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A Comparative Study between the Effect of Simvastatin and Sitagliptin Combined and the Effect of a Large Dose of Each in an Early Treatment of Experimentally Induced Colitis in Mice

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ABSTRACT

Ulcerative colitis is a chronic-relapsing, progressive inflammatory bowel disease characterized by diffuse mucosal inflammation of the colon. Repeated damage and injury of the intestinal surface are key features of inflammatory bowel disease**.** It can be concluded that sitagliptin is partially effective for treatment of experimentally induced ulcerative colitis model in mice. Furthermore, it can be also concluded that effects of simvastatin treatment were mostly dose dependantfor treatment of experimentally induced ulcerative colitis model in mice. Thus, the aim of this study is to evaluate effect of combination of sitagliptin and simvastatin as an early treatment of experimentally induced ulcerative colitis in mice. Furthermore in this study, effect of large dose of either sitagliptin or simvastatin will be compared with combination of both drugs. Thirty mice were equally divided into the following groups: control group, non-treated DSS-induced colitis group, DSS-induced colitis mice treated with simvastatin(50mg/kg/d)group, DSS-induced colitis mice treated with sitagliptin (100 mg/kg/day) group, DSS-induced colitis mice treated with sitagliptin (20mg/kg/d) combined with simvastatin (5mg/kg/d) group. Combination of sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d produced a significant decrease in serum TNF-α, colonic tissue MDA & NO levels.Furthermore, reduced glutathione was significantly increased up to near normal level. As regard colon length, it returned to nearly normal length. Combination of small dose of both drugs showed 66% decrease in DAI. This combination restored normal histological appearance of the colon. This is a very good combination as regards to their small doses and consequently less adverse effects.

Keywords: Sitagliptin, Simvastatin, TNF-α, MDA, Ulcerative colitis

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INTRODUCTION

The inflammatory bowel diseases (IBD), Crohn`s disease (CD) and ulcerative colitis (UC), are immune-mediated disorders resulting in chronic, relapsing inflammation of the gastrointestinal tract. Repeated damage and injury of the intestinal surface are key features of inflammatory bowel disease, and require the constant repair of the epithelium. While no specific etiology has been defined, the complex nature of IBD supports that its origin is likely multi-factorial[1]. Ulcerative colitis is a chronic-relapsing, progressive inflammatory bowel disease characterized by diffuse mucosal inflammation of the colon[2]. Although great advances have been made in the management of the disease with the introduction of immune-modulators and biological agents, a curative therapy does not yet exist. Therefore, it is still challenging to develop novel specific therapies for IBD[3]. It can be concluded that sitagliptin is partially effective for treatment of experimentally induced ulcerative colitis model in mice. In addition, comparing the two doses of sitagliptin, large dose has more curative effect than small dose. The results may reflect the increased proliferation and regeneration of epithelial cells rather than anti-inflammatory effects[4]. Furthermore, it can be also concluded that effects of simvastatin treatment were mostly dose dependantfor treatment of experimentally induced ulcerative colitis model in mice. This is because simvastatin (50 mg/kg/d)rather than(5mg/kg/d)showed highly significant effects on colon length, DAI, histopathological score, NO, MDA and TNF-α levels indicating that the large dose has a more significant anti-inflammatory and antioxidant role in early treatment of DSS induced colitis model. Unfortunately this high dose has no clinical application in human due to toxicity. So it has been advised to use simvastatin with a dose of 5mg/kg as an early treatment of DSS induced colitis model that is a commonly used model for the IBD[5]. Thus, the aim of this study is to evaluate effect of combination of sitagliptin and simvastatin as an early treatment of experimentally induced ulcerative colitis in mice. Furthermore in this study, effect of large dose of either sitagliptin or simvastatin will be compared with combination of both drugs.

MATERIALS AND METHOD

Animals

Thirty female C57BL/6 mice were purchased from (Tudor Bilharz Institute, Cairo, Egypt). This work was done at Mansoura Experimental Research Center (MERC), Faculty of Medicine, Mansoura University. Mice were separated into four groups (n = 6/group) and housed under standard conditions (25 °C and 12-h light–dark cycle, 6 mice per 80 cm2 cage)for at least 1 week to acclimate before starting the experiments. Throughout the experiments the mice were fed with

standard pellet diet ad labium. All protocols were approved by our local committee of Animal Care and Use. At day 5 of the experiment all animals receiving 3% dextran sulphate sodium (DSS) showed clear clinical signs of acute colitis. On showing signs, treatments started from day 5 till day 14.

Induction of DSS colitis

Acute colitis was induced by administration of 3% (w/v) dextran sulphate sodium (TDB consultancy, Sweden; MW 40,000) dissolved in sterile filtered drinking water. The animals had free access to the DSS solution, which was changed every day for 7 days then return to plain water drinking at day 8[6].

Drugs and chemicals

- ***Dextran sodium sulphate (DSS)*:** 3% (w/v) dextran sulphate sodium (purchased from TDB consultancy, Sweden; MW 40,000) dissolved in sterile filtered drinking water. The animals had free access to the DSS solution, which was changed every day for 7 days**.**
- ***Simvastatin (Zocor®):*** it was obtained from MERCK in form of 20 mg tablets. Tablets were suspended in 0.5 % methyl-cellulose solution (vehicle) and administrated once daily by oral gavage.
- ***Sitagliptin (januvia®)*** was obtained from MERCK in form of 100 mg tablets.
- ***PBS(phosphate buffer solution)***

  It was prepared by dissolving the following chemicals into 1000 ml distilled water, used for washing the blood and in tissue homogenization.

  Sodium dibasic phosphate 2.17gm

  Potassium dihydrogen phosphate 0.2gm

  Sodium Chloride 7.1gm

Animal grouping and experimental design

Mice were divided into the following groups (6 in each group):

Group 1: control healthy group received plain filtered water.

Group 2: control non treated group received 3% DSS for 7 days starting from day 1 to day 7 then return to plain water drinking till day 14[7]

Group 3: DSS-induced colitis mice treated with simvastatin by oral gavage once daily (50mg/kg/d) starting from day 5 to day 14[8].

Group4: DSS-induced colitis mice treated with sitagliptin by oral gavage once daily (100 mg/kg/day) starting from day 5 to day 14[9].

Group5: DSS-induced colitis mice treated with sitagliptin (20mg/kg/d) combined with simvastatin (5mg/kg/d) by oral gavage starting from day 5 to day 14[8,10]. For scoring colitis activity, weight changes were recorded daily throughout the experiment. Fecal samples of each animal were visually inspected for signs of diarrhea and rectal bleedings. The disease activity index (DAI) was calculated by summarizing the scores for weight loss, stool consistency, hemoccult positivity (detected by Benzidine test) or gross bleedings (Table 1).DAI value is the combined scores of weight loss, stool consistency, and bleeding divided by 3[11]. At day 14; after the end of each group treatment protocol, animals were anesthetized by thiopental, dissected and postmortem blood was collected by cardiac puncture then allowed to stand for clotting, centrifuged and serum was separated. The entire colon was removed, gently flushed with cold PBS to remove contents and blood clots, placed on an ice-cold plate, cleaned of fat and mesentery and dried between two filter papers then weighed. Each colon was gently stretched; the length of colon was measured from the colocecal junction to the anus as indirect marker of inflammation (rate of colon shortening) for each mouse.

Table 1: Scoring of Disease Activity Index

Score	Weight loss (%)	Stool consistency	Occult/gross bleeding
0	None	Normal	Normal
1	1-5%		
2	5–10%	Loose stools	Occult bleeding
3	10–15%		
4	>15%	diarrhea	Gross bleeding

Preparation of colonic homogenates

Immediately after sacrificing the mice, colons were excised and washed twice with PBS, dried between two filter papers then homogenized in 5 ml PBS per gram tissue, centrifuged at 4000 r.p.m for 15 minutes. Supernatant was removed divided into aliquots and frozen at - 80°C until assayed.

Histopathological examination

Colon segments were fixed in 10% formalin solution for 24-h. Paraffin wax tissue blocks were prepared for sectioning at 4 μm thickness by sledge microtome. The obtained tissue sections were collected on glass slides deparaffinized. Slides were H&E stained, and scored according to the criteria listed in Table 2. Individual scores and the sum of all scores were calculated[12].

Biochemical assay

1-Determination of TNF-α concentration in mouse serum as an inflammatory marker (Mouse TNFα ELISA Kit, Bosterimmunoleader): according to Brenner **et al.,[13].**

2- Oxidative stress markers

A. Colorimetric determination of colonic homogenates reduced glutathione (GSH) according to Beutler et al.,[14].

B. Colorimetric determination of colonic homogenates Nitrite according to Montgomer & Dymock,[15].

***C.* Colorimetric determination of colonic homogenates lipid peroxide (Malondialdehyde)** according to Ohkawa et al.,[16].

Table 2: Histopathological Scores

Grade	Extent of inflammation	Infiltration neutrophils+ Lymphohistio-cytes	Extent of crypt damage	Crypt abscesses	Sub-mucosal oedema	Loss of goblet cells	Reactive epithelial hyperplasia
0	None	None	None	None	None	None	None
1	mucosa	focal	Basal one third	focal	Focal	focal	Focal
2	Mucosa+ submucosa	multifocal	Basal two thirds	multifocal	multifocal	multifocal	Multifocal
3	Mucosa+submucosa+ muscle layer	diffuse	Entire crypt damage		diffuse	diffuse	Diffuse
4	Transmural		crypt damage+ ulceration				

Analytical Statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

ANOVA (analysis of variance):-

Used to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey for multiple comparisons.

Kruskal-Wallis test:

Used to compare between more than two groups of numerical (non- parametric) data. A *P* value <0.05 was considered statistically significant in all analyses.

RESULTS AND DISCUSSION

Effect of sitagliptin (100mg/kg), Simvastatin (50mg/kg) on DAI

DAI is a useful index for the degree of colitis. Mice receiving DSS showed a significant increase in DAI compared to control group ($p < 0.01$). Treatment of DSS induced colitis by sitagliptin (100 mg/kg/d) reduced DAI by 16% as compared to DSS control group. Administration of Simvastatin (50 mg/kg/d) reduced the percentage of DAI by 41% compared to DSS control group (figure 1).

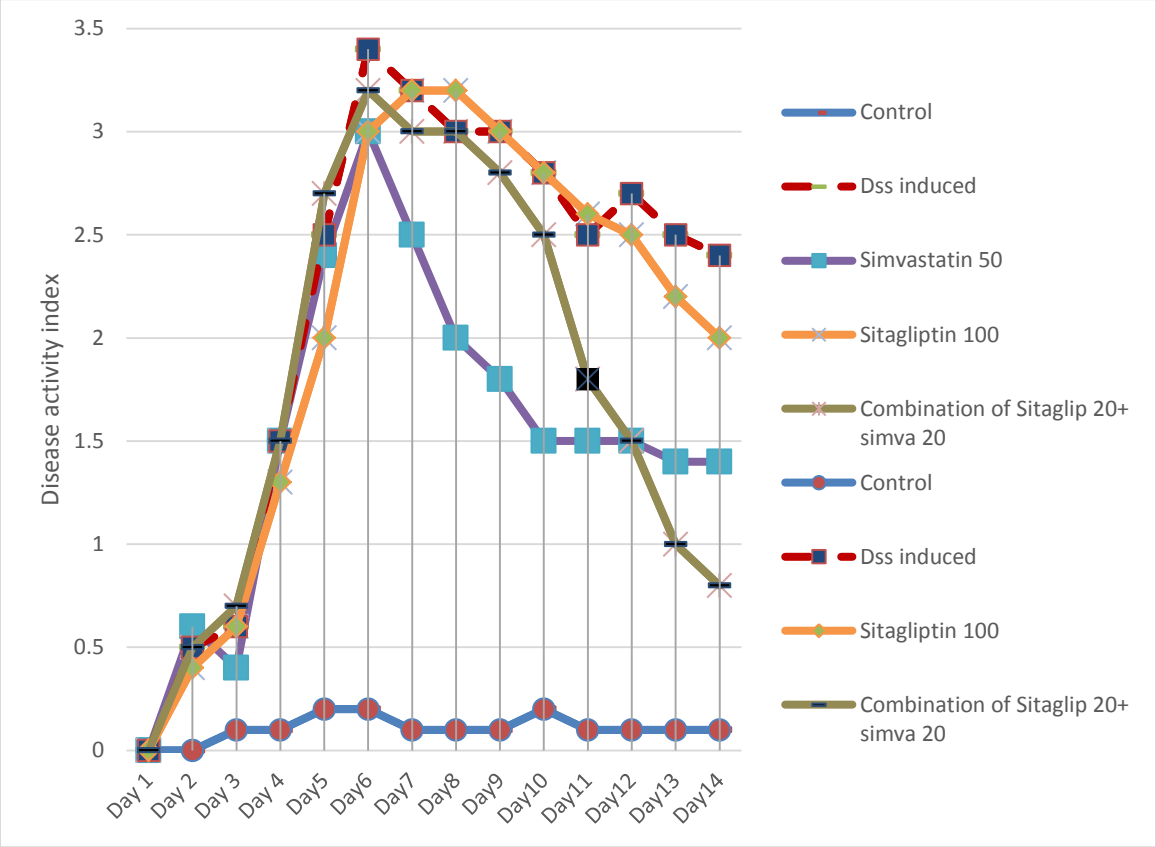

Figure 1: Effect of Simvastatin, Sitagliptin& A Combination Of small dose of Each on Disease Activity Index (DAI) In DSS- Induced Colitis in Mice.

Effect of sitagliptin (100mg/kg), Simvastatin (50mg/kg) on Colonic length

As shown in Table (3) induction of colitis by DSS produced significant shortening of colon length (6.10± 0.56) of mice as compared to the control normal (8.25 ± 0.23) group ($p < 0.001$). Treatment of DSS induced colitis by sitagliptin in doses of (100 mg/kg/d) showed non significant increase (6.47± 0.46) in colon length ($p > 0.05$) compared to DSS induced colitis control group .While simvastatin treated group in doses of (50 mg/kg/d) showed highly significant increase (7.18± 0.28) in colon length of mice ($p< 0.001$) compared to DSS- induced colitis group.

Effect of sitagliptin (100mg/kg), Simvastatin (50mg/kg) on inflammatory markers

As shown in Table (3): induction of colitis by DSS produced a significant increase in serum TNF-α (191.62 ± 58.86) compared with the control normal (30.83 ± 9.91) group ($p < 0.001$). Furthermore, treatment of DSS induced colitis by simvastatin (50 mg/kg/d) reduced (81.03 ± 38.86) TNF-α level significantly ($p < 0.001$) as compared to DSS induced colitis group. Sitagliptin treated groups in doses of (100 mg/kg/d) significantly reduced (83.45 ± 28.63) TNF-α level ($p < 0.001$) as compared to DSS induced colitis group

Effect of sitagliptin (100mg/kg), Simvastatin (50mg/kg)on oxidative stress

Table (4) showed that induction of colitis by DSS produceda significant increase in the MDA (7.63 ± 0.66), NO (45.75 ± 10.47) levels and decreased reduced glutathione (0.67±0.19) level in the colon a ($p < 0.001$) as compared to normal group. Treatment of DSS induced colitis by simvastatin in dose of (50 mg/kg/d) showed significant decrease in NO (24.82 ± 6.73) and MDA (4.57 ± 0.85) levels and significant increase in rGSH level ($p < 0.001$) as compared to DSS induced colitis group. Treatment of DSS induced colitis by sitagliptin (100 mg/kg/d) showed non significant change in colonic NO (29.90 ± 10.38) and rGSH(1.07±0.27) levels ($p >0.05$) as compared to DSS induced colitis group and reduced MDA(5.30 ± 0.63) level($p < 0.01$)as compared to DSS induced colitis group.

Effect of sitagliptin (100mg/kg), Simvastatin (50mg/kg) on histopathological lesion

As compared to Figure. 2A (normal histological appearance of the mouse colon), Figure. 2B showing that histological signs of colonic inflammation were: multifocal mucosal infiltrations of predominantly neutrophils and lympho- histocytes (grade 2) diffuse oedema of submucosa (grade 3). The extent of inflammation involved mucosa, submucosa and muscle layer (grade 4). Extent ofcrypt damage included multiple ulcerations characterised by complete loss of the mucosal epithelium (grade 4). As shown in figure IIC treatment of DSS induced colitis by simvastatin at dose of 50mg/kg/d **(**$p < 0.01$ as compared to DSS control group) reduced the extent of inflammation that involved only mucosa and submucosa (grade 2) with focal mucosal infiltrations of neutrophils and lymphohistocytes (grade 1). Basal one third of the crypts were lost (grade 1). Figure IID showed that sitagliptin at dose of (100 mg/kg/d) led to non significant reduction of DSS induced colitis inflammation showing focal mucosal infiltrations of neutrophils and lymphohistocytes (grade 1) with lost entire crypts (grade 3). Inflammation extended to mucosa and submucosa (grade 2)

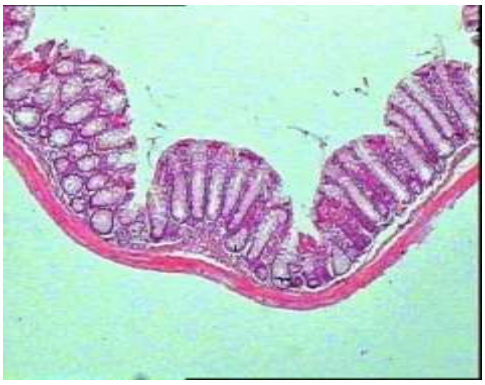

Figure 2A: Histology of the mouse colon in vehicle control tissues, showing normal histological appearance of the mouse colon.

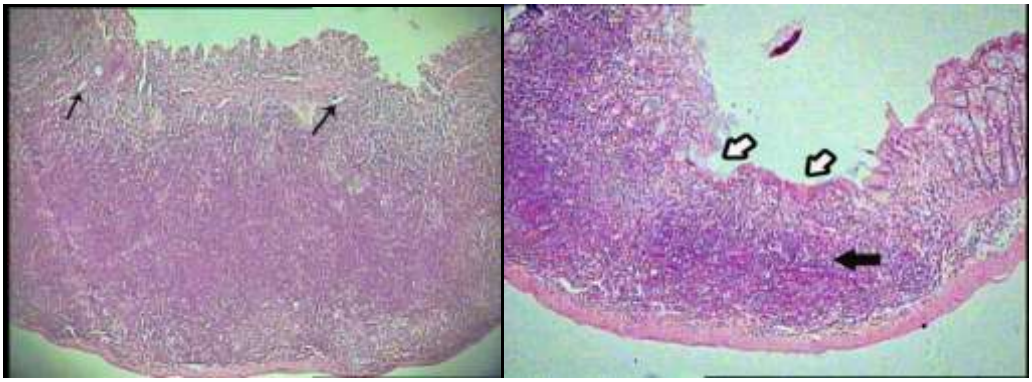

Figure 2B: Histopathological findings of hematoxylin and eosin-stained colonic tissue sections from DSS-treated mice

Open arrows show lost entire crypts with ulceration and diffuses loss of goblet cells. Bold black arrow shows diffuse inflammatory infiltrate involved mucosa, submucosa and muscle layer. Thin arrows show submucosal edema.

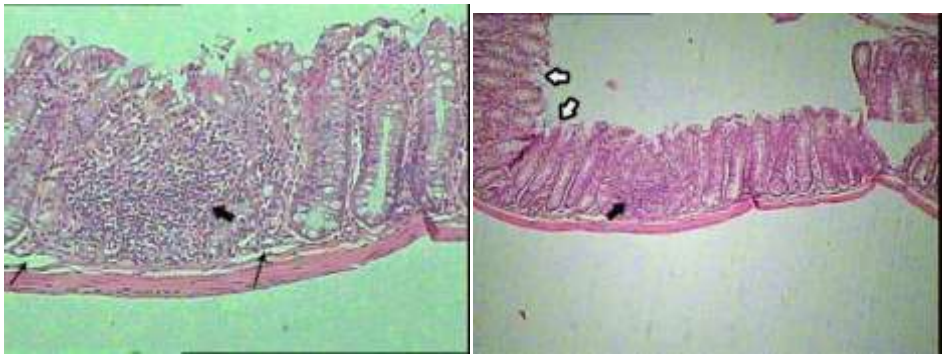

Figure 2C: Histopathological findings of hematoxylin and eosin-stained colonic tissue sections from DSS induced colitis group treated with simvastatin 50mg/kg/d

Open arrows show lost basal one third of the crypts. Bold black arrow show focal infiltrates involving mucosa only. Thin arrows show focal submucosal edema.

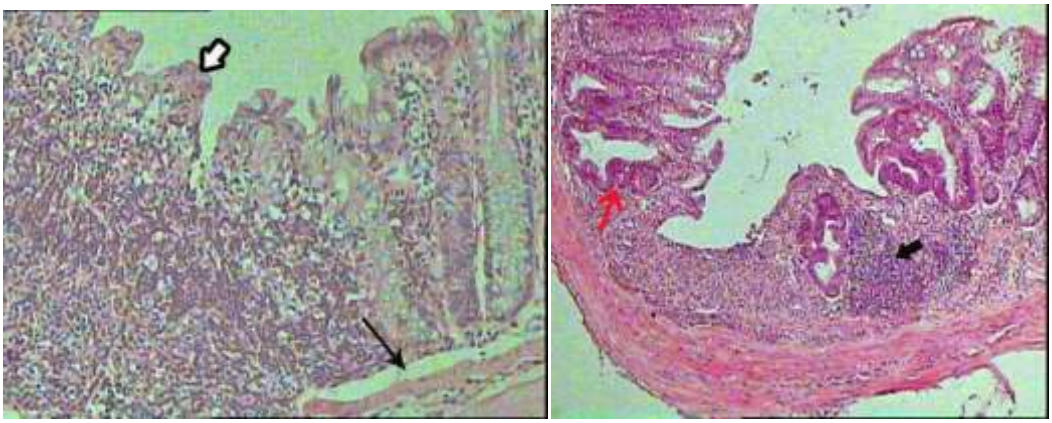

Figure 2D: Histopathological findings of hematoxylin and eosin-stained colonic tissue sections from DSS induced colitis group treated with sitagliptin 100mg/kg/d

Open arrow shows lost basal two thirds of the crypts. Bold black arrow shows focal mucosal infiltrates. Thin arrows show submucosal edema. RED arrow show dysplastic changes

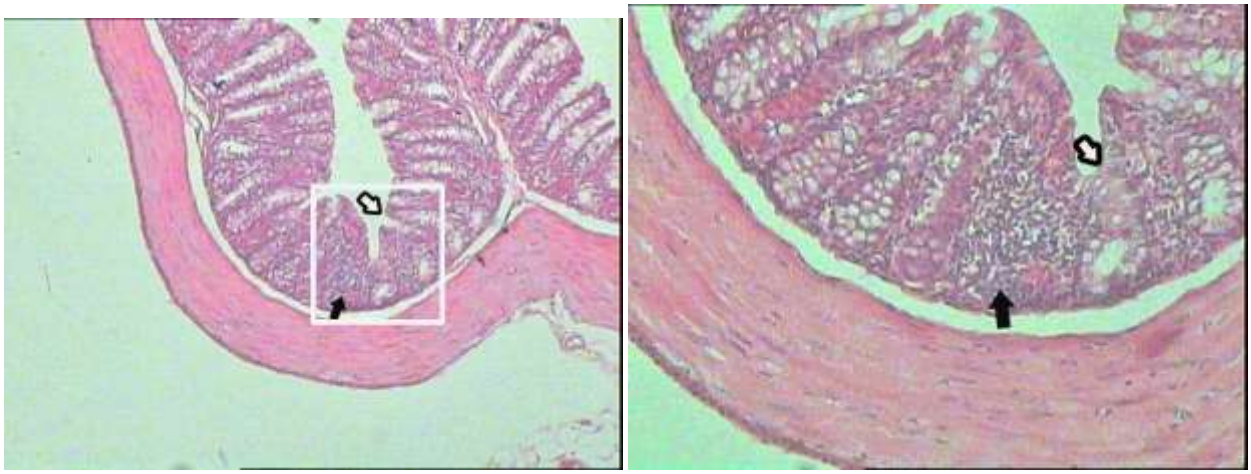

Figure 2E: Histopathological findings of hematoxylin and eosin-stained colonic tissue sections from DSS induced colitis group treated with simvastatin 5mg/kg/d+ sitagliptin 20 mg/kg/d

This section shows only a focal area of lesion with restored histological appearance of the rest of the segment. Open arrow shows lost basal one third of intestinal crypt. Bold black arrows show focal inflammatory infiltrate involved mucosa only with normal submucosa and muscle layer. There was neither submucosal edema nor reactive epithelial hyperplasia (REH)

Effect of combination of Sitagliptin (20mg/kg) and Simvastatin (5mg/kg) on DAI

As shown in figure 1 treatment of DSS induced colitis by Combination of Sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d showed 66% decrease in DAI as compared to DSS control group**.**Combination of small dose of both drugs showed more decrease in DAIas compared tolarge dose of sitagliptin (16%)orlarge dose ofSimvastatin (41%), given individually**.**

Effect of combination of Sitagliptin (20mg/kg) and Simvastatin (5mg/kg) on inflammatory markers

As shown in Table (3): induction of colitis by DSS produced a significant increase in serum TNF-α compared with the control normal group ($p < 0.001$). It noted that DSS induced colitis treated with combination of sitagliptin 20+ simvastatin 5 mg/kg/d produced a significant decrease in serum TNF-α compared with the non treated DSS induced colitis group .The results were non significantly different from the control value(49.85 ± 8.79)as compared to normal control(30.83 ± 9.91) group.

Effect of combination of Sitagliptin (20mg/kg) and Simvastatin (5mg/kg) on Colonic length

As shown in Table (3) induction of colitis by DSS produced significant shortening of colon length of mice as compared to the control normal group ($p < 0.001$). Treatment with combination of

Sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d showed highly significant increase (7.75± 0.33)in colon length of mice(p< 0.001) compared to DSS(6.10± 0.56) induced colitis group**.**Comparing this group to normal control group (8.25 ± 0.23),there was no significant difference (P=0.261).

Table 3: Effect of Simvastatin, Sitagliptin& a Combination of Small Dose Of Each, on Clinical Indices of Inflammation& Serum Tumor Necrosis Factor (TNF) - Alpha in DSS-Induced Colitis In Mice.

Groups *(n=6)*	**Colonic length (cm*)***	**Serum TNF alpha(Pg/ml)**
Control group	8.25 ± 0.23	30.83 ± 9.91
DSS- induced colitis group	6.10±0.56[A]	191.62 ± 58.86 [A]
Simvastatin (50 mg/kg/d) treated group	7.18±0.28[B]***	81.03 ± 38.86[B]***
Sitagliptin (100 mg/kg/d) treated group	6.47±0.46[C]	83.45 ± 28.63[B]***
Combination of Sitagliptin 20mg/kg/d + simvastatin 5 mg/kg/d	7.75±0.33[B]***	49.85 ± 8.79[B]***

A. compared to the normal group (p < 0.001).

B. compared to DSS induced colitis control group (***p < 0.001).

C. compared to DSS induced colitis group (p >0.05).

Effect of combination of Sitagliptin (20mg/kg) and Simvastatin (5mg/kg) on oxidative stress

Table 4 showed that induction of colitis by DSS produceda significant increase in the MDA, NO levels and decreased reduced glutathione level in the colon a (p < 0.001) as compared to normal group. With treatment by combination of sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d, MDA(3.53 ± 0.49), NO(14.00 ± 4.42) levels were non significantly different, as compared to normal (2.95 ± 0.38, 11.12 ±5.43, respectively) control group. However, reduced glutathione was significantly increased up to near normal level.

Table 4: Effect of Simvastatin, Sitagliptin & a Combination of Small Dose of Each on Oxidative Stress Markers activity of Colonic Tissue in DSS- Induced Colitis in Mice.

Groups *(n=6)*	**Colonic Nitric oxide (µmol/L)**	**Colonic MDA (nmol/mg)**	**Colonic r GSH (mmol/gm)**
Control group	11.12 ±5.43	2.95 ± 0.38	1.57±0.36
DSS- induced colitis group	45.75 ± 10.47 [A]	7.63 ± 0.66 [A]	0.67±0.19 [A]
Simvastatin (50 mg/kg/d) treated group	24.82 ± 6.73[B]*	4.57 ± 0.85[B]***	1.43±0.41 [B]*
Sitagliptin (100 mg/kg/d) treated group	29.90 ± 10.38[C]	5.30 ± 0.63[B]**	1.07±0.27 [C]
Combination of Sitagliptin 20mg/kg/d + simvastatin 5 mg/kg/d	14.00 ± 4.42[B]***	3.53 ± 0.49[B]***	1.48±0.45 [B]**

[A], p < 0.001 compared with the normal group

[B], *** p < 0.001 compared to DSS induced colitis control group.

[C], p >0.05 compared to DSS induced colitis control group.

Effect of combination of sitagliptin (20mg/kg) and Simvastatin (5mg/kg) on histopathological lesion

As shown to figure IIE: treatment withcombination of sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d led to highly significant reduction of histopathological scores ($p < 0.001$ as compared to DSS control group) showing focal in filtrations of mucosa only (grade 1) by neutrophils and lympho-histocytes (grade 1) and no crypt damage or loss of basal one third only (grades 0-1). There was no submucosal oedema or goblet cell loss (grade 0). Thus, combination of simvastatin and sitagliptin restored normal histological appearance of the colon segment and shows only a focal area of lesion. The sum of histopathological score of combination group was reduced to 2. In the present study, DSS induced colonic and systemic inflammation in mice when administered for 7 days in drinking water as evident from the DAI. DSS-induced colitis is a well established experimental model that mimics many of the features of human UC, including diarrhea, bloody feces[17] and colonic shortening[18]. These effects of DSS on the colon were explained by the fact that DSS can promote inflammation by many biological pathways including direct cytotoxic effects[19] as well as apoptotic damage of colonic epithelial cells[20]. In the present study, colitis led to a significant increase in the pro-inflammatory markers in the plasma of mice as apparent from increased levels of TNF-α in the plasma of DSS- treated animals as compared to the control animals. This is in consistent with study of ***Nishiyama et al.,***[21] who showed an elevation of the disease activity index score and histological damage score induced by DSS. Based on the changes in tumor necrosis factor-alpha in plasma .Furthermore, ***Oz etal.,***[22] found that Inflammatory cytokine levels like TNF-α was considerably increased in DSS-induced moderately severe colitis in wild type mice. In the present study, inflammation in the colon led to the generation of oxidative stress as indicated from a significant increase in the MDA and NO parameters in the colonic tissue as compared to the control group. Similar findings have been previously reported [23,24]. The production and release of ROS species by immune cells appear to play an important role in the pathophysiology of colitis[25]. Increased MDA level in stress condition is responsible for lipid membrane destruction and tissue injury[26]. NO reacts with O2 -produced by activated neutrophils- to form another potent oxidant, peroxynitrite (ONOO). ONOO administration to the colon results in tissue injury[27]. However, inducible nitric oxide synthesase (iNOS)-derived NO stimulates TNF-α production in the middle and distal colon, which promotes the infiltration of neutrophils for example through stimulation of synthesis of intracellular adhesion molecule (ICAM) and P-selectin, therefore leading to colonic tissue damage[28]. Furthermore, in the current study, there was a reduced GSH level in the colonic tissue when compared to the control group. Depletion of

GSH is considered a crucial event of colonic damage occurring both in human IBD and in animal models[29]. This depletion could be a consequence of enhanced production of free radicals and could represent a specific disorder due to an impaired colitis activity of GSH synthesizing enzyme[30]. Furthermore, the sums of histopathological scores were between 9 and 16 in the non-treated DSS groups in all studies[12]. Histological signs of colonic inflammation were focal mucosal infiltrations of predominantly neutrophils and lympho- histocytes (grade 2), multifocal submucosal oedema (grade 2). The extent of inflammation affected mucosa, submucosa and muscle layer (grade 3).These were evident in figure IIB. The results of the present study are consistent with the previous work of El ***Katary et al.,***[4,5]. It has been advised to use simvastatin with a dose of 5mg/kg as an early treatment of DSS induced colitis model that is a commonly used model for the IBD[5]. As we has concluded previously, thatthe effects of simvastatin treatment were mostly dose dependant as simvastatin (50 mg/kg/d)showed highly significant effects on colon length, DAI, histopathological score, NO, MDA and TNF-α levels. However, unfortunately this high dose has no clinical application in human due to toxicity. Furthermore, it has been concluded that sitagliptin is partially effective for treatment of experimentally induced ulcerative colitis model in mice[4]. The results of the current study showed that:DSS induced colitis treated with combination of sitagliptin 20 mg/kg/d + simvastatin 5 mg/kg/d produced a significant decrease in serum TNF-α. Moreover, treatment by both drugs (simvastatin and sitagliptin) reduced colonic tissue MDA&NOlevels that were non- significantly different from normal group. Furthermore, reduced glutathione was significantly increased up to near normal level. As regard colon length, it returned to nearly normal length. Combination of small dose of both drugs showed 66% decrease in DAI. Interestingly, this combination restored normal histological appearance of the colon segment and shows only a focal area of lesion. The sum of histopathological score of combination group was reduced to 2 showing a great resolution of extent of inflammation and infiltration of neutrophils and repair of damaged crypts. Furthermore, the results of the current study showed that the effect of combination of sitagliptin and simvastatin in those doses is better than the effect of large dose of sitagliptin or simvastatin, individually as regards to colonic nitric oxide, reduced glutathione and histopathological score. This combination has a synergistic effect which can be explained bythat it targets many points of molecular pathophysiological changes occurring in IBD. Sitagliptin have been shown to be effective in limiting the activation processes of immunity and to modify the course of disease associated with an imbalanced T cell response, as in IBD[6, 9, 31] resulting in a decreased secretion of pro-inflammatory cytokines, including tumor necrosis factor (TNF) - α and interferon (IFN)-c as well as an increase in the anti-inflammatory cytokine

transforming growth factor (TGF)-b[32]. Furthermore, Simvastatin is a commonly prescribed statin with antioxidant and anti-inflammatory properties[33, 34]. Simvastatin have pleiotropic effects, including immune-modulatory and anti-inflammatory effects[35]. Simvastatin inhibited monocyte adhesion to endothelial cells induced by TNF-alpha. Moreover, simvastatin protects endothelial progenitor cells from TNF-α- mediated apoptosis[36].

Table 5: Effect of Simvastatin, Sitagliptin& A Combination Of small dose of Each on Histopathological Score of DSS- Induced Colitis in Mice.

Groups *(n=6)*	**Control group**	**DSS-induced colitis group**	**Simvastatin (50 mg/kg/d) treated group**	**Sitagliptin (100mg/kg/d) treated group**	**Combination of Sitagliptin 20+simvastatin 5 mg/kg/d**
Histopathological score	00.00	9.50 ±8.00-16.00 [A]	4.00 ±2.00-9.00[B]**	5.00 ±4.00-12.00[C]	2.00 ±2.00-6.00[B]***

[A],p < 0.001 compared with the normal group

[B], *** p < 0.001 compared to DSS induced colitis control group.

[C], p >0.05 compared to DSS induced colitis control group.

CONCLUSION

It has been concluded that, this study is the first one to demonstrate that the combination of small dose of sitagliptin and simvastatin is more effective than large dose of each individual drug, for treatment of experimentally induced ulcerative colitis model in mice. This is a very good combination as regards to their small doses and consequently less adverse effects.

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