INTRODUCTION

Ischemic injury is a state where there is a lack of oxygen supply to the body tissues superimposed by a reduced rate of washing out undesirable waste products & harmful metabolites that result from tissue metabolism. Reperfusion injury on the other hand is a state where there is a tissue damage as result of regaining the blood flow to a previously ischemic or a rather infracted area or even following an organ transplantation ***(Kinross et al., 2009).***

Among the abdominal organs, the small intestine is probably the most sensitive organ to ischemia reperfusion (IR) injury. It reflects various clinical conditions such as mesenteric artery occlusions, abdominal aneurism surgery, trauma, shock, small intestine transplantation being associated with high rate of morbidity and mortality ***(Conrad et al., 1996)***.

In the context of evaluating the elemental and mineral changes that generally accompany the ischemia reperfusion (IR) injury is through the Energy Dispersive X-ray analysis (EDAX). It is sometimes referred to also as EDS or EDAX analysis. It is a technique used for identifying the elemental composition of the specimen, or an area of interest thereof. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM), and can not operate on its own without the latter ***(Schemberi et al., 2008)***.

Regards the medical management of the ischemia reperfusion (IR) injury of the small intestine. Melatonin possesses an ameliorating effect on both the oxidative and the nitrosative stress in the intestinal ischemia

reperfusion (IR) injury. Furthermore, melatonine has an inhibitory effect on the oxidative injury and histo-pathological changes take place in the small intestine, eventually minimizing the passive repercussions of the ischemia reperfusion injury(IRI) ***(Kesik et al., 2009).***

AIM OF THE WORK

The present study aimed at elucidating the morphological and mineral alterations of the rat intestinal mucosa induced by intestinal ischemic/reperfusion injury. Moreover, comparing between the ameliorating effect of physical preconditioning and the chemical one produced by melatonin administration on the damage to the intestinal mucosa. In addition, verifying the possible role of melatonin as a post-conditioning drug.

HISTOLOGY OF THE SMALL INTESTINE

The small intestine begins at the pylorus the distal limit of the stomach, and ends at the ileocecal valve. At autopsy, it measures about 6 m, but in life it measures only about 3 m long. It is divided into three sections starting from duodenum, passing through jejunum and ending at ileum. Duodenum is the proximal 20-25 cm whereas jejunum begins where the duodenum emerges from behind the peritoneum and extends to an ill-defined junction with the ileum. Eventually, the ileum extends from the jejunum to the ileocecal valve. Being the major absorptive site, small intestine shows architectural modifications to its mucosa and submucosa to increase its surface area, thrown up into numerous folds or plicae arranged circularly around the lumen being most prominent in the jejunum ***(Stevens and Lowe, 2000).***

The surface of the plicae is further arranged into villi which protrude into the intestinal lumen. Tubular glands or crypts extend down from the base of the villi till the muscularis mucosae which in turn attain an external layer of longitudinal and an inner circular muscular layers and finally the submucosa. The small intestinal epithelium can be divided into three functional zones in the form of the villi, the crypts, and the neck zone where villi and crypts merge. The cells of the epithelium are enterocytes, mucous cells, Paneth cells, endocrine cells, lymphocytes and stem cells ***(Mills, 2007).***

The enterocytes constitute the main cell in the villi being absorptive in function**,** they are tall columnar cells with round or oval nuclei in the lower third of the cell. The luminal surface of enterocytes is

bears 2-3000 tightly packed, tall microvilli which are coated by a glycoprotein called glycocalyx**,** which in turn contains a number of enzymes such as lactase, sucrase, peptidases, lipases and alkaline phosphatise. The cytoplasm contains lysosomes and smooth endoplasmic reticulum.Nearer the nucleus there are abundant endoplasmic reticulums, mitochondria and prominent Golgi bodies and many ribosomes ***(Gartner and Hiatt, 2014).***

The enterocytes being the villous columnar cell have three important functions. First, they are specialized for absorption affiliated to their luminal border bears abundant long microvilli another term for which is a brush border. Second, these cells produce certain enzymes that complete the digestion of carbohydrates and proteins. A third is the junctional complexes that interconnect the epithelial lining cells tight junctions seal off all the intercellular spaces from the intestinal lumen, keep the cells from pulling apart under tension ***(Cormack, 1997).***

In the lower part of the crypts are enzyme- producing cells called Paneth cells containing cytoplasmic granules which in turn contain various antimicrobial peptides known as defensives in addition to antibacterial enzymes such as lysozyme and phospholipase A representing antimicrobial agents being the first line of defence against microbes who survived the passage through the stomach. Maturing columnar cells and goblet cells which in turn produce mucin for lubrication of intestinal content and protection of epithelium lie up the crypt. Few enteroendocrine cells are difficult to recognize without special staining. They secrete hormones, including two peptides such as secretin

and cholecystokinin-pancreozymin that stimulate exocrine secretion in the pancreas ***(Young et al, 2014).***

All four kinds of epithelial cells encompassing villous columnar, goblet, Paneth, and enteroendocrine are believed to arise from the same stem cells, the latter lying deep in the crypts dividing continuously and replenish the four types. As soon as new villous columnar cells and goblet cells emerge from the crypts, spread up the villi and are shed from the tips, they are replaced by other cells that follow from the depth, the epithelial cells on villi are renewed about every 5 to 6 days. hence, the intestinal crypts supply new epithelial cells ***(Young et al., 2014).*** In the cores of the intestinal villi are central lymphatic capillaries called lacteals that participate in the absorption of lipids besides also contain a profuse network of fenestrated blood capillaries**,** that are similarly involved in the absorption of nutrients,besides smooth muscle cells extend into them from the muscularis mucosae. lamina propria contains numerous cells, including some lymphocytes, plasma cells, and eosinophils ***(Maronpot et al., 1999).***

Lymphoid nodules are common, particularly in the ileum, smaller lymphoid nodules lie in the lamina propria, but the larger nodules extend into the submucosa as well large aggregates of confluent lymphoid nodules known as Peyer's patches, present in the walls of the ileum opposite its mesentery and less conspicuous aggregates found in other parts of the small intestine ***(Cormack, 1997).***

There are distinguishing aspects among different parts of the small intestine. First, the broad, tongue- like villi of the duodenum whereas those of the jejunum or ileum appear narrower and longer being longest

in the jejunum. Second, prominent submucosal glands called Brunner's glands are characteristic of the duodenum**,** and Lastly, large confluent masses of lymphoid nodules representing Peyer's patches in lamina propria characteristic of the ileum. Peyer's patches act as a source of plasma cells exerting an immune function. Although sharing features with other parts of the small intestine such as the usual four layers; the mucosa with villi and crypts as the mucosa containing a core of connective tissue covered with epithelium yet the ratio of enterocytes to goblet cells remain also characteristic to the ileum. Moreover Paneth cells are more numerous in the ileum than duodenum and jejunum being concentrated at the bases of the crypts side by side with the stem cells which is a ongoing source for renewal of all cell types and maintenance and integrity of epithelium, Hence such stem cells need protection that in fact provided by Paneth cells which in turn signifies the adherence of both type of cells within the crypts The division between jejunum and ileum is arbitrary, as the jejunum has a thicker wall and wider lumen than the ileum. The mesentery of the jejunum is more richly vascularized and contains less adipose tissue than that of the ileum. plicae circularis to be thicker, taller, and more numerous in the jejunum than in the ileum ***(Ovalle and Nahirney, 2013)***.

Physiologically, the small intestine is the principal site for absorption of digestive products. Yet digestion begins in the stomach and is completed in the small intestine the mucosa and submucosa are circularly arranged folds called plicae circularis to increase the surface area of absorption to its at most range. The mucosal surface made up of numerous finger-like projections representing the villi, mucosa between the bases of the villi, called crypts of Lieberkühn. The microvilli present

at the luminal surface of enterocytes, the muscularis mucosae lies immediately beneath the mucosal crypts an separates the mucosa from the submucosa possessing an inner circular and outer longitudinal smooth muscle layers. The latter is responsible for continuous peristaltic activity of the small intestine ***(Fox et al., 2006).***

The ileal mucous secretions is attains high concentration of HC03 that serves as a buffer to protect the mucosa from damage or erosion caused by gastric acid or by digestive enzymes draining into the duodenum from the pancreas. Brunner glands of duodenum also produce, a peptide hormone that inhibits HCI secretion which neutralizes the acidic chyme entering the duodenum from the stomach., goblet cells, secrete alkaline mucus that help to neutralize the chyme. Endocrine cells secrete cholecystokinin and secretin, which stimulate the pancreas to secrete digestive enzymes and pancreatic juice and contraction of the gall bladder to release bile into the duodenum. Such PH of the mixture entering the jejunum is suitable for the digestive enzymes of the small intestine. Thus the jejunum is the major site for absorption reflecting the fact of attaining the longest and thinnest villi ***(Mills, 2007).***

Blood vessels that nourish the intestine and remove absorbed products of digestion penetrate the muscularis forming a large plexus in the submucosa. From the submucosa, branches extend through the muscularis mucosae and lamina propria and into the villi. At the tips of the villi, one or more venules arise from these capillaries and run in the opposite direction, reaching the veins of the submucosal plexus. The lymph vessels of the intestine begin as closed tubes in the cores of villi. These capillaries representing the lacteals. Lacteals anastomose

repeatedly run to the region of lamina propria above the muscularis mucosae, where they form a plexus eventually directed to the submucosa ***(Lewin et al., 1992).***

The rhythmic movement of the villi is the result the contraction of smooth muscle fibres running vertically from the muscularis mucosae to the tip of the villi. These contractions occur at the rate of several strokes per minute and have a pumping action on the villi that propel the lymph to the mesenteric lymphatics. Duodenal villi are usually broad and leaf-shaped. Jejunum, taller finger-shaped villi; the ileum, stubby club-shaped villi. Relative number of goblet cells gradually distally yet the jejunum has the largest surface area for secretion and absorption ***(Mills, 2007).***

ISCHEMIA REPERFUSION INJURY (IRI)

Ischemia/reperfusion –injury (IRI) is defined as injury when restoration of blood flow to an area that had previously experienced ischemia takes place. Successful reperfusion initiates inflammatory responses that may both aggravate local injury as well as induce impairment of remote organ function. Cases under which ischemia/reperfusion injury is encountered include thrombolytic therapy, angioplasty, operative revascularization besides routine surgical procedures in terms of organ transplantation, free-tissue-transfer, cardiopulmonary bypass, vascular surgery major, trauma and shock ***(Dorweiler et al., 2007).***

Moens et al. (2005) stated that ischemia/reperfusion injury in case of myocardial infarction included myocardial necrosis, arrhythmia, myocardial stunning, endothelial- and microvascular dysfunction. The cardiac ischemia/reperfusion injury is induced by high levels of cytoplasmic calcium and/or by low concentrations of ATP. Moreover, renal ischemia/reperfusion (IR) injury damage involves microvascular hemodynamic changes characterized by red blood cell adherence with platelets and leukocytes. In that respect,

Friedewald and Rabb (2004) proved that blocking leukocyte-endothelial interactions had made a significant protection from renal IR injury in experimental models. The authors added that both T-cells and B-cells but not neutrophils directly mediate renal injury. The CD4+ T cell, working both via interferon-ɣ and co-stimulatory molecules appears to be an important modulator of acute renal failure.

Intestinal ischemia is another example for ischemic reperfusion injury being classified into acute and chronic conditions according to the degree to which blood flow is compromised. Acute mesenteric ischemia commonly affects the small intestine, it has a sudden onset and may be affiliated to a blood clot in the form of an embolus that dislodges from the heart and passes systemically to block the superior mesenteric artery or one or more of its branches. This is in fact the most common cause of acute mesenteric artery ischemia and can take place on top of congestive heart failure, arrhythmia or a heart attack. In addition, fatty deposition occurring within the arterial wall can lead to gradual narrowing of the arterial lumen that leads to ischemia and eventually total blockage of the arterial lumen in addition to stiffening of the arterial wall that leads to atherosclerosis as well ***(Ballehaninna et al., 2012).***

On the other hand, non-occlusive arterial hypo-perfusion frequently results from primary splanchnic vasoconstriction that is not affiliated to obstruction in the arterial lumen, being recognized in some cases of heart failure, diminished blood flow due to low blood pressure as a result of various medical conditions such as shock, heart failure, certain medications or chronic renal failure are all considered as other common probable causes of non-occlusive mesenteric ischemia ***(Krämer et al., 2003).***

Furthermore, intestinal ischemic syndromes are caused by visceral artery disease, where there is a simultaneous narrowing of the arteries that supply blood to the intestines, spleen and liver. The narrowing, in turn, is also triggered by atherosclerosis where the elasticity of the arteries is impaired due to the formation of plaque or fatty deposits that adhere to the artery wall. Chronic mesenteric ischemia is considered as one of the most

common presentation of visceral artery diseases, causes abdominal pain after eating and results in weight loss. It can also result in a fatal interruption of blood-flow to the intestines that may lead to gangrenous formation and necrosis of the intestinal tissue, such fatal condition necessitates instantaneous diagnosis and treatment ***(Hebbel, 2014).***

On the other hand, ischemia can- take place in case the venous blood can't be drained out from the intestine. Hence, blood clot can occur in a vein draining deoxygenated blood from the intestine. As the vein is occluded, blood returns back in the intestine leading to swelling and bleeding, such condition actually represents mesenteric venous thrombosis, that may result from acute or chronic pancreatitis, abdominal infection, neoplasm of the digestive system, bowel diseases such as ulcerative colitis, Crohn's disease or diverticulitis and hypercoagulation disorders that in turn leads to increased incidence of blood clotting such as an inherited clotting disorder or receiving a medication such as estrogen that can increase clotting risk and rarely in case of abdominal trauma ***(Harnik and Brandt, 2010).***

Initial ischemic insult causes tissue injury and/or death, according to the degree and duration of the interruption in the blood supply. During sustained ischemia, ATP levels and intracellular pH declines due to anaerobic metabolism and lactate accumulation. Subsequently, ATPase-dependent ion transport mechanisms is impaired, contributing to increased intracellular and mitochondrial calcium levels representing calcium overload. In addition, cell swelling and rupture, cell death by necrotic, apoptotic, and autophagic mechanisms take place as well ***(Hebbel. 2014).***

Kurose et al. (1994) enlightened the role of leukocyte-endothelial cell adhesion and an interrupted metabolism of endothelial cell-derived nitric oxide (NO) in the microvascular dysfunction that accompanied the IR. The authors added NO may reduce the reperfusion-induced increase in venular albumin leakage through influencing leukocyte-endothelial cell adhesion. Leukocyte adherence and emigration as well as albumin extravasation were evident in the postcapillary venules following 20 minutes of ischemia followed by 30 minutes of reperfusion. Superfusion of the mesenteric microcirculation with the NO donors sodium nitroprusside and spermine-NO significantly reduced the IR-induced leukocyte adherence/emigration and albumin leakage in postcapillary venules.

In that respect, ***Cooper et al. (2002)*** mentioned that in the early phase succeeding reperfusion, activated endothelial cells in the microcirculation produce many oxygen radicals, but rather less nitric oxide creating an imbalance between superoxide and nitric oxide in endothelial cells that eventually leads to the production and release of inflammatory mediators such as platelet-activating factor and tumor necrosis factor. Agents capable of establishing the normal balance between NO and reactive oxygen species (ROS) in vascular endothelial cells may prove particularly useful.

Although oxygen concentration is reestablished upon reperfusion, ischemic tissues gets subjected subsequently to rather a gush of harmful reactive oxygen radicals in addition to inflammatory cell infiltration especially neutrophils infiltrate that in fact exacerbate ischemic injury. The pathologic events provoked by IR injury leads to the opening of the mitochondrial permeability transition pores, which represent an end-

effector of the IR induced cell lysis and death**.** Furthermore, in reperfusion injury xanthine oxidase-derived oxidants and leukocytes play a role in the microvascular injury associated with of ischemic intestine. Xanthine oxidase-derived oxidants augments adherence and extravasation of leukocytes in mesenteric venules. One hour of ischemia is further accompanied with substantial adherence and extravasation of leukocytes with reperfusion that in turn seriously augmenting these reactions ***(Fang et al., 2006).***

On the other hand, some drugs such as allopurinol or superoxide dismutase diminished reperfusion-induced leukocyte adherence and extravasation through xanthine oxidase-derived oxidants which recruit the leukocyte infiltration encouraged by reperfusion of ischemic intestine ***(Grisham et al 2005).***

IR associated with thrombolytic therapy, organ transplantation, coronary angioplasty, aortic cross-clamping, or cardiopulmonary bypass results in local and systemic inflammation. In high level injury, the inflammatory response after IR may lead to systemic inflammatory response syndrome or multiple organ dysfunction syndrome (MODS). It is worth to mention that inflammatory mediators secreted after reperfusion stimulate endothelial cells in distant organs that are not subjected to the initial ischemic insult. This remote response to IR injury can result in leukocyte-dependent microvascular injury that manifests itself in the form of the multiple organ dysfunction syndrome ***(Collard and Gelman, 2001).***

Furthermore, ***Cerqueira et al. (2005)*** stated that both ischemia and reperfusion of the small intestine leads to the separation and dissolution of the mucosa barrier, bacterial translocation and the triggering of

inflammatory responses, as well as hydroeletrolytic and acid-alkaline equilibrium disorders which are demonstrated in distant organs. Furthermore, bacterial translocation takes place where passage of viable bacteria through the intestinal mucosa to mesenteric lymphatic nodes and other organs and tissues. The process of translocation includes the preliminary contact of the bacteria with the intestine wall, which alone can triggers the manufacture of cytokines and a subsequent inflammatory response. As the bacteria manage to infiltrate the mucosa, they can be transported to distant organs through the blood circulation inciting subsequent hypoxia that in turn induces alterations in the functioning of the intestine wall, stimulate a cycle of increased permeability leading to the release of toxic mediators resulting in accentuated permeability and the acceleration of bacterial translocation. It is worth mentioning that marked bacteria translocation is more evident as time elapses signifying that time is an important contributing factor for translocation.

As blood perfusion returns, the infiltration of calcium into the intracellular medium increases leading to a substantial increase in phospholipase A_2 activity. Arachidonic acid released by phospholipase A_2 is metabolized during reperfusion by cycloxygenase enzyme that generates prostaglandins, thromboxane, prostacyclins (PGI_2), and by lipoxygenase enzyme that on the other hands generates leukotrienes. These substances such as Thromboxane (TXA_2), Leukotrienes (LTC_4, LTD_4, LTE_4) eventually leads to vasoconstriction while others like Prostacyclin (PGI_2, PGE_1, PGE_2, PGD_2 causes vasodilatation. On the other hand, LTC_4, LTD_4, LTE_4 causes increased vascular permeability whereas LTB_4 can stimulate platelet aggregation and chemotaxia in the polymorphonuclear cells ***(Fang et al., 2006).***