**Abstract**

The thesis deals with Biochemical Approach for Identifying Intestinal Ischemia in Rats. However the approach of the research is to explore the possibility to find out feasible and sensitive parameter/s having clinical applications for the diagnosis of similar conditions in humans, which usually require high suspect of index.

Acute mesenteric ischemia is an abdominal emergency due to inadequate tissue perfusion with a mortality rate between 60 and 100% amongst humans in general. In this regard four patho-physiological phenomenons are present namely
1) superior mesenteric artery embolism
2) superior mesenteric artery thrombosis
3) non-occlusive acute mesenteric ischemia
4) and superior mesenteric venous thrombosis; embolism event is the most frequent.

Clinical diagnosis of mesenteric ischemia requires a high suspect index, especially in elderly patients with cardiovascular problems. Abdominal pain and hemodynamic and/or metabolic changes are the most frequent symptoms. In such cases infarcted bowel is purple, edematous and hemorrhagic. Histologically, transmural necrosis occurs with superficial ulceration and exudation, in extreme conditions perforation in intestine may occur. In general intestinal ischemia many a times accompanied with Multiple System Organ Failure Syndrome (MSOF) and the condition of the patient becomes worse in 4-5 days. Better treatment along with high survival rate is possible when the condition is detectable in an early stage, preferably by simple laboratory test.

The present study was performed on 8-week-old Wistar male rats in triplicate (weight approx 250g) after institutional animal ethical committee’s approval. The rats were randomly distributed in two groups; control sham operated (n>18) and five ischemic test (each n>18) group. Acute mesenteric ischemia (AMI) was created by flush ligation of superior mesenteric artery (SMA) for the duration of 15min., 30min.,
45min., 60min., and 90 min. while in control group SMA was isolated and no ligation was performed. After respective ischemic duration from test as well as control group; blood was withdrawn from Portal Vein (PV), Dorsal Aorta (DA) and Inferior Vena Cava (IVC). Serum was separated immediately and stored at \(-40^\circ\text{C}\) till estimations were carried out. Small part of intestine from all the groups was excised for histopathology and for other biochemical assays.

In this study superoxide dismutase (SOD) and ceruloplasmin was estimated as an indicator of antioxidant activity. Vaso-active intestinal peptide (VIP), a potent vasodilator, which is secreted by entero-endocrine gland and ALT (SGPT), which is an indicator of tissue damage, were also been estimated by using standard kits like ELISA and GPT respectively. Study has shown that in intestinal tissue the level of SOD decreases substantially with initial insignificant increase. It is observed that the VIP increases till 90 minutes of ischemia with initial insignificant decrease. There is significant increase (p<0.01) in the level of ALT (SGPT) in portal blood without insignificant increase in dorsal aorta as compare to control. The Histology showed damage to villus in test group as the duration of ischemia progresses and total villus loss at the end of 60 minutes in test as compare to control during the experiment.

It has been mentioned in literature that endotoxin upregulate alcohol dehydrogenase (ADH) in liver and also ADH has seven isoenzymes located at different organs including the intestine. Therefore the level of ADH in blood was checked at different time interval of ischemia. It is found that ADH increases exponentially in blood samples drawn at all points (PV, DA, IVC) as ischemia progresses with respect to time. ADH was found to be at significantly (p<0.001) higher level in test group as compare to control group. Role of glutathione as a free radical scavenger in oxidative stress has already been mentioned along with intermediary role of glutathione reductase. Glutathione reductase was found to be increasing as ischemia progresses while thiol content was found to be fluctuating throughout the duration in serum. On the other hand the thiol content in ischemic tissue was found to be lesser than normal tissue with simultaneous increase in intestinal luminal contents of the same. Nitric oxide (NO) is key secondary messenger
and its role in vaso-dialation is well known. Level of nitric oxide was estimated in terms of total nitrite and found to be higher in ischemic tissue, which is in agreement with already, reported in literature.

Three more sets of animals (n>18) was performed for induction of segmental small intestinal ischemia (Sli). Sli was created by ligation of 4-5 branches of arteries and veins both in mesenteric arcade. This condition was maintained for 1, 2 and 3 days after suturing of incision made. Simultaneously control group was also kept with same incision made mid ventrally and suturing back for same duration as test group. Food and water was given ad libitum and after respective period of incubation animals were sacrificed for collection of samples as previously mentioned in case of AMI.

Oxidative stress was found to be highest on second day of Sli. Portal vein showed significant increase (p<0.01) of total thiols and total nitrite while free thiols increased in IVC significantly (p<0.01). This indicates the involvement and response of distal organs to ischemic condition in intestine. Ischemic intestine showed significant decrease (p<0.001) in free and total thiols while there is significant (p<0.01) increase in total nitrite in intestinal tissue.

Permeability dysfunction in intestinal barrier was studied in past by many workers using different permeability markers. The permeability dysfunction due to intestinal ischemia is a gradual process and it increases as the period of ischemic condition progresses. The evaluation of dysfunction at an early stage in ischemia can be one of indication of the ischemic condition. The aminoglycoside antibiotics usually remain unabsorbed in gastro-intestinal tract. For this purpose after 8-hour of Sli and after 30 min of AMI, solution of gentamicin (0.1mg/ml) was perfused through intestine at the flow rate 5ml/min. Simultaneously, control group was treated in the same way, without any ischemic condition. Blood was collected from portal vein and dorsal aorta for estimation of gentamycin. It has been found that though tissue damage is taking place as suggested by histopathology the marker molecule i.e. gentamycin is not crossing the intestinal barrier. These results suggests that
permeability dysfunction taken place with respect to certain molecules as reported in literature but still it is selective with respect to certain molecules such as gentamycin.

Use of radiodiagnostic tool for diagnosis of other disorders has already been reported in literature. For the first time 99m-Tc-cysteine was attempted to study intestinal ischemia. Experiments were performed on rats for the induction of AMI (60 min.) and SII (60 and 90 min.) as previously mentioned along with control group. 99m-technisium-cystine chelate was injected intravenously in femoral vein. Distribution and retention of chelate in all tissues including blood was estimated using gamma counter and scintigraphic pictures were taken using gamma camera. Percent retention of chelate was analyzed and correlated with the physiological change in intestine due to ischemia. We got twice retention of chelate in intestine of AMI group while there is three times increase in retention of chelate in SII group. These results suggest that 99mTc-Cysteine chelate has very good potential radiodiagnostic tool.

This study concludes that serum ADH is potential of biochemical marker in early diagnosis of intestinal ischemia. Combination of ADH estimation along with other parameters followed by 99mTc-cysteine chelate will help in diagnosing intestinal ischemia at an early stage.