were noticed in intestinal regions. Hence, based on these evidence, the following study aimed to examine the impact of repeated oral doses (15 and 30d) of MCP in experimentally induced diabetic rats in terms of effect on brush border enzymes, oxidative stress parameters and histology of small intestine.
2. MATERIALS AND METHODS

2.1 Chemicals

Adenosine triphosphate (ATP), bovine serum albumin (BSA), ouabain, paraoxon (O, O-dimethyl-O-p-nitrophenylphosphate), thiobarbituric acid (TBA), quercetin, N,N,N',N'-tetramethylethylenediamine (TEMED), ethylenediaminetetraacetic acid (EDTA), O-dianisidine dihydrochloride, were purchased from Sigma Chemicals Co., (St Louis, MO, USA). Maltose, sucrose, lactose, trehalose, p-nitro phenyl phosphate (PNPP), potassium chloride, magnesium chloride, tris, ammonium molybdate, histidine, ascorbic acid, trichloroacetic acid (TCA), calcium chloride, hydrogen peroxide (H₂O₂), reduced glutathione (GSH), quercetin, 1-chloro-2,4-dinitrobenzene (CDNB), acetylthiocholine iodide (ATCI) and dinitrobenzoic acid (DTNB) were procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Glucose estimation kit was procured from Span Diagnostics Limited (Mumbai, India). Technical grade Monocrotophos (76%) was a gift from Hyderabad Chemicals Limited, Hyderabad, India. All other reagents used were of analytical grade.

2.2 Pesticide solution

Monocrotophos (MCP) was dissolved in distilled water and was administered orally to rats at a volume of 1.0 ml/kg b.w. to achieve the doses described in the experimental section.

2.3 Animal care

Adult male rats (CFT – Wistar strain, 8 weeks old, 180 ± 10 g) used for the study were obtained from the Central Food Technological Research Institute animal house. They were housed in polypropylene cages (two per cage) at room temperature (25 ± 2°C) with relative humidity of 50–60% and on a 12 h light–darkness cycle. They had free access to food and water ad libitum. The rats were acclimatized to the commercial diet (Saidurga Feeds and Food, Bangalore, India) for seven days prior to the start of the experiment. All procedures with animals handling conducted strictly in accordance with guidelines approved by
the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept to a minimum.

3.0 EXPERIMENTAL DESIGN

3.1 Impact of multiple doses of MCP (15d and 30d) on small intestinal structure and enzymes in experimentally-induced diabetic rats

Rats were grouped by randomized design into four groups (n=12) as follows:

Group I: Control (Distilled water)

Group II: MCP - 0.9 mg/kg b.w/d (1/20 LD$_{50}$)

Group III: STZ - 60 mg/kg b.w

Group IV: STZ - 60 mg/kg b.w + MCP - 0.9 mg/kg b.w/d

Rats of Group III and IV were administered a single dose of streptozotocin (STZ, 60 mg/kg b.w i.p) freshly prepared in 100mM citrate buffer of pH 4.5 (Singh et al., 2005). Since STZ was capable of producing hypoglycemia as a result of massive pancreatic release of insulin, STZ treated rats were given 5% glucose water for 24h. Blood glucose was monitored in rats (48h after STZ administration) by Accu-check Sensor Glucometer to confirm the induction of diabetes and those rats showing the blood glucose levels above 250 mg/dl were selected as ‘diabetic rats’. Rats of group II and IV were administered MCP at 0.9 mg/kg b.w/d for 15d, 48h after administration of STZ. One set of rats (n=6, from each group) were sacrificed after 15d while the other set of rats were sacrificed after 30d.

One set of rats (n=6, from each group) were sacrificed after the treatment schedule. Blood was collected by cardiac puncture into tubes; serum was separated and used for blood glucose estimation. Entire small intestine was
excised and flushed with ice cold saline. Length and weight of small intestine was measured and cut into duodenum, jejunum and ileum. Another set of rats (n=6, from each group) were sacrificed after the treatment schedule and used for histopathology and SEM.

3.2 Tissue processing

After sacrificing rats, small intestine was excised, flushed with chilled 0.9% (w/v) NaCl solution. The length and weight was recorded. Duodenum, jejunum and ileum were separated and processed as described earlier (Chapter I, Section 3.3).

3.3 Preparation of homogenates

Mucosal scrapings from different regions of intestine i.e. duodenum, jejunum and ileum was processed for assaying the activities of disaccharidase, dipeptidase and alkaline phosphatase, Na⁺, K⁺-ATPase, AChE, PON, SOD, CAT and GST and for measuring the extent of lipid peroxidation (LPO) and GSH content as described earlier (Chapter I, Section 3.3).

4.0 ASSAY METHODS

4.1 Body weight and unit weight of intestine

Body weight of rats was monitored at regular intervals during the treatment period and also weight and length of the small intestine was recorded after sacrificing rats.

4.2 Blood glucose

Blood glucose level was quantified employing the commercial kit (Span Diagnostics Ltd., Mumbai) which is based on the GOD–POD method and the results were expressed as mg glucose/dl.
4.3 Brush border enzymes

**Disaccharidases – (sucrase, maltase, lactase and trehalase)**

Disaccharidases activity was measured as described previously (Chapter I, Section 4.1) and results were expressed as nmol glucose released/min/mg protein.

**Alkaline phosphatase**

Alkaline phosphatase estimation was done as described previously (Chapter I, Section 4.3) and results were expressed as nmol p-nitrophenol (PNP) formed/min/mg protein.

**Na\(^+\), K\(^+\) -ATPase**

Na\(^+\), K\(^+\) - ATPase activity was measured as described earlier (Chapter I, Section 4.4) and activity was expressed as nmol of Pi released/min/mg protein using Pi standard graph.

**Acetylcholinesterase**

Acetylcholinesterase activity was measured as described previously (Chapter I, Section 4.6) using acetylthiocholine iodide as the substrate. The amount of enzyme producing a change of 0.001 units of absorbance per minute was considered as one unit of enzyme and the results were expressed as units/mg protein.

**Paraoxonase 2 (PON 2)**

Paraoxonase activity in the intestinal homogenate was assayed as described earlier (Chapter I, Section 4.7) Rate of hydrolysis of paraoxon was calculated and the results were expressed as nmoles of paraoxon hydrolysed per min per ml using p-nitrophenol standard graph.
4.4 Redox state markers

Lipid peroxidation products, Reduced glutathione levels, CAT, SOD and GST activities were measured as described earlier (Chapter I, Section 4.8).

4.5 Histopathology

Intestinal regions from all rats of all the groups were processed for histopathology as described earlier (Chapter II, Section 4.12). The sections were examined under light microscope at 100x and 400x magnifications.

4.6 Scanning electron microscopy (SEM) (Cassidy et al., 1981)

Intestinal regions from all rats of all the groups were processed for SEM as described earlier (Chapter II, Section 4.13). The ultrastructure of the intestine was examined with particular reference to villi morphology.

4.7 Protein Estimation (Bradford, 1976)

Protein content in intestine homogenate/ brush border membrane was estimated using Bovine Serum Albumin (BSA) as standard.

4.8 Statistical analysis

Mean and standard error (SE) were determined for all parameters and the results were expressed as mean ± SE. The data were analyzed employing analysis of variance (ANOVA) followed by Tukey's HSD test for comparison of means to determine the significance of differences among the groups. P values below 0.05 were considered as significantly different.
5. RESULTS

5.1 Impact of multiple doses of MCP (15d and 30d) on small intestinal structure and enzymes in experimentally-induced diabetic rats

5.1.1 Body weight and intestine weight

A significant decrease (30-40%) in body weight was evident among rats of both STZ and STZ + MCP groups after 15d and the body weights were further decreased (50-55%) after 30d. MCP treated rats showed a marginal decrease in body weight, while their relative and unit weight of intestine were comparable to that of control rats. The relative weight of the intestine was significantly increased (2-fold) in both STZ and STZ + MCP groups (15 and 30d). Marked increase in unit weight of the small intestine was evident in STZ treated (24-37%) rats. Interestingly, STZ + MCP rats showed further increase in the unit weight of intestine (15d -44%; 30d -50%) (Table 3.1).

5.1.2 Blood glucose

STZ administered rats showed a marked elevation (nearly 4 fold) in blood glucose levels (Fig. 3.1). MCP treatment (15 and 30d) resulted in a marginal increase in blood glucose levels. Interestingly, rats of STZ + MCP groups showed significantly elevated levels of blood glucose (33% over STZ rats) both after 15 and 30d.

5.1.3 Disaccharidases

Significant increase in activities of all the four disaccharidases (maltase, sucrase, lactase and trehalase) was evident in all the treatment groups. MCP induced an increase (15d-66%; 30d- 34%) in maltase activity in jejunum. Similar increase was also observed in STZ rats (Fig. 3.2A and Fig. 3.2B). The enzyme activity was further increased (29 and 38 %) in STZ+MCP rats compared to STZ rats in 15d and 30d treatment respectively. In 30d study, increased disaccharidase activity was observed in STZ+MCP group in duodenum and ileum also.
Sucrase activity was increased by MCP (49%) in jejunum after 15d. Similar increase was also evident in STZ rats (Fig. 3.3A). In STZ+MCP rats, 2-fold increase in sucrase activity was evident in jejunum, while in duodenum and ileum marked increase in sucrase activity was evident (38 and 73% compared to STZ group in duodenum and ileum respectively). Similar trend in increase were seen in jejunum after 30d (Fig. 3.3B). In duodenum, MCP per se induced an increase (78%) in activity and in STZ rats, the enzyme activity was further increased (2-fold increase compared to control). In ileum, MCP per se did not alter the activity but augmented the enzyme activity in STZ rats.

MCP and STZ induced marked increase in lactase activity (56% and 76% respectively) in jejunum after 15d (Fig. 3.4A). MCP treatment increased the enzyme activity further (by 20%) in STZ- rats compared to STZ per se group. In duodenum, 60% increase in enzyme activity was seen in STZ+MCP compared to control. Similar results were observed in jejunum after 30d (Fig. 3.4B). In duodenum, increased activity was seen in STZ (70%) and STZ+MCP rats (180%). 2-fold higher activity was induced in ileum by MCP in STZ rats.

Trehalase activity was found to be increased in jejunum in rats of all treatment groups after 15d (Fig. 3.5A). MCP per se increased the activity by 66% and further augmented the activity in STZ rats. Similar results were evident in duodenum although extent of increase in activity was less compared to that in jejunum. Marginal increase in activity was induced by MCP in jejunum and significantly higher activity was evident in STZ and STZ+MCP rats after 30d (Fig. 3.5B). Similar increase in enzyme activity was also seen in duodenum and in ileum. However, marked increase was observed (76% compared to control) in rats of STZ+MCP group.

5.1.4 Alkaline phosphatase

ALP activity was significantly elevated in MCP treated as well as STZ rats (50-60%) in both duodenum and jejunum after 15d (Fig. 3.6A). An additional increase in enzyme activity was observed in STZ + MCP rats. Similar pattern of
Increase in ALP activity was observed in 30d treatment, although the extent of increase was higher (Fig. 3.6 B). In addition, ALP activity was also elevated in ileum of rats of STZ and STZ+MCP group.

5.1.5 Na⁺, K⁺-ATPase

In jejunum, a marked increase (50%) in the enzyme activity was evident as a result of MCP treatment and a similar degree of increase were observed in STZ rats after 15d (Fig. 3.7A). However, the enzyme activity was dramatically enhanced (2-fold) in rats of STZ + MCP group. Further, the enzyme activity was also significantly increased in ileum of rats of STZ (34%) and STZ + MCP (59%) groups. Similar results were evident in jejunum after 30d (Fig. 3.7B). Besides, increased activity was induced by MCP alone in ileum after 30d.

5.2 Acetylcholinesterase and Paraoxonase 2 activities

Rats treated with MCP (15d) showed significant inhibition of intestinal AChE activity (29-34%) (Fig. 3.8A). However, diabetic rats exhibited a marked increase in AChE activity (up to 54%), while rats of the STZ + MCP group showed a marked decrease in AChE activity in all the three intestinal regions. Similar results were also obtained with MCP treatment for 30d (Fig. 3.8B). Interestingly, the extent of inhibition of AChE activity by MCP in STZ rats was comparable to inhibition caused by MCP alone.

There was no change in PON 2 activity in any region of the intestine as a result of MCP (15 d) treatment (Fig. 3.9A). However, in STZ and STZ + MCP groups, there was a decrease in PON activity in jejunum and ileum (27-33%). A decrease in the enzyme activity (32-35%) was observed in jejunum and ileum as a result of MCP treatment for 30d (Fig. 3.9B). Reduced enzyme activity was also evident in STZ and STZ+MCP rats after 30d.
5.3 Redox state markers

5.3.1 Lipid peroxidation

The extent of lipid peroxidation was significantly increased (2-fold) in the MCP and STZ groups and further increase (2.5-fold) was noticed in MCP + STZ group in jejunum after 15 d. Similar results were evident in ileum and duodenum also, but the extent of increase was lesser compared to that in jejunum. Marked increase in increase in LPO (70-90%) was evident in all treatment groups in the three intestinal regions (Table 3.2).

5.3.2 Reduced glutathione

MCP treatment (15d) decreased GSH level (by 24%) in duodenum and jejunum and the extent of reduction was similar in STZ rats (Table 3.3). Greater depletion in GSH content (33-42%) was evident in STZ +MCP group. In 30d treatment, the extent of depletion in GSH content in all treatment groups was similar to that seen in 15d treatment (Table 3.3).

5.3.3 Antioxidant enzymes: Catalase and SOD

Significant decrease in catalase activity was observed in MCP treated rats after 15d in both jejunum and duodenum (32-42%) (Table 3.4). A decrease in CAT activity was also observed in all the intestinal regions of STZ rats. MCP treatment further decreased the CAT activity in STZ rats. Similar results were observed after 30d (Table 3.4). However, SOD activity was significantly elevated in jejunum of MCP treated rats after 15d (Table 3.5). Up to 50% increase in SOD activity was noticed in all the intestinal regions of STZ rats. MCP did not augment the activity of SOD in STZ rats. The pattern of results was similar after 30d (Table 3.5).

5.3.4 Glutathione-S-transferase

MCP treatment induced an increase (39%) in GST activity in jejunum after 15d (Table 3.6), while STZ rats showed 28-55% increase in GST activity in the intestinal regions. MCP treatment further increased the GST activity in STZ
group (in duodenum and jejunum). MCP per se enhanced GST activity (31%) in both duodenum and jejunum after 30d (Table 3.6). Similar increase (upto 56%) was noticed in STZ rats while further increase in GST activity was induced by MCP in STZ rats after 30d (Table 3.6).

5.4 Histopathology

Duodenum

Increased villi length was observed in duodenum of rats treated with MCP for 15d (Fig 3.10A, B- ii). However, no visible changes were discernible in duodenal villi of STZ and STZ+MCP rats after 15d (Fig 3.10A, B- iii and iv). Areas of necrosis were visible in duodenum of MCP treated rats after 30d (Fig 3.11, B- ii) and inflammatory cell infiltration was evident in STZ (Fig 3.11A, B-iii) and STZ + MCP rats (Fig 3.11A, B-iv).

Jejunum

Mild degree of congestion, hydropic degeneration and infiltration of inflammatory cells was discernible in jejunum of MCP treated rats after 15d (Fig. 3.12B and Fig. 3.13A). Jejunum of STZ rats revealed increased villi length along with the infiltration of inflammatory cells, mild degree of congestion and hemorrhage. Goblet cell hyperplasia was also observed in STZ rats (Figure 3.12C and Fig.3.13B). STZ + MCP showed greater pathology in jejunum including focal areas of necrosis (Fig. 3.12D and 3.13C, D). Jejunum of rats treated with MCP for 30d showed congestion and erosion of villi and infiltration of inflammatory cells (Fig. 3.14A, B-ii). STZ + MCP showed greater pathology in jejunum including focal areas of necrosis and distorted villi (Fig. 3.14A, B-iv).

Ileum

Erosion of villi epithelium was seen in rats treated with MCP for 15d (Fig. 3.15A, B-ii). Similar pathology was evident in ileum of rats of STZ + MCP group (Figure 3.15A, B-iv). In rats of STZ group, infiltration of inflammatory cells was evident (Fig. 3.15A, B-iii).
However, MCP induced several changes in ileum after 30d which included congestion and increased length of villi, erosion of villi epithelium and infiltration of inflammatory cells (Fig. 3.16A, B-ii). But, not many changes were seen in ileum of STZ rats. Changes induced by STZ+MCP were similar to that caused by MCP alone (Fig. 3.16A, B-iv).

5.5 Scanning electron microscopy

**Duodenum**

Hyperplasia of goblet cells was evident in duodenum of MCP rats (15d) (Fig. 3.17B, b) and STZ rats (Fig.3.17C, c). Similar results were evident after 30d in duodenum of MCP rats (Fig. 3.18B, b). Loss of villus epithelium and necrosis of villi tip were observed in duodenum of STZ+MCP rats after 30d (Fig. 3.18D, d).

**Jejunum**

The villi of control rats appeared intact while MCP treatment induced increase in the number of goblet cells (hyperplasia) in villi in 15d study (Fig. 3.19B, b). In diabetic rats, the villi appeared broader and revealed necrosis at villi tip (Fig. 3.19C, c). In jejunum of rats of MCP + STZ group, the number of goblet cells was increased enormously and villi epithelium was damaged (Fig. 3.19D, d). Jejunum of rats of all the treatment groups revealed goblet cell hyperplasia after 30d (Fig. 3.20).

**Ileum**

MCP (15d) induced an increase in the number of goblet cells in villi (Fig. 3.21B, b). Not many changes were observed in ileum of rats of STZ and STZ + MCP group (Fig. 3.21C, D). MCP treatment for 30d induced a loss of villus epithelium in ileum (Fig. 3.22B, b). In ileum of STZ rats, villi appeared thick and goblet cell number was increased (Fig. 3.22C, c) while in STZ + MCP group, loss of villus epithelium was observed (Fig. 3.22D, d).
Table 3.1 Effect of multiple doses (15 d and 30d) of MCP on body weight, relative and unit weight of small intestine in experimentally induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of MCP treatment</th>
<th>15d</th>
<th>30d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>Relative weight (g)</td>
<td>Unit weight (mg/cm)</td>
</tr>
<tr>
<td>Control</td>
<td>247.3 ± 6.3</td>
<td>2.7 ± 0.1</td>
<td>83.6 ± 2.9</td>
</tr>
<tr>
<td>MCP</td>
<td>217.3 ± 4.2</td>
<td>3.1 ± 0.1</td>
<td>84.3 ± 1.0</td>
</tr>
<tr>
<td>STZ</td>
<td>171.5 ± 8.8</td>
<td>6.0 ± 0.3</td>
<td>103.8 ± 4.9</td>
</tr>
<tr>
<td>STZ+MCP</td>
<td>148.6 ± 8.9</td>
<td>7.7 ± 0.6</td>
<td>120.8 ± 4.9</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n = 6).
Data analyzed by one way ANOVA - Tukey’s HSD test for comparison of means to determine the significance of differences among the groups. P values below 0.05 were considered as significantly different.

a - Control v/s STZ; b - Control v/s STZ + MCP;  
c - MCP v/s STZ + MCP; d - STZ v/s STZ + MCP
Fig. 3.1 Effect of MCP (A - 15d, B - 30d) on blood glucose level in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6). Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05).

- Control v/s STZ
- MCP v/s STZ
- Control v/s STZ + MCP
- MCP v/s STZ + MCP
- STZ v/s STZ + MCP
Fig. 3.2 Effect of MCP (A - 15d, B - 30d) on intestinal maltase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6). Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP  
b - Control v/s STZ  
c - Control v/s STZ + MCP  
d - MCP v/s STZ + MCP  
e - STZ v/s STZ + MCP
Fig. 3.3 Effect of MCP (A - 15d, B - 30d) on intestinal sucrase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP
b - Control v/s STZ
c - Control v/s STZ + MCP
d - MCP v/s STZ + MCP
e - STZ v/s STZ + MCP
Fig. 3.4 Effect of MCP (A - 15d, B - 30d) on intestinal lactase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analysed by one way ANOVA-Tukey's HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

- a - Control v/s MCP
- b - Control v/s STZ
- c - Control v/s STZ + MCP
- d - MCP v/s STZ + MCP
Fig. 3.5 Effect of MCP (A - 15d, B - 30d) on intestinal trehalase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP
b - Control v/s STZ
c - Control v/s STZ + MCP
d - MCP v/s STZ + MCP
e - STZ v/s STZ + MCP
Fig. 3.6 Effect of MCP (A - 15d, B - 30d) on intestinal alkaline phosphatase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP
b - Control v/s STZ
c - Control v/s STZ + MCP
d - MCP v/s STZ + MCP
e - STZ v/s STZ + MCP
Fig. 3.7 Effect of MCP (A - 15d, B - 30d) on intestinal Na⁺, K⁺ ATPase activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analysed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP
b - Control v/s STZ
c - Control v/s STZ + MCP
d - MCP v/s STZ + MCP
Fig. 3.8 Effect of MCP (A - 15d, B - 30d) on intestinal AChE activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey's HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

- a - Control v/s MCP
- b - Control v/s STZ
- c - Control v/s STZ + MCP
- e - STZ v/s STZ + MCP

Values are mean ± SE (n=6). a p<0.01 for control v/s MCP; b p<0.01 for control v/s STZ + MCP; c p<0.01 STZ v/s MCP + STZ.
Fig. 3.9 Effect of MCP (A - 15d, B - 30d on intestinal PON 2 activity in experimentally induced diabetic rats

Values are expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups at p<0.05 for each intestinal region (D, J & I)

a - Control v/s MCP
b - Control v/s STZ
c - Control v/s STZ + MCP
d - MCP v/s STZ + MCP
Table 3.2 Effect of multiple doses (15 d & 30d) of MCP on lipid peroxidation (LPO) in small intestinal regions in experimentally induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (nmol MDA/g tissue)</th>
<th>Duodenum (D)</th>
<th>Jejunum (J)</th>
<th>Ileum (I)</th>
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<tr>
<td></td>
<td></td>
<td>15d</td>
<td>30d</td>
<td>15d</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>9.4 ± 1.2</td>
<td>10.2 ± 0.3</td>
<td>16.0 ± 0.4</td>
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<tr>
<td>MCP</td>
<td></td>
<td>15.7 ± 0.7</td>
<td>17.7 ± 0.7</td>
<td>31.2 ± 0.4</td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td>17.3 ± 1.2</td>
<td>18.1 ± 1.0</td>
<td>31.4 ± 2.2</td>
</tr>
<tr>
<td>STZ+MCP</td>
<td></td>
<td>17.5 ± 1.5</td>
<td>20.1 ± 0.8</td>
<td>39.8 ± 1.3</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05) in each intestinal region (D, J & I).

a - Control v/s MCP; b - Control v/s STZ

c- Control v/s STZ + MCP; d- MCP v/s STZ + MCP
Table 3.3 Effect of multiple doses (15 d & 30d) of MCP on reduced glutathione (GSH) content in small intestinal regions in experimentally induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (nmol/g tissue)</th>
<th>Duodenum (D)</th>
<th>Jejunum (J)</th>
<th>Ileum (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15d</td>
<td>30d</td>
<td>15d</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>363.3 ± 22.0</td>
<td>403.3 ± 20.2</td>
<td>453.4 ± 18.3</td>
</tr>
<tr>
<td>MCP</td>
<td></td>
<td>277.3 ± 10.0</td>
<td>327.7 ± 11.3</td>
<td>352.8 ± 17.6</td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td>260.9 ± 15.1</td>
<td>301.5 ± 13.1</td>
<td>286.6 ± 20.0</td>
</tr>
<tr>
<td>STZ+MCP</td>
<td></td>
<td>211.7 ± 11.4</td>
<td>274.2 ± 10.0</td>
<td>292.1 ± 15.6</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05) in each intestinal region (D, J & I).

a - Control v/s MCP; b - Control v/s STZ

c - Control v/s STZ + MCP; d - MCP v/s STZ + MCP
Table 3.4 Effect of multiple doses (15 d and 30d) of MCP on catalase (CAT) activity in small intestinal regions in experimentally induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (units/mg protein)</th>
<th>Duodenum (D)</th>
<th>Jejunum (J)</th>
<th>Ileum (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15d</td>
<td>30d</td>
<td>15d</td>
</tr>
<tr>
<td>Control</td>
<td>20.7 ± 1.9</td>
<td>16.2 ± 0.8</td>
<td>21.2 ± 0.5</td>
<td>19.0 ± 0.6</td>
</tr>
<tr>
<td>MCP</td>
<td>12.0 ± 0.5</td>
<td>9.0 ± 0.4</td>
<td>14.5 ± 0.7</td>
<td>11.5 ± 0.6</td>
</tr>
<tr>
<td>STZ</td>
<td>10.8 ± 0.7</td>
<td>8.9 ± 0.2</td>
<td>12.1 ± 0.6</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>STZ + MCP</td>
<td>10.2 ± 0.5</td>
<td>9.2 ± 0.6</td>
<td>10.9 ± 0.7</td>
<td>12.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05) in each intestinal region (D, J & I).

a - Control v/s MCP; b - Control v/s STZ

STZ + MCP; MCP v/s STZ + MCP
Table 3.5 Effect of multiple doses (15 d & 30d) of MCP on superoxide dismutase (SOD) activity in small intestinal regions in experimentally induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (units/mg protein)</th>
<th>Duodenum (D)</th>
<th>Jejunum (J)</th>
<th>Ileum (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15d</td>
<td>30d</td>
<td>15d</td>
</tr>
<tr>
<td>Control</td>
<td>122.3 ± 3.6</td>
<td>138.1 ± 6.1</td>
<td>108.3 ± 2.7</td>
<td>121.4 ± 3.6</td>
</tr>
<tr>
<td>MCP</td>
<td>139.6 ± 4.2</td>
<td>152.8 ± 7.0</td>
<td>133.9 ± 3.7</td>
<td>168.9 ± 4.2</td>
</tr>
<tr>
<td>STZ</td>
<td>174.0 ± 6.0</td>
<td>204.0 ± 7.2</td>
<td>162.3 ± 5.4</td>
<td>171.0 ± 4.8</td>
</tr>
<tr>
<td>STZ+MCP</td>
<td>171.4 ± 3.3</td>
<td>201.6 ± 9.6</td>
<td>153.2 ± 7.2</td>
<td>185.1 ± 6.9</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05) in each intestinal region (D, J & I).

a - Control v/s MCP; b - Control v/s STZ

C- Control v/s STZ + MCP; d- MCP v/s STZ + MCP
Table 3.6 Effect of multiple doses (15 d and 30d) of MCP on Glutathione-S-Transferase (GST) activity in small intestinal regions in experimentally induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>GST (units/mg protein)</th>
<th>Duodenum (D)</th>
<th>Jejunum (J)</th>
<th>Ileum (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15d</td>
<td>30d</td>
<td>15d</td>
</tr>
<tr>
<td>Control</td>
<td>290.9 ± 16.9</td>
<td>274.2 ± 12.6</td>
<td>203.6 ± 15.5</td>
<td>231.6 ± 11.5</td>
</tr>
<tr>
<td>MCP</td>
<td>338.3 ± 13.8</td>
<td>361.3 ± 13.1</td>
<td>283.8 ± 17.0</td>
<td>303.2 ± 12.2</td>
</tr>
<tr>
<td>STZ</td>
<td>371.0 ± 25.8</td>
<td>389.7 ± 16.5</td>
<td>316.5 ± 19.7</td>
<td>326.7 ± 12.7</td>
</tr>
<tr>
<td>STZ+MCP</td>
<td>418.4 ± 26.5</td>
<td>447.1 ± 17.3</td>
<td>324.1 ± 23.0</td>
<td>353.4 ± 18.4</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE (n=6).

Data analyzed by one way ANOVA-Tukey’s HSD test for comparison of means to determine the significance of differences among the groups (p<0.05).

a - Control v/s MCP; b - Control v/s STZ

c- Control v/s STZ + MCP; d- MCP v/s STZ + MCP
Fig. 3.10 Photomicrographs of duodenum of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (H&E; A - 100x, B - 400x)

A (i) & B (i) - Control - normal duodenal villi
A (ii) & B (ii) - MCP - increased villi length
A (iii) & B (iii) – STZ - no visible changes in villi
A (iv) & B (iv) – STZ + MCP - no visible changes in villi
Fig. 3.11 Photomicrographs of duodenum of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (H&E; A - 100x, B - 400x)

A (i) & B (i) - Control - intact villi
A (ii) & B (ii) – MCP - necrotic areas (blue arrows) in villi
A (iii) & B (iii) – STZ - inflammatory cell infiltration (black arrows) in villi
A (iv) & B (iv) – STZ + MCP - inflammatory cell infiltration (black arrows) in villi
Fig. 3.12 Photomicrographs of jejunum of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (H&E; 100x)

A - Control - Intact jejunal mucosa with finger like projections of villi
B - MCP - Congestion of villi
C - STZ - Increase in villi length and congestion
D - STZ + MCP - Increase in length of villi
Fig. 3.13 Photomicrographs of jejunum of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (H&E; 400x)

A - MCP - Infiltration of inflammatory cells (blue arrow)
B - STZ - Goblet cell hyperplasia (black arrow) and inflammatory cells (blue arrow)
C - STZ + MCP - Focal areas of necrosis rats (yellow arrow) at the base of villi
D - STZ+MCP - Inflammatory cell infiltration (blue arrow)
Fig. 3.14 Photomicrographs of jejunum of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (H&E; A - 100x, B - 400x)

A (i) & B (i) – Control - intact villi

A (ii) & B (ii) – MCP - congestion of villi, erosion of villus epithelium and inflammatory cells

A (iii) & B (iii) – STZ - not many changes in villi

A (iv) & B (iv) – STZ + MCP - Villi appear disorted and focal areas of necrosis (yellow arrow) at the base of villi
Fig. 3.15 Photomicrographs of ileum of in experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (H&E; A - 100x, B - 400x)

A (i) & B (i) – Control - intact villi
A (ii) & B (ii) – MCP - erosion of villi epithelium
A (iii) & B (iii) – STZ - inflammatory cell infiltration (black arrow)
A (iv) & B (iv) - STZ + MCP - erosion of villi epithelium
Fig. 3.16 Photomicrographs of ileum of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (H&E; A - 100x, B - 400x)

A (i) & B (i) – Control - intact villi

A (ii) & B (ii) - MCP - congestion and increased length of villi, erosion of villus epithelium and inflammatory cells

A (iii) & B (iii) – STZ - not many changes visible in villi

A (iv) & B (iv) – STZ + MCP - villi length increased and inflammatory cells seen in the lumen of the villi
Fig. 3.17 Scanning electron micrographs of duodenum villi of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure.
B, b - MCP - Hyperplasia of goblet cells (black arrow)
C, c - STZ - Not many changes visible in villi
D, d - STZ + MCP - Hyperplasia of goblet cells (black arrow)
Fig. 3.18 Scanning electron micrographs of duodenum villi of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure.
B, b - MCP - Hypertrophy of goblet cells (white arrow)
C, c - STZ - Not many changes visible in villi
D, d - STZ + MCP - Loss of villus epithelium (yellow arrow) and necrosis of villi tip
Fig. 3.19 Scanning electron micrographs of jejunum villi of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure.
B, b - MCP - Hyperplasia of goblet cells (white arrow)
C, c - STZ - Necrotic villi tip
D, d - STZ + MCP - Hyperplasia of goblet cells (white arrow) and loss of villus epithelium (blue arrow)
Fig. 3.20 Scanning electron micrographs of jejunum villi of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure.
B, b - MCP - Hyperplasia of goblet cells (yellow arrow)
C, c - STZ - Villi appear thick with Hyperplasia of goblet cells (yellow arrow)
D, d - STZ + MCP - Hyperplasia of goblet cells (yellow arrow)
Fig. 3.21 Scanning electron micrographs of ileum villi of experimentally induced diabetic rats exposed to multiple (15d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure
B, b - MCP - Hyperplasia of goblet cells (yellow arrow)
C, c - STZ - Not many changes visible in villi
D, d - STZ + MCP - no visible changes in villi
Fig. 3.22 Scanning electron micrographs of ileum villi of experimentally induced diabetic rats exposed to multiple (30d) doses of MCP (A, B, C & D - 500x; a, b, c & d - 1000x)

A, a - Control - intact villus structure
B, b - MCP - Loss of villus epithelium
C, c - STZ - Villi appear thick with hyperplasia of goblet cells
D, d - STZ + MCP - Loss of villus epithelium
6. DISCUSSION

The primary objective of this investigation was to delineate the effects of repeated oral doses of MCP on structural and biochemical alterations in the small intestine of experimentally induced diabetic rats.

In the present study, rats administered with STZ exhibited significant hyperglycemia accompanied by weight loss, indicating their diabetic condition. Oral doses of MCP (at 1/20 \(LD_{50}\)) in both 15d and 30d study caused a marginal increase in blood glucose levels. However, at the same dose, MCP potentiated the hyperglycemic condition among diabetic rats as evidenced by significantly enhanced levels of blood glucose. This observation corroborates with the previous findings from our laboratory (Begum and Rajini, 2011a), wherein MCP besides marginally increasing the blood glucose levels in rats, enhanced the hyperglycemic outcome in diabetic rats by disrupting both gluconeogenesis and glycogenolysis pathways in the liver, thus intensifying diabetic complications.

In the present model, a significant increase in the relative and unit weight of the small intestine of diabetic rats was noticed in both 15d and 30d study. Previously, increase in the small intestinal enterocyte mass has been reported in rat models of diabetes with a concomitant increase in intestinal proliferation and longer villi (Adachi et al., 2003). Small intestinal hyperplasia and hypertrophy observed in experimentally induced diabetic rats is speculated to be due to suppression of apoptosis (Noda et al., 2001). In a recent study, Sen et al., (2011) attributed the mucosal hyperplasia to hyperphagia, overproduction of gastrin, growth hormone and increased level of glucagon-like peptide-2, up-regulation of cell proliferation and inhibition of cell death via apoptosis. Several experimental studies have shown that cell proliferation increases significantly in experimentally induced diabetes due to gut trophic hormones or to neuronal loss (Sen et al., 2011). Interestingly, although MCP did not increase the unit weight of intestine, it seemed to increase the unit weight of intestine in STZ rats. Impact
of OPI on intestinal mass is hitherto not reported. Hence, the mechanism by which MCP affected the intestinal weight in diabetic rats is not clear.

Diabetes is reported to stimulate the functional activity of the intestinal brush border membrane with enhancement of both hydrolytic enzyme activity and membrane transport systems (Fedorak, 1990). Up-regulation of the activities of disaccharidases and glucose carriers in the intestinal mucosa is also reported to contribute to the hyperglycemia in diabetes (Fedorak, 1990). Abnormally high activities of disaccharidases and monosaccharide transporters have been observed in the small intestine of diabetic patients and experimental diabetic animals (Hamden et al., 2011). Increase in the activities of disaccharidases has also been suggested to be one of the major factors resulting in postprandial hyperglycemia in diabetic states (Dyer et al., 2002). Increase in the total and specific enzymatic activity of several membrane-bound hydrolases including sucrase, maltase, lactase, trehalase, leucyl naphthylamidase, and alkaline phosphatase have been reported (Olsen and Rogers, 1971; Younoszai and Schedl, 1972; Caspary et al., 1972).

In the present study, we observed increase in the activities of all the four disaccharidases (maltase, sucrase, lactase and trehalase) upon MCP treatment (in both 15 and 30d regime) and the extent of increase was similar to that of diabetic rats. The activities of these disaccharidases were further increased in STZ+MCP rats. Elevated steroid hormone levels have been reported in experimental diabetes (Doell and Kretschmer, 1964) and also in rats treated with MCP (Joshi and Rajini, 2012). Hence, an increase in disaccharidase activities in STZ and STZ+MCP rats may be attributed to the elevated steroid levels as has been suggested earlier (Caspary et al., 1972). Increased glucose absorption has been reported in diabetic rats, which has been ascribed to the increased intestinal disaccharidase activities (Schedl and Wilson, 1971). Studies have shown that diabetes causes both structural and functional changes in the intestine such as hyperplasia and increase in intestinal glucose absorption as well as in the activities of brush border disaccharidases (Tuin et al., 2009),
which in turn may be responsible for the increased monosaccharide absorption. Our results also show that MCP elicits a similar response in rats in terms of both increased intestinal disaccharidase activities and increased blood glucose levels, thereby complicating the pathophysiology of diabetes.

Recent studies (both in vitro and in vivo) have clearly demonstrated that insulin deficiency induces a marked increase in intestinal disaccharidase activities and expression under diabetic states (Liu et al., 2011). Interestingly, OPI have been demonstrated to disrupt beta cell function by oxidative/nitrosative stress in islets of Langerhans, and thereby induce insulin insufficiency (Hectors et al., 2011). Further, a recent study from our laboratory had shown that MCP treated rats (at 0.9 mg/kg b.w/d for 150d) exhibited marked insulin deficiency (up to 50%) (Nagaraju et al., 2014). Based on these facts, we speculate that MCP induced insulin deficiency may be responsible for the increased intestinal disaccharidase activities.

Intestinal alkaline phosphatase plays multiple biological roles in the maintenance of intestinal homeostasis (Lalles, 2010). In the present study, an increase in activity of ALP was evident as a result of MCP treatment and STZ treated rats also showed elevated alkaline phosphatase activity as has been reported earlier (Caspary et al., 1972). A marked upregulation of ALP activity has been often reported in the inflamed intestine and, both infiltrating leukocytes and intestinal epithelial cells appear to contribute to this phenomenon in vivo (Sanchez de Medina et al., 2004). Increased intestinal alkaline phosphatase activity has also been reported in certain inflammatory disorders of intestine (Tormo et al., 2002). Hence, elevated levels of intestinal alkaline phosphatase, both in MCP and STZ+MCP rats might indicate inflammatory response.

Na\(^+\), K\(^+\)-ATPase plays a key role in the absorption of electrolytes, water, and nutrients from the small intestine. In diabetic condition, Na\(^+\), K\(^+\)-ATPase activity and numbers of sodium pumps have been reported to be increased in the small intestine (Barada et al., 1994; Wild et al., 1999). The alterations in the Na\(^+\), K\(^+\)-ATPase expression in small intestine in chronic diabetic state appear to
involve alterations in transcriptional and posttranscriptional events (Wild et al., 1999), and in particular increase in mRNA encoding the α 1 and β1 subunits of Na\(^+\), K\(^+\)-ATPase (Barada et al., 1994). In the present study, the activity of Na\(^+\), K\(^+\)-ATPase was marginally increased in the intestine of rats treated with MCP while a significant increase in the activity was evident in both in STZ and STZ+MCP rats. This increase in the enzyme activity may probably represent an adaptive response which leads to increased Na\(^+\)-coupled monosaccharide absorption as has been suggested earlier (Wild et al., 1999).

It is well known that OPI exert their toxicity mainly through the inhibition of acetylcholinesterase (AChE). In the present study, AChE activity was reduced significantly in the small intestine of MCP treated rats. However, AChE activity was markedly elevated in STZ rats in 15d study, whereas no change was observed in 30d study. Enhanced activity of AChE is commonly observed in tissues of diabetic rats (Lincoln et al., 1984). Earlier studies from our laboratory (Begum and Rajini, 2011b) also reported elevated levels of AChE activity in the brain of STZ treated rats. Acetylcholine (ACh) is a major neurotransmitter in the enteric nervous system. It plays a major role in rhythmic spontaneous contractions of rat intestinal smooth muscle and also in the absorption of nutrients from colon (Keely, 2011). ACh also regulates proliferation, mucus secretion, cytokine production and migration at the level of epithelium (Keely, 2011). Thus, ACh cannot be considered only as a neurotransmitter but rather as an important intercellular messenger that is likely to be important in integrating many different aspects of intestinal physiology in health and disease. In the present study, the activity of AChE was reduced in MCP treated rats and increased in diabetic rats. Overall, it seems that this might have an impact on intestinal functions both in terms of motility and nutrient absorption. This aspect merits further investigation.

PON 2 is expressed widely in a number of tissues, including the liver, lungs, brain, heart and intestine (Mochizuki et al., 1998). The distribution of PON 2 in many tissues suggests the possibility of its role as an antioxidant (Draganov
et al., 2000). PON 2 appears to be a good candidate for preventing oxidative stress locally within cells (Horke et al., 2007). In the present study, MCP per se did not change the activity of PON 2 in 15d study though the other oxidative stress markers were altered, the activity of PON 2 was reduced in 30d study. This might indicate that in addition to its role in protecting against oxidative stress, PON 2 has many other roles to play in the intestine which are not yet investigated completely. The fate of PON 2 is not reported so far in the diabetic state especially in the small intestine. In the diabetic state, the decreased activity observed in jejunum and ileum regions might indicate the oxidative stress.

It is well recognized that diabetes induces oxidative stress and that the resulting oxidative damage has a key role in the development of diabetic complications (Son et al., 2004). Occurrence of oxidative damage has been clearly demonstrated in the small intestine of STZ-induced diabetic rats (Bhor et al., 2004). Lipid peroxidation is regarded as one of the primary molecular mechanisms involved in OPI-induced toxicity (Abdollahi et al., 2004). In the present study, the extent of lipid peroxidation was markedly increased in MCP and also in diabetic rats. Depletion of hepatic and renal GSH levels demonstrated among diabetic rats clearly suggests the increased utilization as a result of oxidative stress (Rosa and Catala, 1998). Similar results were found in the present study, where in MCP per se decreased the level of GSH and also in diabetic rats. The altered balance of the antioxidant enzymes in the intestine of all the treatment groups may be responsible for the inadequacy of the antioxidant defences in combating oxidative damage. The extent of alterations in the activity of these two enzymes was augmented in diabetic rats by MCP.

Glutathione S-transferases (GSTs) are a family of phase II detoxification enzymes that eliminate electrophilic compounds by conjugating them to GSH. Hence, induction of GST observed in the intestine of MCP treated rats may be perceived as a counter-regulatory response to eliminate MCP. Depletion in reduced GSH levels observed by MCP treatment (per se and in STZ rats) may
be partly attributed to induction in GST activity. These results suggest the involvement of oxidative stress in the small intestine during diabetes, and it is possible in some of the accompanying functional alterations in the intestine.

Diabetes is reported to be associated with numerous changes within the small intestine, including hyperplasia and hypertrophy of epithelial cells (Zoubi et al., 1995), increased absorption of sugars and amino acids (Fedorak, 1990) and increased endogenous cholesterol synthesis (Feingold et al., 1982). Inflammatory cytokines are also increased in diabetes (Esposito et al., 2002). In the present study, increase in relative weight and unit weight of the small intestine in both STZ and STZ+MCP rats was accompanied by hyperplasia of goblet cells as evident in the scanning electron micrographs and histological observation of different intestinal regions. Since goblet cells are mucus-producing cells, an increase in unit weight of intestine in treatment groups might be a result of increased production of mucus and diabetes induced stimulation of intestinal mucosal mass is believed to be a response to elevated physiological demand (Bhor et al., 2004). Increase in the small intestinal mucosal mass, protein and DNA content is commonly reported in STZ diabetic rats, which are suggested to be due to increase in the total number of intestinal epithelial cells (Tormo et al., 2002). Sloughing of the surface epithelium of the villi and necrosis both at the tip and base of villi observed among STZ and STZ+MCP rats could be due to decreased blood supply as a result of diabetic micro angiopathy (Abo Gazia and Hasan, 2012). Scanning electron micrographs also showed wide marked convolutions in villi of diabetic rats. These convolutions of the villous surface may be due the disturbance in maturation of absorptive cells at the villus tip as has been reported earlier (Abo Gazia and Hasan, 2012). Increase in the length of villi was observed in MCP and STZ rats. Increase in villi length in diabetic rats is already reported (Adachi et al., 2003; Abo Gazia and Hasan, 2012). Infiltration of inflammatory cells observed in all treatment groups and augmented inflammatory response by MCP in STZ rats necessitates further study in this line.
Collectively, the present study provides evidence for the potential of MCP to augment dysfunctions in the small intestine of diabetic rats. Besides affecting glucose homeostasis as reported earlier from our lab, the current work substantiates that MCP also has an effect on intestinal brush border enzymes and the structure of the small intestine, which might contribute towards postprandial hyperglycemia. Further, these results clearly demonstrate that the intestine of diabetic rats are prone to further structural, functional and oxidative damage by MCP, which might result in exacerbated intestinal dysfunction.

7. SUMMARY

1. Significant decrease in body weight, increase in relative and unit weight of small intestine were observed in rats of STZ and STZ+MCP groups both after 15 and 30d.

2. STZ rats exhibited marked increase in blood glucose level and MCP exposure further increased the level (by 33%).

3. Activities of all the disaccharidases (maltase, sucrase, lactase and trehalase) were significantly elevated in intestine of rats of all treatment groups (MCP, STZ and STZ+MCP groups). MCP augmented the disaccharidases activity in STZ rats. Majority of the change was noticeable in jejunum.

4. A similar pattern of response was also noted with the activity levels of alkaline phosphatase in intestinal regions.

5. The activity of membrane bound enzyme, \( \text{Na}^+, \text{K}^+ \)-ATPase activity was increased in jejunum of rats of all treatment groups and the activity was markedly increased in jejunum of STZ + MCP rats.

6. AChE activity was significantly decreased by MCP (\textit{per se and in diabetic condition}) in all regions of small intestine. However, AChE activity was increased in intestinal regions of STZ rats.
7. MCP treatment did not affect the PON 2 activity in intestinal regions after 15d, while the activity in STZ rats was increased. After 30d, MCP significantly reduced the PON 2 activity in jejunum and ileum and the extent of decrease was similar to that of STZ rats.

8. Majority of the redox state markers exhibited significant alterations in all treatment groups, both after 15 and 30d and changes were predominant in the jejunum.

9. Histopathological studies of intestinal regions also revealed that major changes were discernible in jejunum (followed by ileum and duodenum). MCP (per se and in diabetic condition) induced salient changes such as increased villi length, infiltration of inflammatory cells, goblet cell hyperplasia and congestion of villi and similar changes were noticeable among STZ rats. MCP dosage resulted in focal areas of necrosis in STZ rats.

10. Most changes in treatment groups were evident in jejunum irrespective of the duration of MCP treatment. Increase in the number of goblet cells, necrosis at villi tip and loss of villus epithelium were the characteristic alterations noticed in all treatment groups.

11. Collectively, the present study provides evidence for the potential of MCP to augment dysfunctions in the small intestine of diabetic rats. Further, these results clearly demonstrate that the intestine of diabetic rats are prone to further structural, functional and oxidative damage by MCP which might result in exacerbated intestinal dysfunction.