Journal of Experimental Research

March 2022, Vol 10 No 1

Email: editorinchief.erjournal@gmail.com editorialsecretary.erjournal@gmail.com

Received: 16th Jan., 2022

Accepted for Publication: 2nd March, 2022

MULTI-ORGAN METABOLIC PERTURBATION STUDY OF DEXTRAN SULFATE SODIUM-INDUCED ULCERATIVE COLITIS USING GLYCOLYTIC AND MITOCHONDRIAL METABOLIZING ENZYMES AS INDICES

Kehinde SA[1], Ore A[1], *Olajide AT, Fatoki JO[2], Faokunla O[3]

[1]Department of Chemical Sciences, Faculty of Natural Science, Ajayi Crowther University, Oyo, Oyo State, Nigeria.
[2]Department of Medical Biochemistry, School of Basic Medical Sciences, Osun State University, Osogbo, Osun State. Nigeria.
[3]Department of Biochemistry, Faculty of Science, Federal University, Lokoja, Kogi State, Nigeria.

***Author for Correspondence:** at.olajide@acu.edu.ng.

ABSTRACT

Ulcerative colitis is an inflammatory bowel disease, which includes chronic inflammation of the gastrointestinal tract. Recent studies have suggested that the etiology of inflammatory bowel disease is multifactorial, resulting from the interplay of immunological, molecular, genetic, microbial, diet, drug use-related, and environmental factors. This study was designed to investigate multiple organ toxicity of Dextran sulfate sodium-induced (DSS) ulcerative colitis using glycolytic and mitochondrial metabolizing enzymes as indices**.** Twelve mice were divided into two groups of six mice each. Group A (Control) received normal drinking water while group B was fed with 2.5% DSS for 7 days in their drinking water, and the dextran sulfate sodium solution was replenished daily. The liver, kidney, colon, spleen was excised from the mice after the last administration of DSS, glycolytic and mitochondrial metabolizing enzymes were assessed in all the organs and lymphocytes. Activities of glycolytic enzymes lactate dehydrogenase and NADase were down-regulated in all the organs. Hepatic hexokinase activity significantly reduced as opposed to the increase observed in other organs, while aldolase activities were up-regulated in all the organs. Furthermore, DSS administration caused perturbation in the activities of mitochondrial metabolizing enzymes in all the organs. Activities of succinate dehydrogenase, malate dehydrogenase, Combined Complexes I+III, II+III, and IV were down-regulated. All observations are relative to control. ***D***ata from this study demonstrated that administration of DSS induced ulcerative colitis which invariably perturbs the glycolytic enzymes while mitochondrial metabolizing enzymes are down-regulated leading to decreased energy availability for cellular processes during ulcerative colitis pathological condition.

Keywords: Glycolysis, Mitochondria, Toxicity, Colitis, Inflammation.

INTRODUCTION

Human inflammatory bowel disease (IBD), ulcerative colitis, and Crohn's disease being the most important entities are chronic, relapsing, and remitting inflammatory conditions that result from chronic dysregulation of the mucosal immunesystem in the gastrointestinal tract (Stephens et al. 2013). Ulcerative colitis (UC) is an inflammatory bowel disease (IBD), which includes chronic inflammation of the gastrointestinal tract (Tanideh et al. 2016). The primary symptoms of UC are acute and chronicinflammation of the mucosa, diarrhea, and rectal bleeding (Tanidehet al. 2016).Recent studies have suggested that the etiology of IBD is multifactorial, resulting from the interplay of immunological, molecular, genetic, microbial, diet, drug use-related, and environmental factors (Stephens et al. 2013). Obtaining about 70% of the blood supply from the portal vein, which is the direct venous outflow of the intestine, the liver is the first and main organ exposed to gut-derived substances, such as ingested nutrients and bacterial products (Adawi et al. 1999). IBD is considered to affect liver homeostasis (Sartini et al. 2018).

Among various chemical-induced colitis models, the DSS-inducedcolitis model is widely used because of its simplicity and many

similarities with humanulcerative colitis. Dextran sulfate sodium, a polymer of sulfated polysaccharide is widely used for the induction of ulcerative colitis. It is thought to induce mucosal injury and inflammation through a direct toxic effect on epithelial cells with subsequent activation of macrophages and T-lymphocytes resulting in cytokine-mediated cytotoxicity. DSS is also known to be a chemical colitogen with anticoagulant properties (Sartini et al. 2018). However, there is a lack of comprehensive studies investigating the correlation between the severity of ulcerative colitis and perturbation of glycolytic and mitochondrial metabolizing enzymes of the liver, kidney, colon, spleen, and lymphocytes. Thus, this study aims to evaluate the severity of ulcerative colitis in relation to perturbation of glycolytic and mitochondrial metabolizing enzymes in mice.

MATERIAL AND METHODS

Chemicals and Reagents

DSS (molecular weight, 40 kDa) was obtained from ICN BiomedicalsInc, Cleveland, OH, USA. The diagnostic kit for lactate dehydrogenase was a product of CYPRESS® Diagnostics, Langdrop, Belgium. Mannitol, sorbitol, sucrose, glucose-6-phosphate dehydrogenase, ethylenediaminetetraacetic acid (EDTA), nicotinamide adenine dinucleotide (NADH), fructose-1,6-bisphosphate, succinate, phosphoenolpyruvate, oxaloacetate, rotenone, cytochrome C, and tris (hydroxymethyl) aminomethane (Trizma base) were products of Carlroth GMBH, Karlsruhe, Germany. All other reagents used were of the purest grade available and were products from British Drug House (BDH) Chemicals Limited, Poole, England, and Sigma-Aldrich, Missouri, USA.

Experimental Animals and Treatment

Twelve BALB/c mice weighing 20-23g were obtained from the central animal house, College of Medicine, University of Ibadan, Ibadan. The mice were kept in plastic cages on a 12-h light:12-h dark cycle, at room temperature of 22–24°C at the animal house, Ajayi Crowther University, Oyo for the period of acclimatization and treatment. The mice were acclimatized for a period of one week and were allowed free access to food and water. Handling of the experimental animals was consistent with international principles on the care and use of experimental animals (National Research Council., 2011).This study was approved by the Faculty of Natural Sciences Ethical Review Committee (FNS / ERC / 2021/ 007), Ajayi Crowther University, Oyo, Oyo State, Nigeria. Animals were assigned into two groups of six mice each: Group I animals were the control that were administered normal drinking water only, while group II received DSS (2.5%) orally.

Ulcerative Colitis was induced by replacing normal drinking water with a 2.5% (w/v) solution of DSS (molecular weight, 40 kDa; ICN Biomedicals, Inc., Aurora, OH) dissolved in water. Assessment of inflammation in DSS-treated mice was carried out Daily with clinical assessment including measurement of drinking water volume and body weight, evaluation of stool consistency, and the presence of blood in the stools by the guiac paper test (ColoScreen, Helena Laboratories, Beaumont, TX). Control mice received normal drinking water.

Analytical Methods:

Preparation of Tissue Homogenate

Animals were sacrificed and blood was collected into heparinized tubes from the abdominal artery under light ether anesthesia after an overnight fast and rapidly dissected to excise the liver, kidney, colon, and spleen. Organs were washed in ice-cold 1.15% KCL, blotted, and weighed. Organs collected were homogenized in 10 volumes/weight of ice-cold0.1M phosphate buffer pH 7.4 and homogenized using a potter-elevehjem homogenizer. The homogenate was centrifuged at 10,000g for 15 minutes at 4^{o}C and the homogenate was stored at -4^{0}C and utilized for the biochemical assays.

Preparation of lymphocytes and mitochondrial isolates from organs

Lymphocytes were isolated by differential centrifugation using Ficollpaque as described by Boyum, 1996 from an aliquot of the blood, and samples were stored at 4^{0}C. This was done within 24 hours of blood collection.

Isolation of mitochondria was carried out using the method described by Erika et al.(2010).

Assay of Glycolytic Enzymes

The following glycolytic enzymes; hexokinase, aldolase, lactate dehydrogenase and NADase were assayed in the liver, kidney, colon, spleen, and lymphocytes.

Determination of Hexokinase Activity

Determination of the activity of hexokinase followed the procedure described by Colowick (1973). The assay is based upon the reduction of the NAD^+ through a coupled reaction with glucose-6-phosphate dehydrogenase (G-6-P-D) and was determined spectrophotometrically by measuring the increase in absorbance at 340 nm.

Determination of Aldolase Activity

Determination of the activity of aldolase followed the procedure described by Jagannathan et al. (1956) which was based on Boyer's modification of the hydrazine assay in which 3-phosphoglyceraldehyde reacts with hydrazine to form a hydrazone that absorbs at 240 nm.

Determination of the Activity of Lactate Dehydrogenase

LDH catalyzes the reduction of pyruvate by NADH, the rate of decrease in the concentration of NADH, measured spectrophotometrically at 340nm, is proportional to the catalytic concentration of lactate dehydrogenase present in the sample.

Determination of NADase Activity

NADase activity was assayed according to a procedure described by Kaplan (1995) in which cyanide reacts with the quaternary nitrogen form of NAD^+ to form an addition product with a maximum absorbance at 340 nm.

Determination of Total Protein Concentration

The concentration of total protein was determined by the method of Gornall et al. 1949in all the compartments. Briefly, the reaction mixture involves 1 mL of Biuret reagent and 100 µL of the sample. The mixture was allowed to incubate for 10 min at room temperature and the absorbance of color developed, corresponding to the total protein concentration was measured at 546 nm against reagent blank.

Assay of Mitochondrial Metabolizing Enzymes

Enzymes of the tricarboxylic acid pathway (Succinate dehydrogenase and Malate dehydrogenase), electron transport chain (combined complexes I+III, II+III, and complex IV) were assayed in the liver, kidney, colon, spleen, and lymphocytes.

Determination of Succinate Dehydrogenase (SDH) Activity

Succinate dehydrogenase catalyzes the conversion of succinate to fumarate. The activity of SDH was determined in the liver, kidney, colon, spleen, and lymphocytes following the procedure described by Veeger (1964)**.**

Determination of Malate Dehydrogenase (MDH) Activity

The interconversion of L-malate and oxaloacetate using nicotinamide adenine dinucleotide (NAD) as a coenzyme is catalyzed by MDH. The activity of MDH was estimated in the brain and liver using the procedure described by Thorne (1962). This was done by measuring the decrease in absorbance at 340nm resulting from the oxidation of NADH.

Determination of Combined Complexes I & III (NADH Cytochrome C Oxidoreductase) Activity

Activity of Complex I & III was assessed by the method of Medjaet al.(2009). This activity was assessed following the increase in absorbance of reduced cytochrome C at 550nm. These complexes transfer electrons from NADH which is oxidized to NAD, to cytochrome C.

Determination of Combined Complexes II & III (Succinate Cytochrome C Oxidoreductase) Activity

Complex II & III catalyze the transfer of electron from succinate to cytochrome C. Activity of Complex II & III was determined following the increase of absorbance of reduced cytochrome C at 550nm. Subsequent oxidation

of the reduced cytochrome C was inhibited by the addition of cyanide in the reaction medium. The method used was described by Medjaet al.(2009).

Determination of Combined Complexes IV (Cytochrome C Oxidase) Activity

In the respiratory chain, Complex IV transfers electron from reduced cytochrome C to oxygen. The activity of Complex IV was determined following the decrease in absorbance of reduced cytochrome C at 550nm using the method of Medjaet al.(2009).

Statistical Analysis

Results were expressed as mean ± SD. Data were analyzed by subjecting data obtained to F-test (ANOVA) using Graphpad Prism (V 8.01). P values less than 0.05 were considered statistically significant.

RESULTS

Data from this study showed that DSS altered the activities of both the glycolytic and mitochondrial metabolizing enzymes in the various organs studied (liver, kidney, colon, spleen, and lymphocyte).

Hepatic hexokinase activity significantly reduced ($p<0.05$) as opposed to the increase observed for hexokinase activity in other organs studied (Fig. 1a-e).

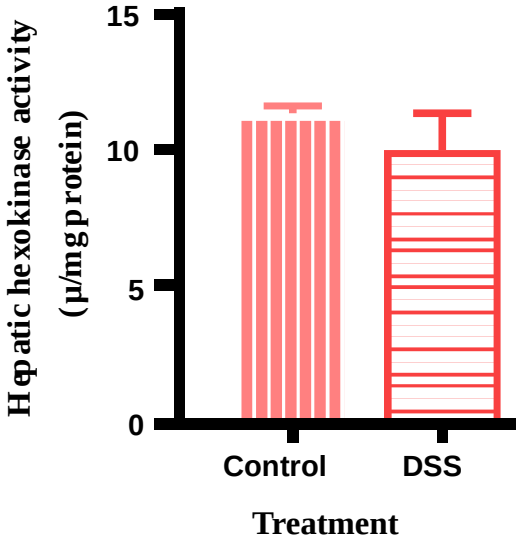

Figure 1a: Effect of DSS on hexokinase activity in the liver. *Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).*

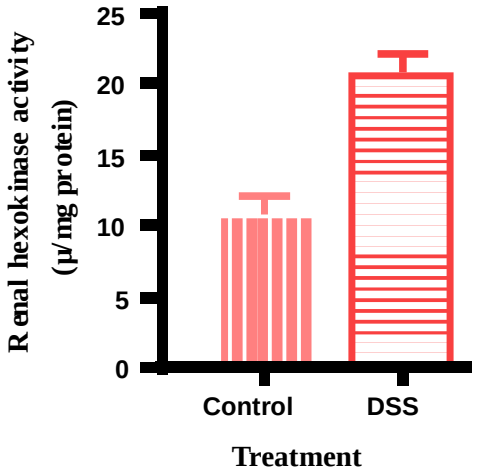

Figure 1b: Effect of DSS on hexokinase activity in the kidney. Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

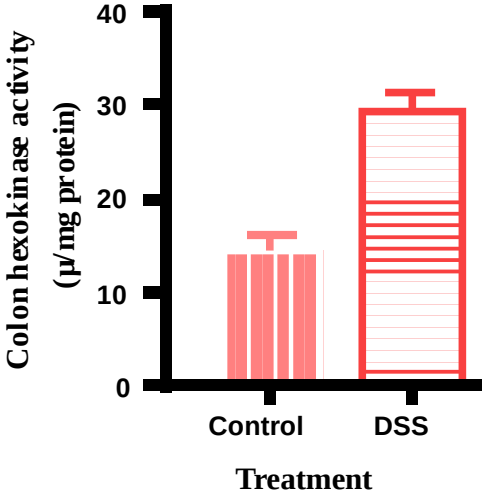

Figure 1c: Effect of DSS on hexokinase activity in the colon. Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

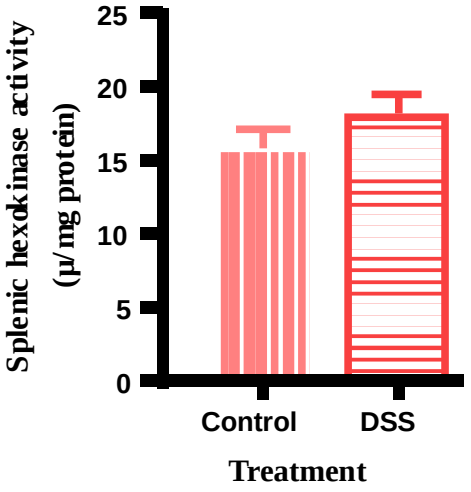

Figure 1d: Effect of DSS on hexokinase activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

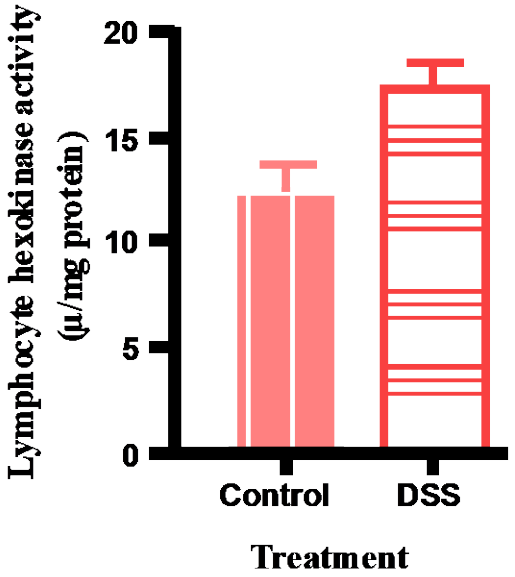

Figure 1e: Effect of DSS on hexokinase activity in the lymphocyte. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

Renal, colon, splenic and lymphocyte hexokinase activity significantly increased (p<0.05) with 52, 55, 12, and 25% respectively. However, aldolase activities were up-regulated in all the organs (Fig. 2a-e) when compared with control.

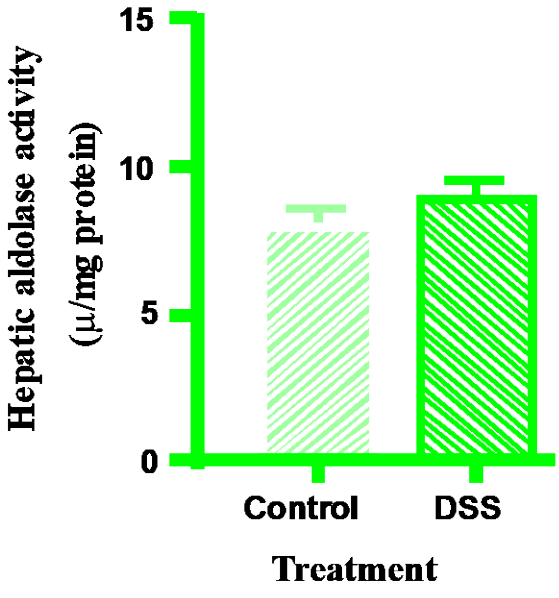

Figure 2a: Effect of DSS on aldolase activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

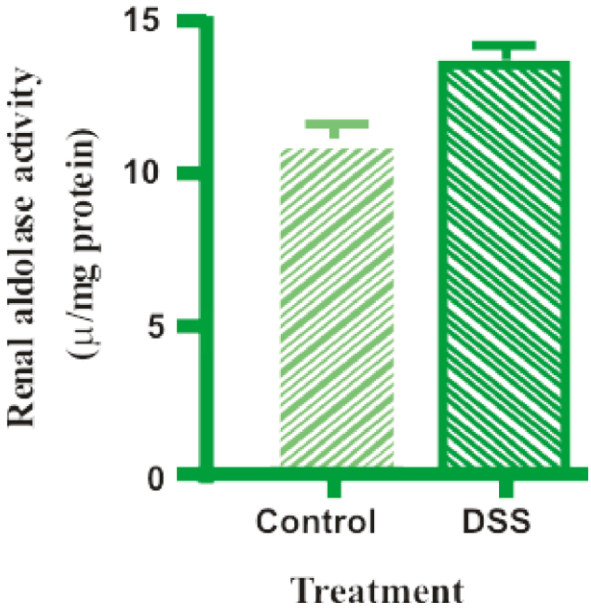

Figure 2b: Effect of DSS on aldolase activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

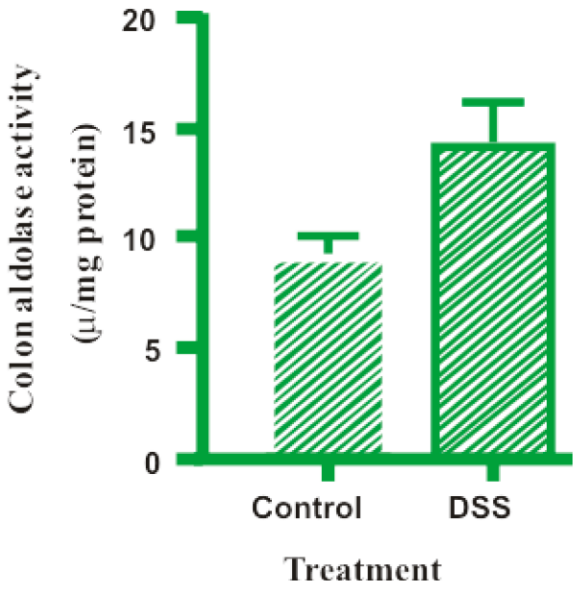

Figure 2c: Effect of DSS on aldolase activity in the colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

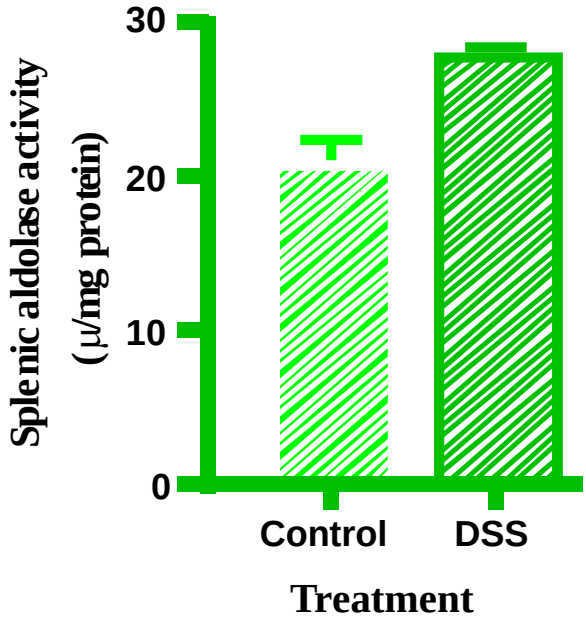

Figure 2d: Effect of DSS on aldolase activity in the spleen. *Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).*

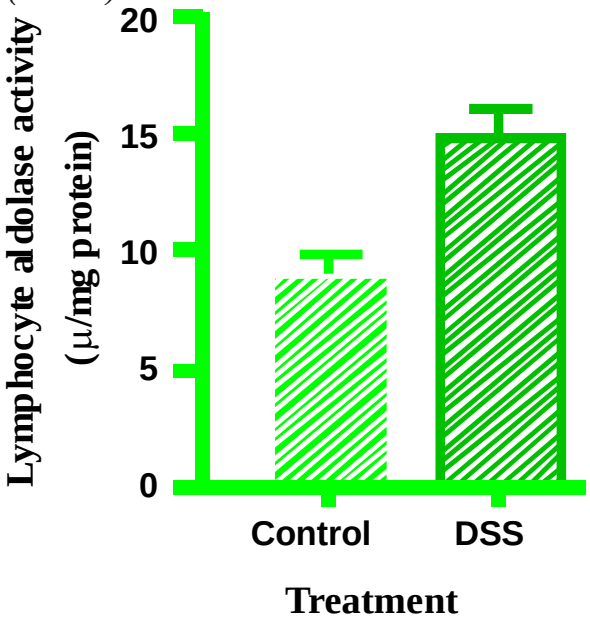

Figure 2e: Effect of DSS on aldolase activity in the lymphocyte. *Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).*

Activities of glycolytic enzymes LDH and NADase were down-regulated significantly ($p<0.05$) in all the organs when compared with the control (Fig. 3a-e and 4a-e). Renal and splenic LDH experienced a profound decrease (62.5 and 51.75% respectively) relative to control. Also, renal and lymphocyte NADase have a common signature of profound down-regulation (71 and 55% respectively) like LDH ($p<0.05$).

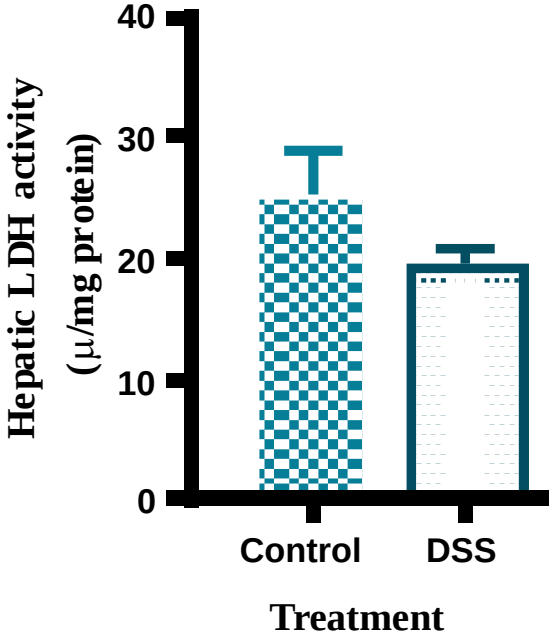

Figure 3a: Effect of DSS on lactate dehydrogenase (LDH) activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

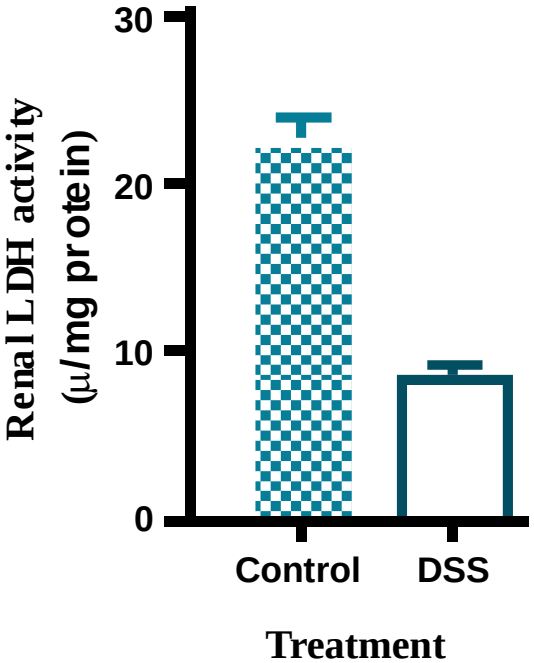

Figure 3b: Effect of DSS on lactate dehydrogenase (LDH) activity in the Kidney. Each bar represents the mean ± SD. significantly different when compared with control ($P<0.05$).

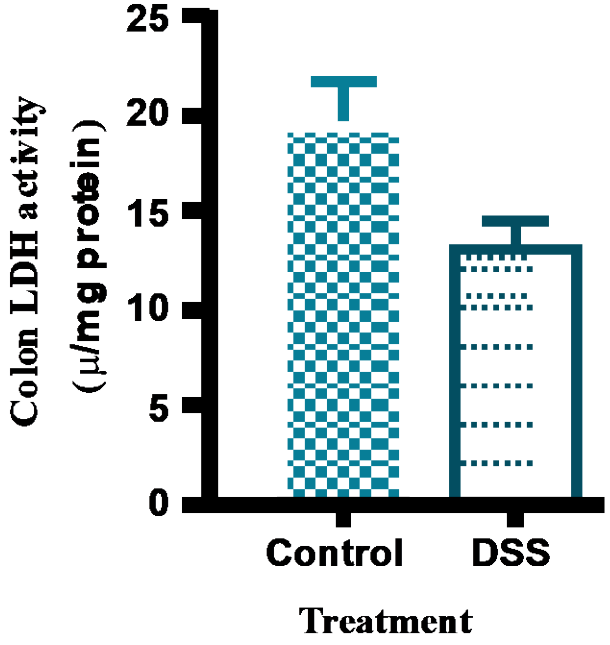

Figure 3c: Effect of DSS on lactate dehydrogenase (LDH) activity in the Colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

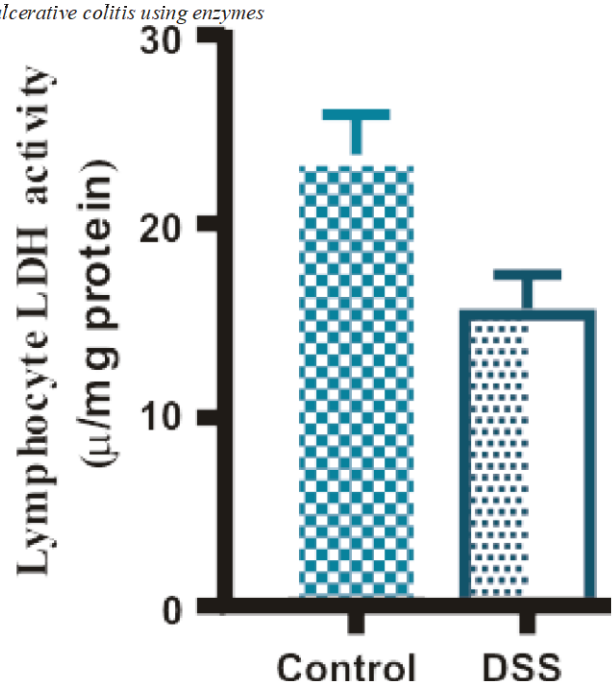

Figure 3e: Effect of DSS on lactate dehydrogenase (LDH) activity in the lymphocyte. Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

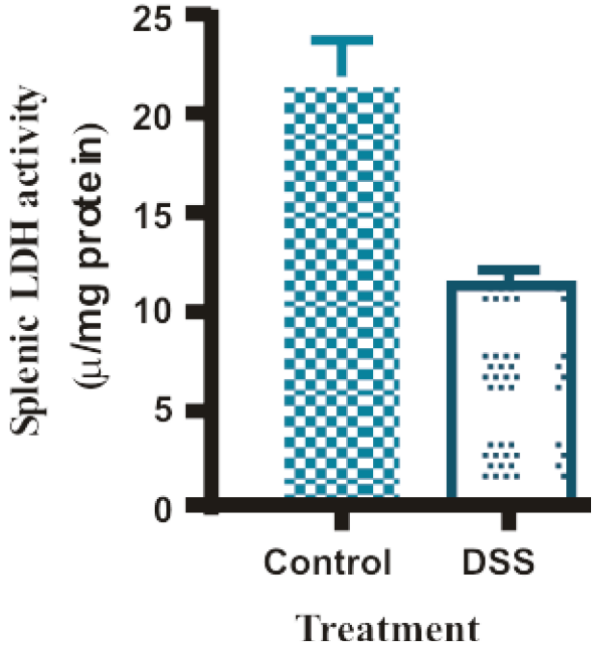

Figure 3d: Effect of DSS on lactate dehydrogenase (LDH) activity in the spleen. *Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).*

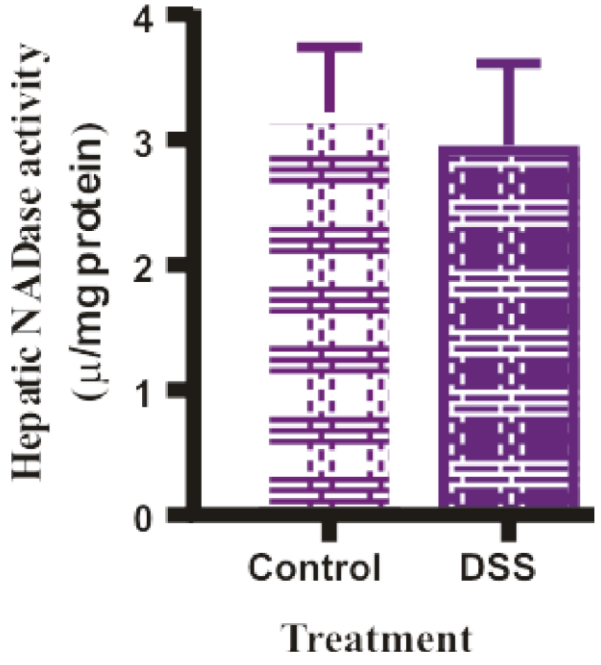

Figure 4a: Effect of DSS on NADase activity in the liver. Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

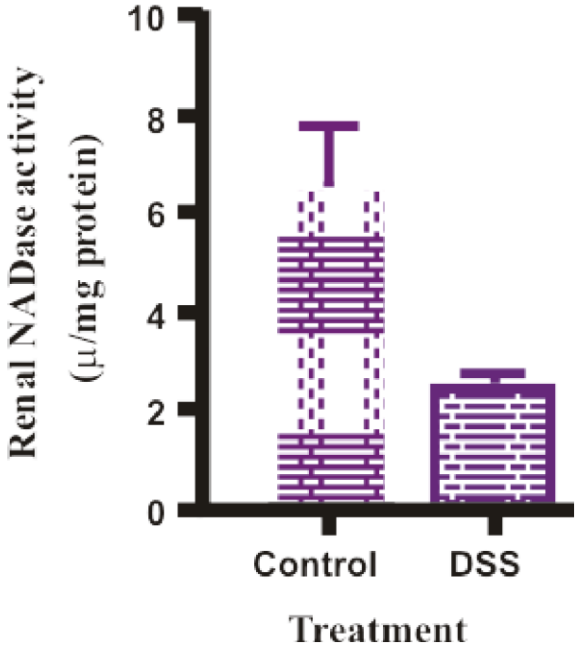

Figure 4b: Effect of DSS on NADase activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

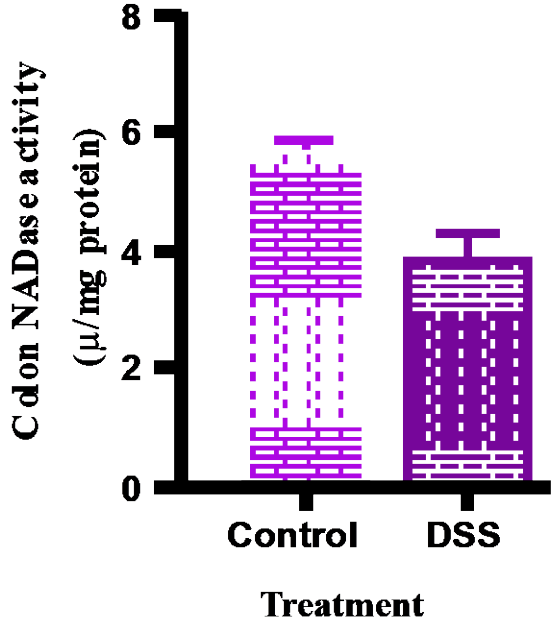

Figure 4c: Effect of DSS on NADase activity in the colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

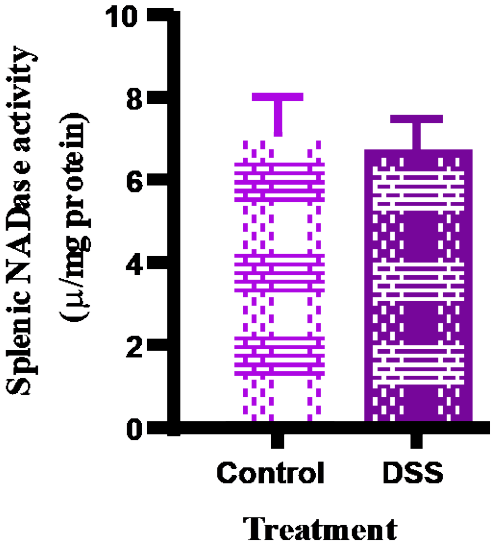

Figure 4d: Effect of DSS on NADase activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

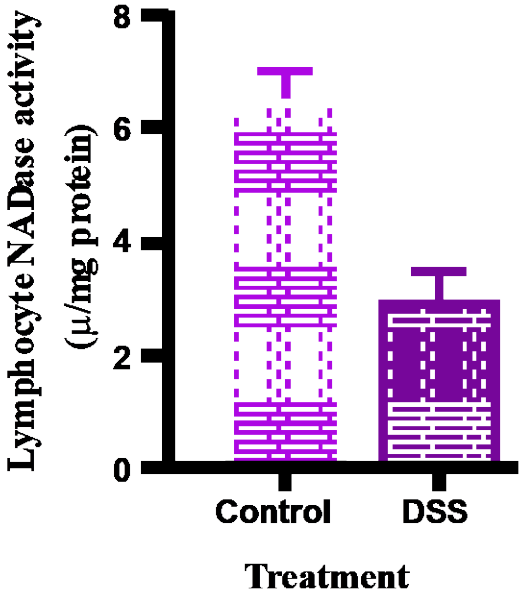

Figure 4e: Effect of DSS on NADase activity in the lymphocyte. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

Furthermore, DSS-induced ulcerative colitis caused perturbation in the activities of mitochondrial metabolizing enzymes in all the organs relative to control. Activities of SDH (Fig. 5a-e) were significantly reduced ($p<0.05$) in the liver, spleen, colon, kidney, and lymphocytes (60, 27, 23, 55, and 9% respectively).

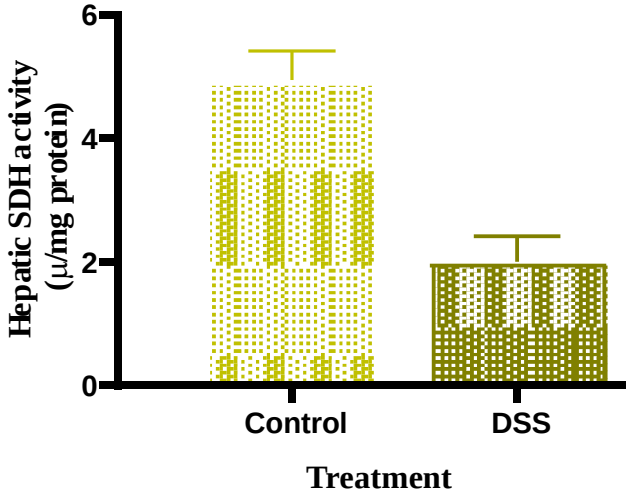

Figure 5a: Effect of DSS on succinate dehydrogenase (SDH) activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

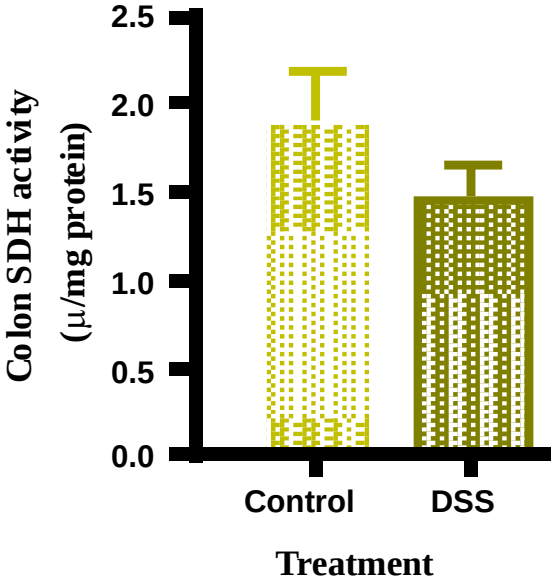

Figure 5c: Effect of DSS on succinate dehydrogenase (SDH) activity in the colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

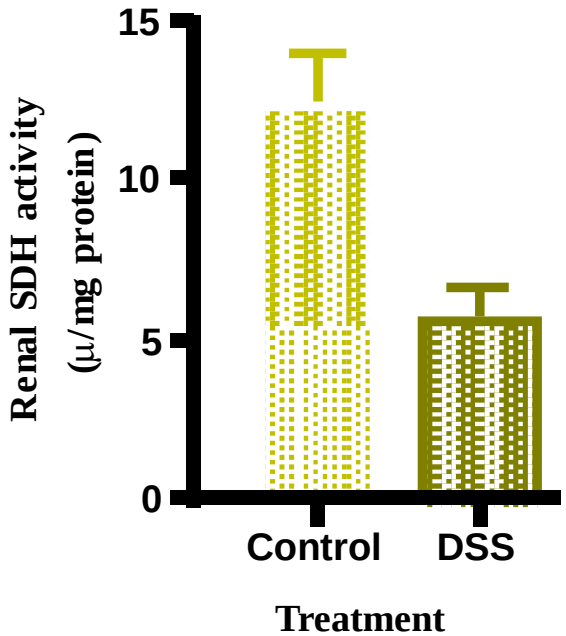

Figure 5b: Effect of DSS on succinate dehydrogenase (SDH) activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

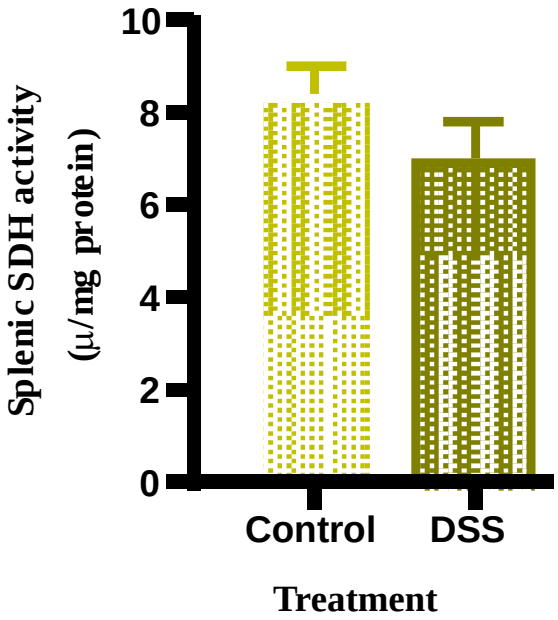

Figure 5d: Effect of DSS on succinate dehydrogenase (SDH) activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

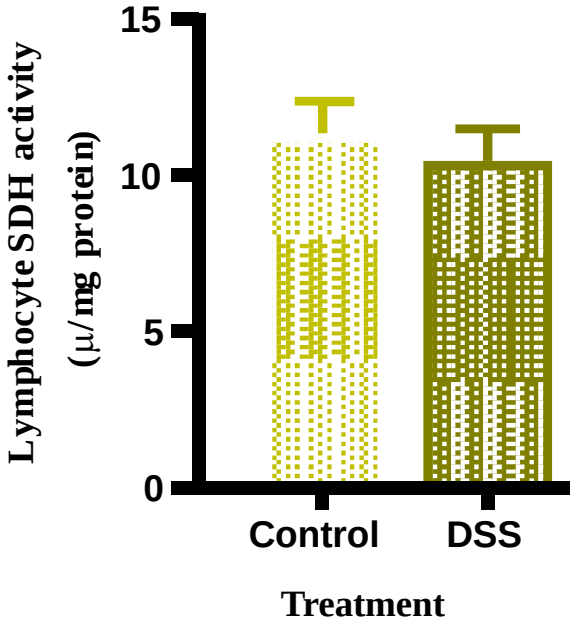

Figure 5e: Effect of DSS on succinate dehydrogenase (SDH) activity in the lymphocytes. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

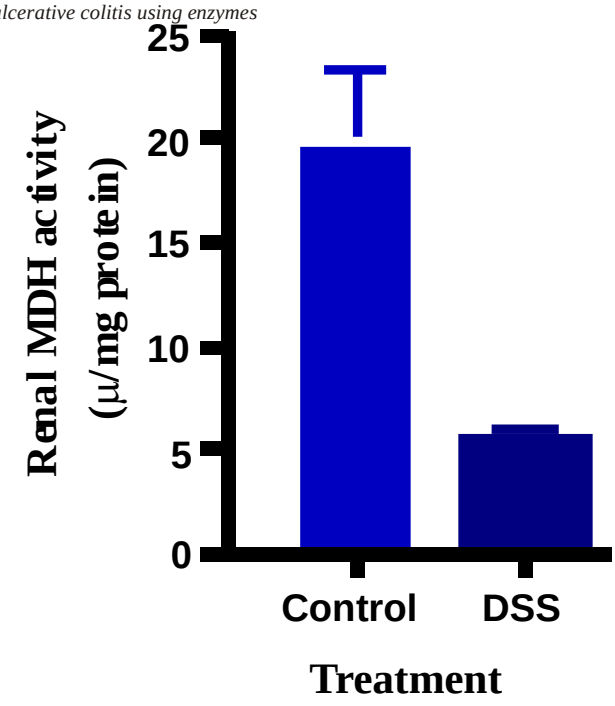

Figure 6b: Effect of DSS on malate dehydrogenase (MDH) activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

Likewise, MDH activity (Fig. 6a-e) was down-regulated ($p<0.05$) in the liver (47%), spleen (65%), colon (45%), Kidney (72%), and lymphocytes (64%) when compared with control.

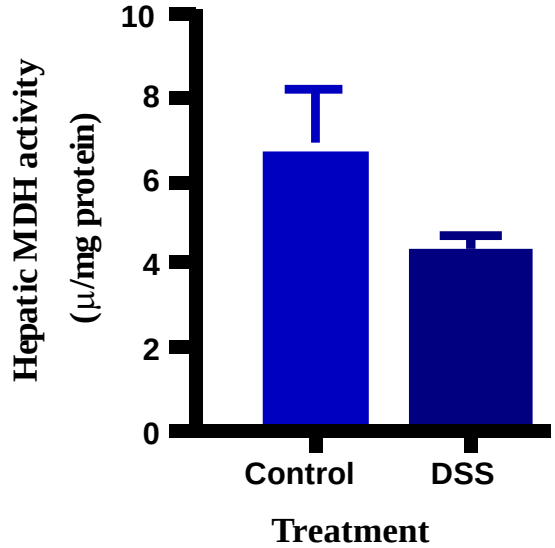

Figure 6a: Effect of DSS on malate dehydrogenase (MDH) activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

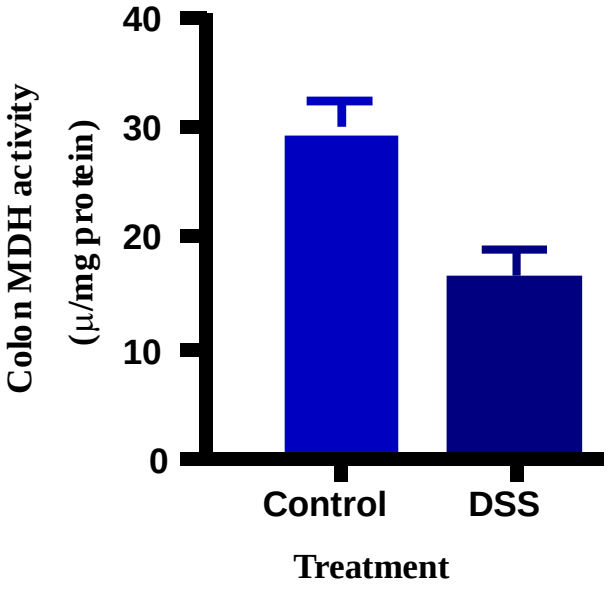

Figure 6c: Effect of DSS on malate dehydrogenase (MDH) activity in the Colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

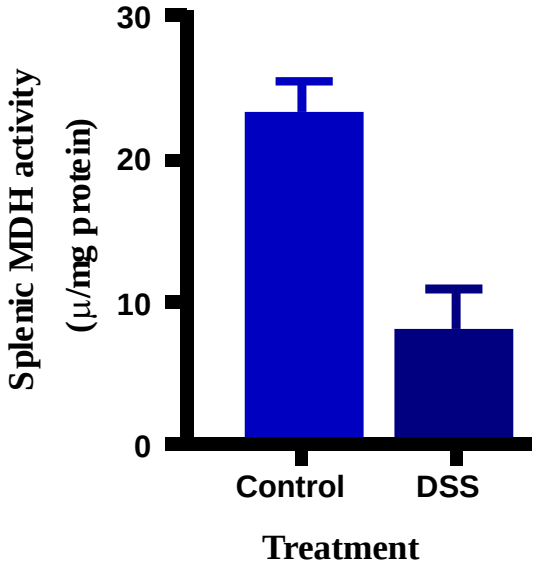

Figure 6d: Effect of DSS on malate dehydrogenase (MDH) activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

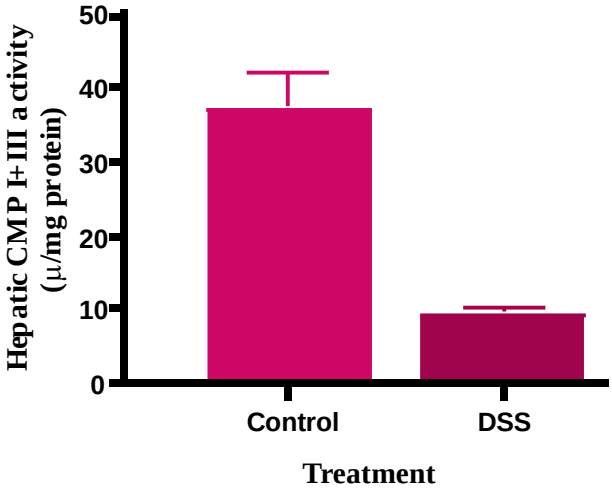

Figure 7a: Effect of DSS on combined complex I+III (CMP I+III) activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

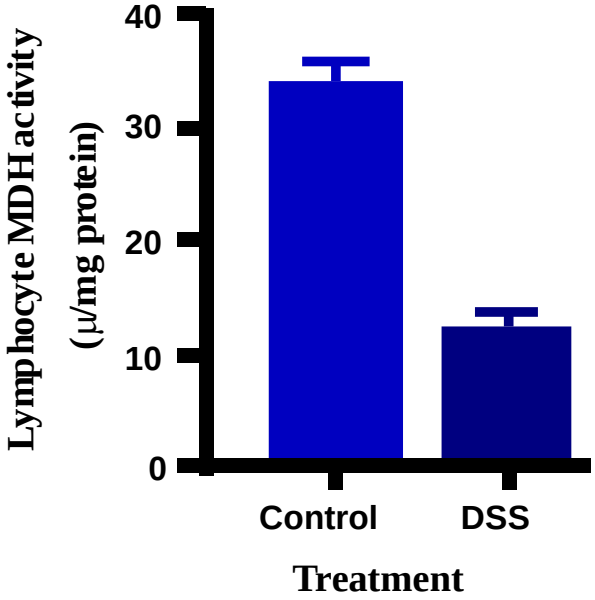

Figure 6e: Effect of DSS on malate dehydrogenase (MDH) activity in the lymphocytes. ***Each bar represents the mean ± SD. significantly different when compared with***

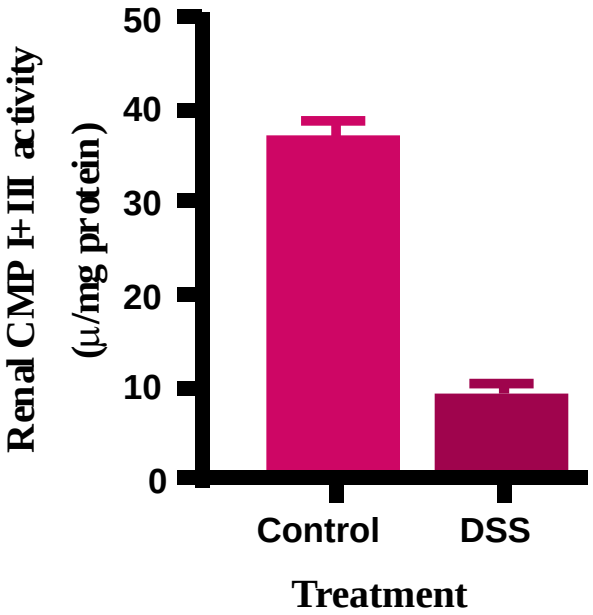

Figure 7b: Effect of DSS on combined complex I+III (CMP I+III) activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

Renal, splenic, colonic, renal, and lymphocyte Combined CMP I+III (Fig. 7a-e) activities were significantly reduced (75, 7, 15, 75, and 53% respectively) when compared with control ($p<0.05$).

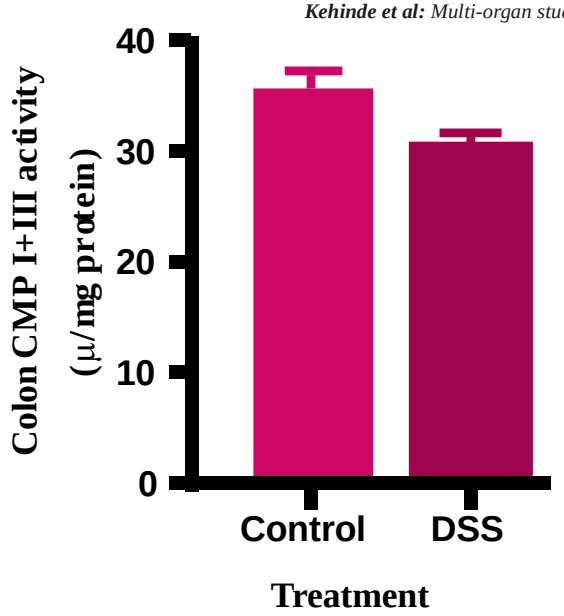

Figure 7c: Effect of DSS on combined complex I+III (CMP I+III) activity in the colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

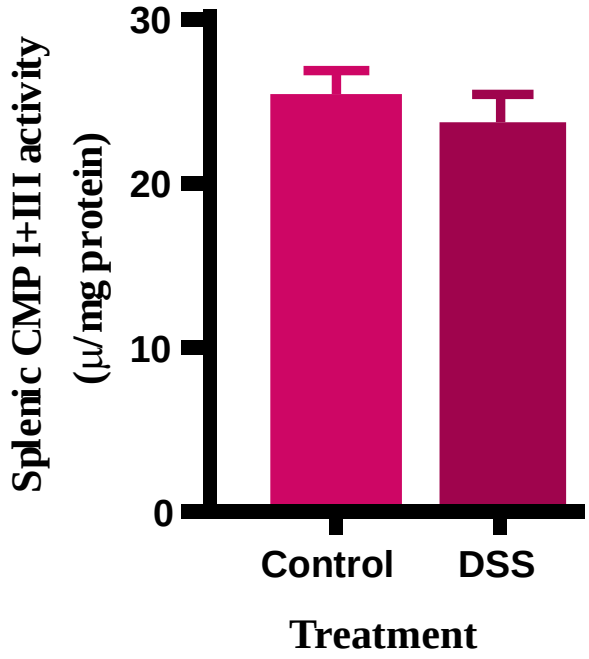

Figure 7d: Effect of DSS on combined complex I+III (CMP I+III) activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

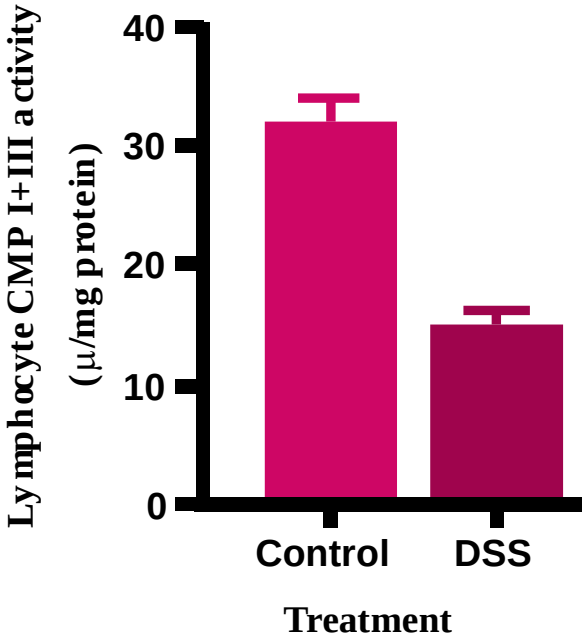

Figure 7e: Effect of DSS on combined complex I+III (CMP I+III) activity in the lymphocytes. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

Combined CMP II+III activities (Fig. 8a-e) in the liver, spleen, colon, kidney, and lymphocyte were also down-regulated (20, 30, 7, 32, and 55% respectively) throughout the study ($p<0.05$) when compared with control and CMP IV activities were also significantly decreased (Fig. 9a-e) as observed in the kidney, spleen, colon, kidney and lymphocyte (28, 30, 15, 3 and 5% respectively) relative to control.

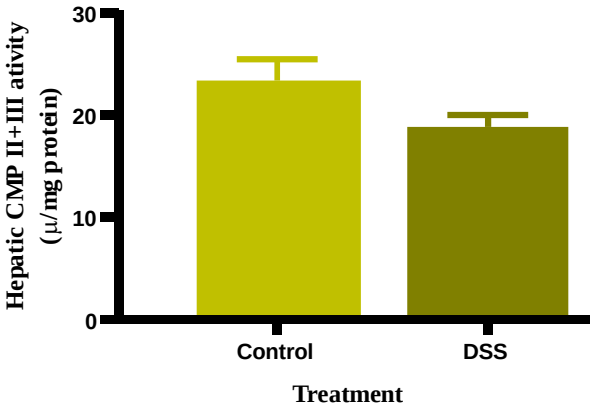

Figure 8a: Effect of DSS on combined complex II+III (CMP II+III) activity in the liver.
Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

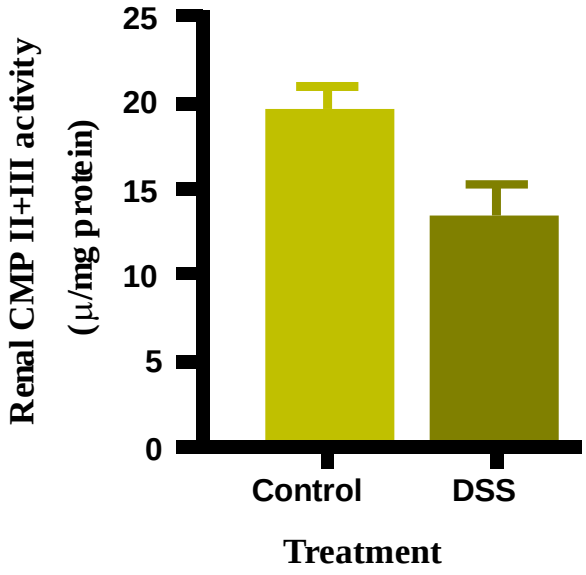

Figure 8b: Effect of DSS on combined complex II+III (CMP II+III) activity in the kidney.
Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

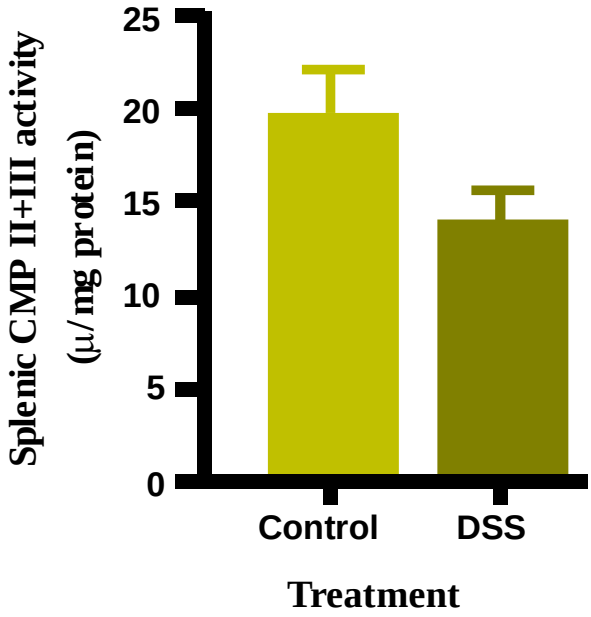

Figure 8d: Effect of DSS on combined complex II+III (CMP II+III) activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

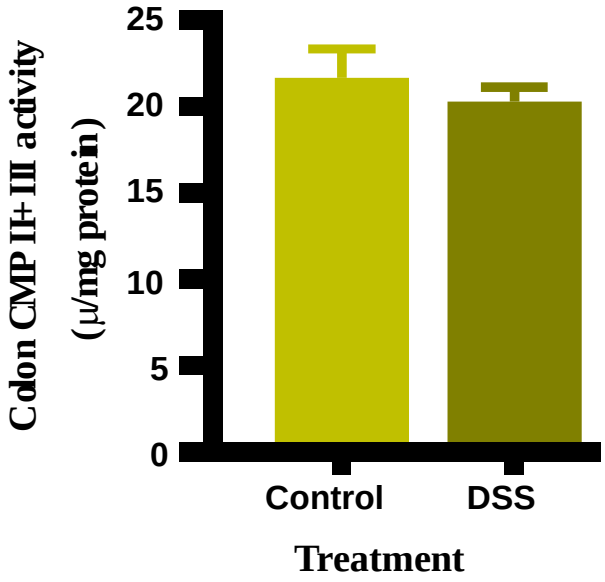

Figure 8c: Effect of DSS on combined complex II+III (CMP II+III) activity in the colon.
Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

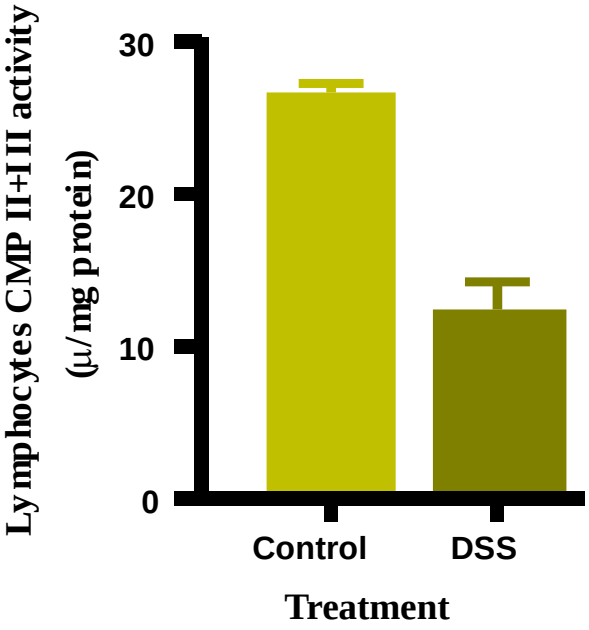

Figure 8e: Effect of DSS on combined complex II+III (CMP II+III) activity in the lymphocytes.
Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).

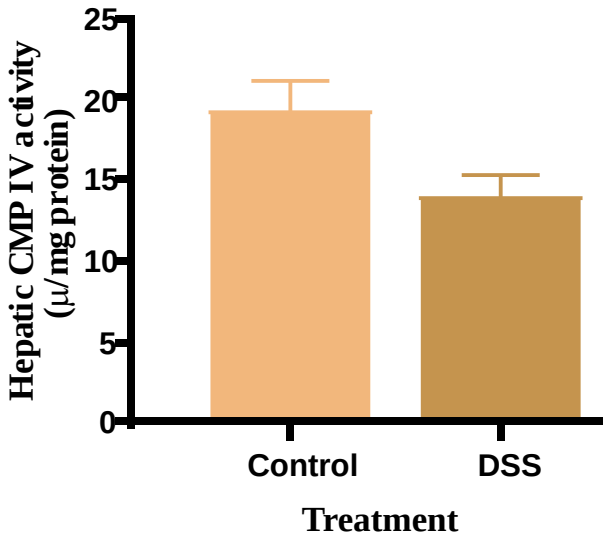

Figure 9a: Effect of DSS on combined complex IV (CMP IV) activity in the liver. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

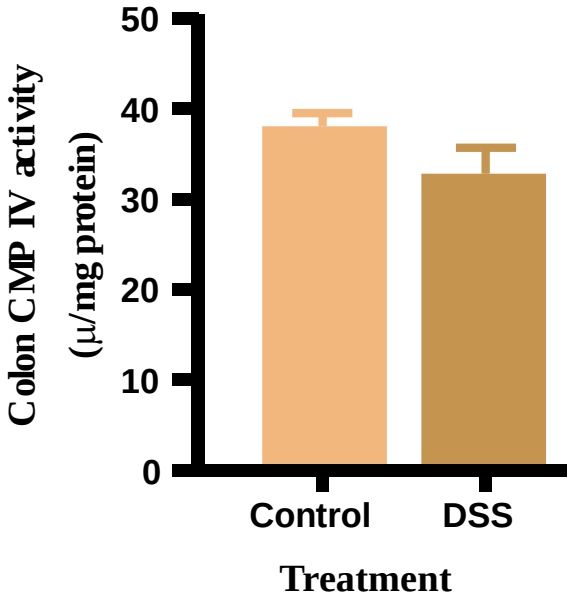

Figure 9c: Effect of DSS on combined complex IV (CMP IV) activity in the colon. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

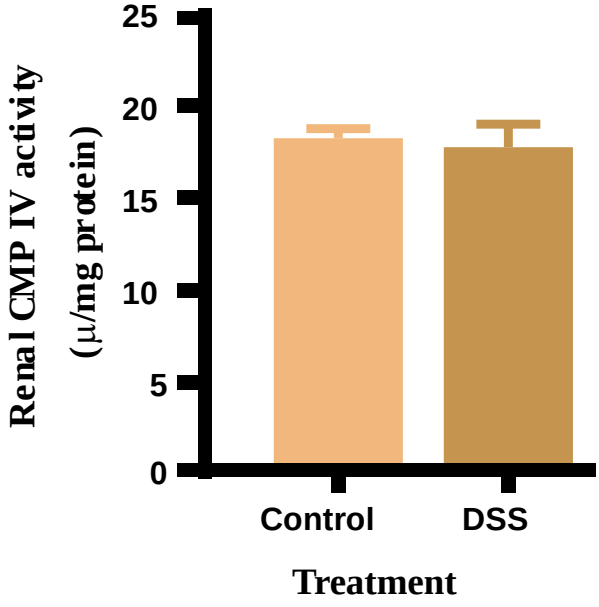

Figure 9b: Effect of DSS on combined complex IV (CMP IV) activity in the kidney. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

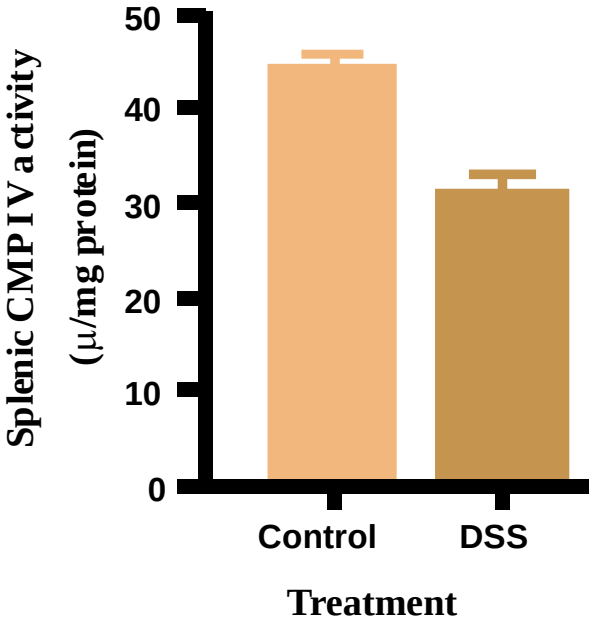

Figure 9d: Effect of DSS on combined complex IV (CMP IV) activity in the spleen. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

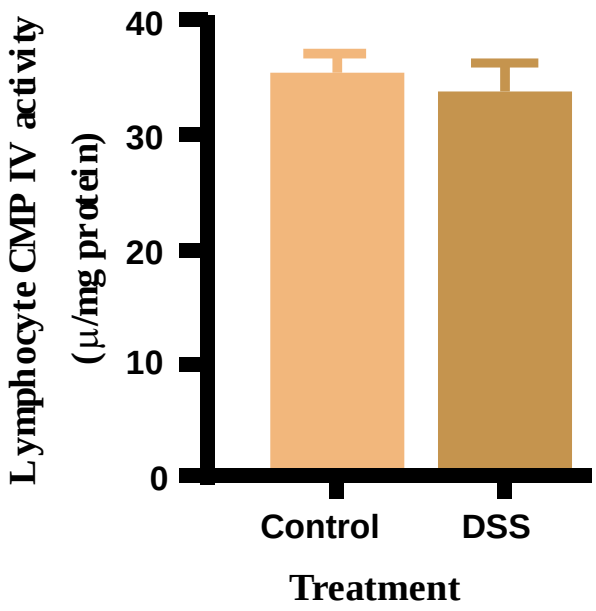

Figure 9e: Effect of DSS on combined complex IV (CMP IV) activity in the lymphocyte. ***Each bar represents the mean ± SD. significantly different when compared with control (P<0.05).***

DISCUSSION

Dextran sulphate sodium, a polymer of sulphated polysaccharide is widely used for the induction of ulcerative colitis. It is thought to induce mucosal injury and inflammation through a direct toxic effect on epithelial cells with subsequent activation of macrophages and T-lymphocytes resulting in cytokine-mediated cytotoxicity (Sartini et al. 2018). DSS is also known to be a chemical colitogen with anticoagulant properties (Sartini et al. 2018). Glycolytic pathways as well as mitochondrial localized enzymes are crucial for providing the cells with its energy need for biological processes. Mitochondrial dysfunction has been shown to be associated with different toxicities and degenerative disorders. The defect(s) in these metabolic pathways can present serious deleterious cellular response and consequences (Chaudhry and Varacallo, 2018).

The findings of this study indicated that pathological condition of ulcerative colitis induced by administration of DSS significantly altered both glycolytic and mitochondrial metabolizing enzymes of the liver, kidney, colon, spleen and lymphocytes.

The liver is the principal site of xenobiotic metabolism immediately after their absorption from the gastrointestinal tract. The liver also has the highest supply of biotransformation enzymes of all organs in the body and therefore, has a key role in xenobiotic detoxification and protection against chemical toxicity (Parkinson and Ogilvie, 2008). From the results, with an exception to aldolase activities in all the organs studied, all hepatic glycolytic and mitochondrial metabolizing enzymes showed decreased activities when compared with control.

IBD has been demonstrated to be associated with extra intestinal manifestations (Mendoza et al. 2005; Trikudanathan et al. 2014) and of which hepatobiliary manifestations is one of the most common (Mendoza et al. 2005). It has been reported that hepatobiliary manifestations are most commonly associated with ulcerative colitis than crohn"s disease (Mendoza et al. 2005; Venkatesh et al. 2011). This may be parallel with pathophysiological changes seen with IBD including Cholelithiasis, portal vein thrombosis and hepatic abscess. According to a study published in 2019 by American Gastoenterological Association, the prevalence of non-alcholic liver diseases in patient with ulcerative colitis was more than double of the general population (Navaneethan, 2014).

Synmously, having reported the fact that there is possibility of liver-gut crosstalk, it is not surprising that there is a reduced activity of hepatic hexokinase, lactate dehydrogenase and NADase in the pathological condition of ulcerative colitis, thus invariably leads to a decrease in energy production since most of the key enzymes of glycolytic and mitochondrial has been inhibited and most commonly reported symptom of ulcerative colitis include fatigue or low energy. Considering the increase risks, it makes sense to actually take precautions and it's recommended that people with UC have their liver enzymes checked regularly.

The kidney on the other hand plays a critical role in the excretion of many metabolic waste products and xenobiotics. During the process of excretion, the kidney may become vulnerable to toxicity. The pathway for xenobiotics elimination in the kidney includes glomerular filtration and tubular secretion, drugs smaller

than 50 to 60kDa pass through the glomerulus in the unbound form (Brater, 2000). Renal involvement has been considered also as an extraintestinal manifestation in both Crohn's disease and ulcerative colitis. The most common frequent renal involvements in patients with IBD diseases are nephrolithiasis, tubulointestinal nephritis, glomerulonephritis and amyloidosis (Domerico and Claudio, 2016). Kidney pathologies in IBD have been associated as either depending on immunological mechanisms that determines intestinal inflammatory diseases directly related to intestinal activity or related to metabolic disorders developed in the IBD (Schreiber et al. 1997). From the results, a perturbation in energy metabolism in the sense that an observed decrease in activity of renal LDH, NADase, SDH, MDH, Combined Complex I+III, II+III and IV relative to control were observed while significant increases were observed only with renal hexokinase and aldolase. All aforementioned pointed to the fact that there was an initial concomitant commitment of glucose into the cell via increased hexokinase activity while its transduction to energy was inhibited by decreased activities of other enzymes of glycolysis, TCA and ETC relative with control (Yoo et al. 2014). Luke and Staurt, 2018 reported a cross-organ sensitization between the colon and the bladder and in such circumstances, colonic inflammation may result in profound changes to the sensory pathways innervating the bladder resulting in severe renal dysfunction.

Furthermore, the spleen is an organ of the body that filters the blood. This is done by detecting any red blood cells that are old and damaged, it also plays a role in the immune responses by detecting pathogens and producing white blood cells in response. While the lymphocytes (about one-quarter) are stored in the spleen. A higher neutrophil-lymphocyte ration (NLR) have been suggested to be an indicator of active ulcerative colitis. Although the site of DSS expression is basically in the colon as it induces ulcerative colitis, nevertheless, organs like the liver, kidney and spleen maybe associated with DSS toxicity. Also, increased lymphocytes levels have been demonstrated in ulcerative colitis. In left-sided UC, inflammation occurs only to the left-side of the colon towards the splenic flexure (Guo et al. 1994). One of the characteristic macroscopic changes associated with DSS-induced UC is enlargement of the spleen (Morteau et al. 2000). Several studies suggested a close correlation between spleen size and severity of UC (Guo et al. 1994). However, the role of spleen during intestinal inflammation has been poorly reported.

Lactate dehydrogenase (LDH) is an important enzyme of the anaerobic metabolic pathway, it also belongs to the class of enzyme referred to as oxidoreductases. LDH catalyze the reversible conversion of lactate to pyruvate with the reduction of NAD^+ to NADH and vice versa (Schuma et al. 2002). Increase in LDH has been attributed to liver disease, muscle trauma, heart attack, cancers and infections such as meningitis, encephalitis. Though LDH is regarded as a cytoplasmic enzyme, its mitochondrial presence also has been demonstrated (Passarella and Schurr, 2018). From this study, it was observed that activities of LDH in the mitochondrial isolate of the organs of interest (liver, kidney, spleen, colon) and lymphocytes were down-regulated when compared with the control. This indicate that administration of DSS inhibits the anaerobic metabolic pathway of energy utilization because for cells that lack mitochondria, LDH activity represents an index for determining not just the membrane permeability but also as a sensor for energy homeostasis (Miranda *et al.* 2018).

Activity of the glycolytic enzyme NADase was also significantly reduced. NADase belongs to the family of hydrolases, the glycosylases that hydrolyse N-glycosyl compound. NADase also participate in nicotinate and nicotinamide metabolism and calcium signalling pathway. NADase uses the substrates NAD^+ and H_2O and produces ADP-ribose and nicotinamide as products. From this study, lymphocytes NADase activity was drastically inhibited when compared to the control. The cytosolic concentration of NAD^+ is minimal; and given that NAD^+ is electron acceptor in different oxidative pathways, its concentration and rate of breakdown is tightly regulated. The observed down-regulation of NADase in the tissues may indicate that the rate of its breakdown is lowered.

However, its availability to accept electrons and be reduced to NADH does not translate to ATP synthesis. It is not yet clear how TiO_2 NPs induced its inhibitory effect on the NADase activity.

Furthermore, hexokinase, the initial enzyme of glycolysis catalyses the phosphorylation of glucose by ATP to glucose-6-phosphate. Hexokinase is known to be the rate limiting enzyme of glycolysis (John, 2003). Hepatic hexokinase activity significantly reduced relative to control as opposed the increase observed in other mitochondrial isolates of the organs and lymphocytes while aldolase activities were up-regulated in all the mitochondrial isolates of the organs and lymphocyte used for this study.

Aldolase, also known as fructose-bisphosphate aldolase is a cytoplasmic enzyme that is involved in glucose and fructose metabolism. Aldolase catalyses the reversible reaction of converting fructose-1,6-bisphosphate into dihydroxyacetone phosphate. In the case of liver or muscle damage, there is an increased level of aldolase (Du et al. 2014). Aldolase activities were upregulated in all the isolates of the organs and lymphocyte used for this study. The up-regulation of aldolase activity following UC -induction exposure is not surprising as Mamczur *et al* (2013) showed that elevated activity of aldolase is associated with highly proliferating cells. This suggests that following induction of UC, the response of the lymphocytes and other organs indicates that the UC as a pathological condition has one way or the other modulated the energy transduction through a mechanism which is not yet clear. But according to Lew and Tolan (2013), a line of evidence has been accumulated that enzymes of carbohydrate metabolism display diverse cellular functions from stabilization of cytoskeleton to regulation of mitochondrial functions. This implies that the increased activity of aldolase in the tissues represent a subtle way which the living system tends to use in order to stabilize the cellular functions.

In addition, DSS administration caused perturbation in the activities of mitochondrial metabolizing enzymes in the mitochondrial isolates of the organs and lymphocyte used for this study. Activities of SDH, MDH, CMP I+III, II+III and IV were down-regulated.In this present study, TCA and ETC enzymes of liver, kidney, colon, spleen tissues and lymphocyte responded to the administration of DSS. TCA and ETC enzymes were all down-regulated. There are numerous evidences showing modifications of mitochondrial dynamics in organs involved in energy metabolism. The liver also plays an important role in glucose homeostasis and development of metabolic alterations (Naon et al. 2016).

Succinate dehydrogenase (SDH) is known to be a key enzyme in intermediary metabolism and aerobic energy production in living cells. Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate in the Krebs cycle with the concomitant reduction of ubiquinone to ubiquinol using flavin adenine dinucleotide (FAD) as the electron acceptor (Kruckeberg et al. 2004). There was observed an inhibitory effect of UC on SDH activity in different organs. Taken together, it may be inferred inhibition/modulation of SDH as metabolic adaptation to metabolic stress induced by UC on the different tissues/organs. Malate dehydrogenase (MDH) catalyzes the interconversion of malate to oxaloacetate in the citric acid cycle. This reaction occurs through the oxidation of hydroxyl group on malate and reduction of NAD^+. It should be noted that MDH has both cytosolic and mitochondria isoforms but the mitochondrial isozyme is linked to the electron transport chain as electron donor in a process that culminates in adenosine triphosphate (ATP) synthesis (Voet et al. 2015). In this study, MDH activity in the lymphocyte dose-dependently nose-dived after the induction of UC when compared to the control rats further affirming the immunotoxic downplay UC.

The electron transport chain (ETC) comprises of a series of complexes that transfer electrons from electron donors to electron acceptors via redox reaction. This electron transfer is coupled with the transfer of protons (H^+) across a membrane. The electrochemical gradients of protons generated as a result of its movement across the membrane is used to drive the synthesis of ATP. This electron transport chain is made up of 4 complexes: I, II, III and IV. Electrons are harnessed from NAD^+ and FAD-linked enzymes of the tricarboxylic acid (TCA)

cycle and the proton-motive force generated is thus used to synthesize ATP. Electrons move from complex I directly to complex III via ubiquinone while electrons from FAD-linked SDH passes from complex II-III (Lyall, 2010; Kracke et al. 2015). In the present study, lymphocytes combined complexes I+III was markedly down-regulated when compared with control. The observed inhibitory effect of UC on the electron transport chain complexes might be as a result of significant reduction in viable cells and reduced proliferative activity of the lymphocytes. It is of note that complex I+III supplies the electrons and (H^+) to the respiratory chain, inhibition could lead to uncoupling of the electron transport chain from oxidative phosphorylation leading to reduced ATP synthesis (Teodoro *et al.* 2011) Taking together, compromised mitochondrial transmembrane potential and possible uncoupling of electron transport chain from oxidative phosphorylation in different tissues through the inhibition of complex I+III may be the hallmark of UC multi-organ pathologies.Results from this study showed that UC caused a very pronounced inhibition of complex IV activity at all the investigated doses and duration in the lymphocytes and other organs investigated which eventually leads to the suspected disruption of mitochondrial and possible cell death. Notably, the inhibition could also lead to reduced or attenuated ATP synthesis which can lead to necrosis.

CONCLUSION

Taking all aforementioned observations made from indices obtained from DSS-induced UC animals into consideration; this study indicates a down-regulation of energy metabolism and transduction. It is of note that TCA and ETC are the major energy-producing pathways and when inhibited, insufficient energy is being produced which may not be enough to sustain life and day-to-day activities. It is clear that UC pathological condition can cross tour other organs being characterized by mitochondronopathy and depleted ATP synthesis which tend to shift the metabolic need for ATP from the mitochondrial pathway towards glycolysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adawi D, Molin G, Jeppsson B. (1999). Gut–liver axis. Hpb. 1: 173–186.

Brater DHS. (2000). Disposition and dose requirements of drugs in renal insufficiency. In: Seldinand DGG, editor. The kidney. Lippincott Williams and Wilkins; Philadephia.2923-2942.

Chaudhry R, Varacallo M. (2018). Biochemistry, Glycolysis.

Du S, Guan Z, Hao L, Song Y, Wang L, Gong L, Liu L, Hou Z, Shao S. (2014). Fructose-bisphosphate aldolase is a potential metastasis-associated marker of lung squamous cell carcinoma and promotes lung cell tumorigenesis and migration. PLOS ONE. 9(1).

Guo W, Andersson R, Willen R, Ljungh A, Wang, X, Liu X, Bengmark S. (1994) Bacterial translocation after intraperitoneal implantation of rubber fragments in the splenectomized rat. J. Surg. Res. 57: 408.

John EW. (2003). Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. J Exp Biol. 206(12): 2049-2057.

Kracke F, Vassilev I, Krömer JO. (2015). Microbial electron transport and energy conservation - the foundation for optimizing bioelectrochemical systems. Frontiers in Microbiology. 6: 575.

Kruckeberg A, Dickinson JR. (2004). Carbon metabolism. In: Dickinson, J.R., Schweizer, M. editors. The metabolism and molecular physiology of Saccharomyces cerevisiae. London: CRC, 42103.

Lew CR, Tolan DR. (2013). Aldolase sequesters WASP and affects WASP/Arp2/3- stimulated actin dynamics. Journal of cellular biochemistry. 114(8): 1928-1939.

Lyall F. (2010). "Biochemistry". Basic Science in Obstetrics and Gynaecology. pp. 143–171.

Mamczur P, Gamian A, Kolodziej J, Dziegiel P, Rakus D. (2013). Nuclear localization of aldolase A correlates with cell proliferation. *BiochimicaetBiophysicaActa (BBA)-Molecular Cell Research*. *1833*(12): 2812-2822.

Medja F, Allouche S, Frachon P, Jardel C, Malgat M, de Camaret BM, Slama A, Lunardi J, Mazat JP, Lombès A. (2009). Development and implementation of standardized respiratory chain spectrophotometric assays for clinical diagnosis.

Mitochondrion.

Mendoza JL, Lana R, Taxonera C. (2005). Extra-intestinal manifestations in inflammatory bowel disease: differences between Crohn's disease and ulcerative colitis. *Med Clin (Barc)* 125:297–300.

Miranda RR, Gorshkov V, Korzeniowska B, Kempf SJ, Neto FF, Kjeldsen F (2018). Co-exposure to silver nanoparticles and cadmium induce metabolic adaptation in HepG2 cells. *Nanotoxicology*. *12*(7): 781-795.

Morteau O, Morham SG, Sellon R, Dieleman LA, Langenbach R, Smithies O, Sartor RB. (2000). Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. J. Clin. Invest. 105: 469.

Naon D, Zaninello M, Giacomello M, Varanita T, Grespi F, Lakshminaranayan S. (2016). Critical reappraisal confirms that mitofusin 2 is an endoplasmic reticulum-mitochondria tether. *ProcNatlAcadSci* U S A. 113(40):11249–54.

Navaneethan U. (2014). *Hepatobiliary manifestations of ulcerative colitis: an example of gut-liver crosstalk. Gastroenterology Report, 2(3), 193–200.*

Pardi DS, Tremaine WJ, Sandborn WJ, McCarthy JT. (1998). Renal and urologic complications of inflammatory bowel disease. Am J Gastroenterol. 93:504–514.

Parkinson A, Ogilvie BW. (2008). Biotransformation of xenobiotics. In: Casarett L.J, and Doull J. editors. Casarett and Doull's Toxicology: the basic science of poisons. 7. McGraw-Hill; New York. 161-304.

Passarella S, Schurr A. (2018). I-Lactate transport and metabolism in mitochondria of Hep G2 cells- the cori cycle revisited. *Front Oncol*. 8:120.

Sartini A, Gitto S, Bianchini M, Verga MC, Di Girolamo M, Bertani A, Del Buono M, Schepis F, Lei B, De Maria N. (2018). Non-alcoholic fatty liver disease phenotypes in patients with inflammatory bowel disease. *Cell Death Dis*. 9: 87.

Schreiber S, Hamling J, Zehnter E, Howaldt S, Daerr W, Raedler A, Kruis W. (1997). Renal tubular dysfunction in patients with inflammatory bowel disease treated with aminosalicylate. Gut. 40:761–777

Schuman G, Bonora R, Ceriotti F, Clerc-Renaud P, Ferrero CA, Ferard G, Franck PF, Gella, FJ, Hoelzel W, Jorgensen PJ, Kanno T, Kessner A, Klauke R, Kristiansen N, Lessinger JM, Linsinger TP, Misaki H, Panteghini M, Pauwels J, Schimmel HG, Vialle A, Weidemann G, Siekmann L (2002). IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. Part 3. Reference procedure for the measurement of catalytic concentration of lactate dehydrogenase. *ClinChem Lab Med.* 40(6): 643-648.

Stephens NS, Siedeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM. (2013**).** Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. *J. Crohns Colitis.* 7: 42–48.

Tanideh N, Bahrani M, Khoshnood-Mansoorkhani MJ, Mehrabani D, Firoozi, D, Koohi-Hosseinabadi O, Iraji A. (2016**).** Evaluating the Effect of Melillotusofficinalis L. Aqueous Extracts on Healing of Acetic Acid-Induced Ulcerative Colitis in Male Rats. *Ann. Colorectal Res*. 4.

Teodoro JS, Simões AM, Duarte FV, Rolo AP, Murdoch RC, Hussain SM, Palmeira, CM. (2011). Assessment of the toxicity of silver nanoparticles in vitro: a mitochondrial perspective. *Toxicology In vitro*. *25*(3): 664-670.

Testa B, Kramer SD. (2007). The biochemistry of dug metabolism- an introduction: part 3. Reactions of hydrolysis and their enzymes. *Chemistry and Biodiversity.* 4: 2031-2122.

Thorne CJR. (1962) *Biochim. Biophys. Acta*. 69: 624.

Trikudanathan G, Venkatesh PG, Navaneethan U. (2012) Diagnosis and therapeutic management of extra-intestinal manifestations of inflammatory bowel disease. *Drugs.* 72:2333–49.

Veeger C, Dervartanian D. (1964). Studies on Succinate Dehydrogenase. I. Spectral Properties of The Purified Enzyme and Formation of Enzyme-Competitive Inhibitor Complexes. *BiochimBiophysActa*. 22: 92:233-247.

Venkatesh PG, Navaneethan U, Shen B. (2011) Hepatobiliary disorders and complications of inflammatory bowel disease. *J Dig Dis.* 12:245–56.

Voet D, Voet JG, Pratt CW. (2015). Fundamentals of Biochemistry: Life at the Molecular Level (4th ed.). Hoboken, NJ: Wiley. pp. 574–575.

Yoo YJ, Chung SY, Gu DH. (2014) A case of late onset acute tubulo-interstitial nephritis with infliximab and mesalazine treatment in a patient with Crohn's disease. Korean J Gastroenterol. 63(5):308-12