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ANATOMICAL AND MORPHOMETRIC CHANGES OF INTESTINAL VILLI AND ABSORPTIVE EPITHELIAL CELLS OF MICE INTESTINE AFTER ADMINISTRATION OF FENOTEROL

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ABSTRACT

To demonstrate drug treatment anatomically the functional difference in each intestinal part of male mice after treatment with beta-agonist fenoterol at 7,14,21 and 28 days. The villous height and the fine structure of absorptive epithelial cells in the duodenum, jejunum and ileum were compared after drug treatment. Duodenum had the highest villi at the initial stage followed by undeveloped lower jejunum villi, while the latter showed a marked growth rate up to last stages of investigation. The outstanding morphological feature of the tube like small intestine is enormously increased absorptive surface which is achieved by three particularities: a) Valvulaeconniventes (Kerkrings) which are prominent mucosal folds in duodenum and jejunum, less so in the upper ileum ; b) Finger like villi, projections of the mucosal layer which is more frequent in duodenum and jejunum than ileum; c) Striated border of the upper part of the absorptive epithelium, now known to consist of microvilli. The mucosal epithelial layer providing this huge surface is the vital barrier through which absorption takes place. Epithelial layer lines the villi and consist of the primary absorptive columnar cells and a number of mucous secreting cells, both of which are constantly renewed in the crypts. The histological characterizations revealed that the wall of the intestine is composed of the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Intestinal mucosa displays many villi and a simple columnar epithelium in associated with goblet cells and intraepithelial lymphocytes. The columnar cells of the intestine have microvilli towards the lumen and are joined together at the apical surfaces by the junctional complexes. Fenoterol, on the other hand, may directly affect the epithelial cells counteracting any villus alterations or degeneration of fibers. The administration of fenoterol has been associated with the relaxation of the small intestine with broader and longer villi, thick mucosa, expanded Brunner's gland. Splitting of fibers and changes in the shape of cells, fibrolysis, hypertrophy and degeneration of muscle fibers, have been reported in experimental animals. The severity of intestinal morphological changes appears to increase with dose and time. The morphology of gut of normal animals differs from that of treated one. The aim of the study is to ascertain the effect of fenoterolon small intestine. We have administered equimolar dose of fenoterol to mice for 28 days to see its effects on small intestine in order to test the hypothesis that fenoterol would produce powerful anabolic and ergogenic effects.

Key Words:- Beta –agonist, Fenoterol, Villi, Epithelial cells, lumen.

INTRODUCTION

The overall gastrointestinal morphology is related to different feeding habits including the nature of the food and frequency of food intake, as well as to taxonomy, body size and shape (Domeneghini *et al*., 1999). The morphology and function of small intestine are known to be altered by its nutrional condition. The growth of an animal depends in part on its capacity to digest and

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assimilate ingested macromolecules, and any impairment of this is expected to constrain growth. Postnatal intestinal growth, the initial appearance and development of intestinal digestive hydrolases, and the capacity of the intestines to absorb nutrients are important factors when characterizing how animals are able to assimilate ingested macromolecules (King *et al*., 2000). Hydrolases and nutrient transporters anchored on the brush border of enterocytes play significant roles in the final stages of digestion and assimilation of nutrients, and the capacity of an animal in this regard dictates that Intestinal epithelium is characterized by fast cellular renewal with continuous proliferation of stem cells inside Lieberkühn crypts, cellular migration along the crypt-villi axis, cellular differentiation, polarization, apical apoptosis and luminal loss(Zbar*et al*., 2004). It has been shown that the autonomic nervous its ability to attain conditions of optimal growth when nutrients are provided in adequate amounts. The objective of this study was to investigate the change in small intestine. The weight, length, surface area, and mucosa weight of the small intestine were measured when mice were 7, 14, 21, and 28 days of age. Body weights increased from 22g at 7 wk to 30 g at 28 days postnatal. Body weight gains were greater ($P< 0.05$) from day 14 to 28 than from day 7 to 14. Weights of the small intestine and of the intestinal mucosa increased faster ($P< 0.05$) from 21 to 28 days than from14 to 21 days, the slowest increase occurred from 7 to 14days. In summary, increases in BW during the first week of postnatal growth in mice are accompanied by significant developmental changes in digestive capacity including intestinal weights, length, and surface area. In the current study, the observations are extended for morphometry of small intestine of mice between the ages of 7 to 28 days. Therefore, the objective of this study was to investigate developmental patterns of digestive capacity in growing mice using weights, linear and area dimensions of each section of the small intestine, duodenum, jejunum and ileum as response criteria after fenoterol treatment.

MATERIAL AND METHODS

The present investigation has been carried out on smooth muscle (small intestine) of mice. Adult sexually mature male mice of Balb – C strain were obtained from Central Research Institute (CRI), Kasauli (H.P). These were housed in flat bottomed polypropylene cages and were maintained in the animal house of department of Biosciences of Himachal Pradesh University under suitable hygienic conditions with 16 hours day light and temperature 24 ± 2^0C. The animals were provided feed (Hindustan Lever Ltd.) and water *ad libitum.*

The experimental animals were divided in to two groups-a) Control, b) Animals of second group were given daily oral administration of fenoterol (1.5mg/ kg body wt.) for 28 days. Small intestine was excised immediately after sacrificing the animals. Small tissue pieces were fixed in aqueous bouin's fixative. These were washed in running tap water till excess of picric acid got washed away, dehydrated, cleared in xylene and embedded in paraffin wax. 5 µ thin sections were cut on a rotary microtome and subjected to haematoxylin-eosin staining.

Haematoxylin-eosin staining

Ribbons of tissue sections were cut and stretched on albuminised slides. These were subjected to dewaxing at 37^0C overnight and hydrated by passing in descending grades of alcohol 100% to 30% (30 min each) and then finally in the distilled water. After that, slides were subjected to dehydration in ascending grades of alcohol (30-90%) for 30 min each. After that sections were stained in Haematoxylin stain for 30 minutes. Make a dip in acid water and alkali water for 1 minute. Counterstaining was done in 2% alcoholic eosin for 2 min. and excess of stain was removed in 90% alcohol. Sections were dehydrated in absolute alcohol and then subjected to xylene for clearance. The sections were mounted directly in DPX. The permanent slides were dried, scanned and photographed.

Morphometric Analysis

Morphometric study is conducted on lumen diameter, submucosa diameter, distance between mucosa and submucosa, villi length and width of villi as per the method of Soni and Katoch (1997). It is done with the help of micrometer. The diameter of the respective fiber populations are calculated in µm. Since the muscle fibers did not show regular outline, dimensions of each fiber are recorded from the two different angles perpendicular to each other. The data obtained is statistically analysed and standard error of mean is calculated. To determine the significance of changes in respective fiber number and diameter, student t-test and analysis of variance are used to determine the significance of changes in respective fiber and diameter.

RESULTS

Histological findings showed that the basic organization of intestinal wall is similar to that in other vertebrates and is formed by tunica mucosa with a loose connective tissue lamina propria, tunica submucosa, tunica muscularis and tunica serosa layers (Fig. 1). Neither muscularis mucosae between the lamina propria and the submucosae, nor any mucosal tubular glands were observed in the tunica mucosa. A thick layer of densely packed acidophilic

connective tissue, the stratum compactum, separates the mucosa from submucosa (Fig. 4). The mucosal surface has numerous projections (villi), decreasing in length towards the posterior intestine and they lined by simple single-layered tall columnar cells with a basal nucleus containing a nucleolus, an apical brush border and acidophilic cytoplasm that are interspersed with goblet cells and intra-epithelial lymphocytes (IELs) (Fig. 2 and 3). The goblet cells exhibited a supranuclear region characterized by a swollen distal region that contained a translucent cytoplasm and a basal region with associated nuclei.

On the height of villi in each intestinal part, the normal control mice had developed high villi in the duodenum (107.63µm), the undeveloped lower ones in the jejunum (68.35µm) and height of villi in the ileum is (122.60µm). Mice subjected to fenoterol treatment showed a remarkable increase in the duodenal villous height and for the next three stages reveals the marked increase.

Duodenum

At 7 days stage the normal duodenum part of small intestine shows, villi length (107.63µm) and width of the villi (26.67µm),distance between the villi is (6.30 µm), crypt depth is (93.61µm). The diameter of the lumen is (257.21µm). The thickness of the mucosa and submucosa11.39 µm and 38.06 µm is observed in the control mice.

The villous height of the fenoterol treated mice is 211.92 µm at 7 days stage. The width of the villi is 66.81 µm, distance between the villi is 10.42 µm and crypt depth is 145.84 µm, the diameter of the lumen is 269.94µm, thickness of submucosa is 41.18 µm while that of mucosa is 15.20 µm.

At14 days stage, the height of the villi further increases to 245.07 µm and width of the villi increases to 68.89 µm, distance between the villi is 11.86 µm and that of crypt depth is 168.60 µm. The diameter of the lumen is found to be 274.42 µm, thickness of submucosa is 45.11 µm and of mucosa is 24.95 µm.

At 21 days stage the height of the villi increases to 252.24 µm, the width becomes 72.64 µm, distance between the villi is 72.64 µm and the distance between the villi is 12.26 µm, crypt depth is found to be170.34 µm, the diameter of the lumen is 276.60 µm. thickness of submucosa is 48.29 µm and thickness of mucosa is 26.02 µm.

After fenoterol treatment at 28 days stage the villi height increases to 254.68µm, width of the villi is 74.26µm, the distance between the villi is 13.40µm and crypt depth is 172.64 µm, diameter of the lumen is 279.86µm, thickness of submucosa is 50.02µm and that of mucosa is 29.62µm.

Jejunum

Normal jejunum have 68.35 µm villous height and width of the villi is found to be 151.09 µm, the distance between the villi is 11.15 µm and diameter of the lumen is 169.66 µm. Thicknesss of submucosa is 81.02 µm and thickness of mucosa is found to be 53.65 µm.

Treated jejunum at 7 days

Jejunum after fenoterol treatment at 7 days stage shows increase in the villous height which is found to be 124.66 µm. Width of the villi is found to be 154.26 µm and distance between the villi is 16.73 µm. The diameter of the lumen is 172.62 µm, thickness of submucosa is 83.39 µm and thickness of mucosa is 42.56 µm.

Fenoterol administration causes increase in the villous length to 187.64 µm and width of the villi reaches to 157.65 µm, distance between the villi is found to be 20.84 µm, diameter of the lumen further increases to 186.24 µm. The thickness of the submucosa and mucosa also get altered and becomes 86.34 µm and 40.46 µm respectively at 14 days stage.

After fenoterol administration to Swiss albino male mice at 21 days stage, there occur change in villous height, width, diameter and thickness of mucosa and submucosa is witnessed. Villous height increases to 220.46 µm and width of the villi becomes 160.12 µm, distance between the villi is found to be 22.7 2µm. The diameter of the lumen is 188.48 µm. Thickness of submucosa and mucosa is 88.20 µm and 32.54 µm.

Villous height further increases to 223.49 µm, width of villi is 162.67 µm, distance between the villi is found to be 24.68 µm, the diameter of the lumen is 190.82 µm. Thickness of submucosa is 90.56 µm and thickness of mucosa becomes 30.58 µm after drug treatment.

Ileum

The villi length of normal ileum is found to be 122.60 µm, diameter of the lumen is found to be 128.29 µm, thickness of submucosa and mucosa is found to be 52.34 µm and 18.41 µm. The villous length after fenoterol treatment increases to 133.89 µm, diameter is 144.26 µm, thickness of submucosa and mucosa is found to be 68.78 µm and 58.61 µm. At 14 days stage the villi length increases to 146.28 µm, diameter is 148.64 µm, while the thickness of submucosa and mucosa is found to be 74.18 µm and 64.16 µm.

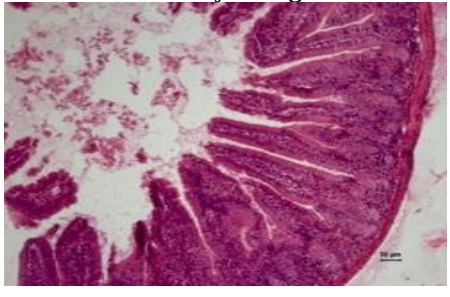
Fenoterol administration causes hypertrophy of the smooth muscle. The villous length further increases at the 21 days stage. It now becomes 152.32 µm, diameter is

152.42 µm, the thickness of submucosa and mucosa is found to be 78.96 µm and 66.31 µm respectively. At 28 days stage, villous length reaches to 154.25 µm, diameter is 195.38 µm, thickness of submucosa and mucosa is 80.02 µm and 67.24 µm.

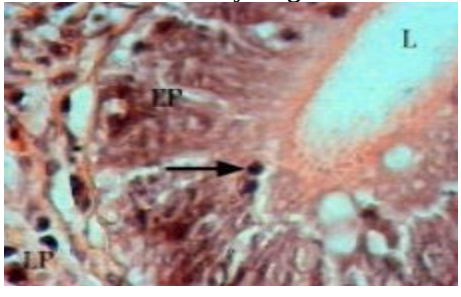
Emergence of different fiber subpopulation and their diameter, length, width, lumen (µm) in drug treated mice (1.5 mg/kg body wt.) for 28 days. Fibers were hypertrophic and atrophic depending upon increase or decrease in fiber diameter as compared to normal counterparts. Values are mean ± SEM from 6 animals each n = number of sections counted.

Tissue type	Length (µm)	Width (µm)	Diameter of lumen (µm)
Duodenum (Normal)	107.63 ± 4.61 n = 10	26.67 ± 4.08 n = 10	257.21 ± 6.52 n = 10
Duodenum (Treated 7 days)	211.92 ± 5.90 n = 10	66.81 ± 4.28 n = 10	269.94 ± 3.65 n = 10
Duodenum (Treated 14 days)	245.07 ± 3.59 n = 10	68.89 ± 3.12 n = 10	274.42 ± 3.50 n = 10
Duodenum (Treated 21 days)	252.24 ± 4.08 n = 10	72.64 ± 2.52 n = 10	276.60 ± 6.24 n = 10
Duodenum (Treated 28 days)	254.68 ± 4.72 n = 10	74.26 ± 3.93 n = 10	279.86 ± 4.03 n = 10
Jejunum (Normal)	68.35 ± 2.73 n = 10	151.09 ± 4.73 n = 10	169.66 ± 3.82 n = 10
Jejunum (Treated 7 days)	124.66 ± 2.78 n = 10	154.26 ± 3.18 n = 10	172.62 ± 5.20 n = 10
Jejunum (Treated 14 days)	187.64 ± 4.38 n = 10	157.65 ± 3.20 n = 10	186.24 ± 2.88 n = 10
Jejunum (Treated 21 days)	220.46 ± 4.84 n = 10	160.12 ± 2.05 n = 10	188.48 ± 3.73 n = 10
Jejunum (Treated 28 days)	223.49 ± 4.64 n = 10	162.67 ± 1.98 n = 10	190.82 ± 2.68 n = 10
Ileum (Normal)	122.60 ± 1.68 n = 10	115.27 ± 2.23 n = 10	128.29 ± 1.98 n = 10
Ileum (Treated 7 days)	133.89 ± 2.08 n = 10	117.76 ± 3.55 n = 10	144.26 ± 2.20 n = 10
Ileum (Treated 14 days)	146.28 ± 3.54 n = 10	122.20 ± 2.56 n = 10	148.28 ± 2.52 n = 10
Ileum (Treated 21 days)	152.32 ± 1.56 n = 10	126.42 ± 2.09 n = 10	152.42 ± 2.66 n = 10
Ileum (Treated 28 days)	154.25 ± 1.38 n = 10	128.36 ± 2.74 n = 10	195.38 ± 3.89 n = 10

D.C 7days Fig. 1

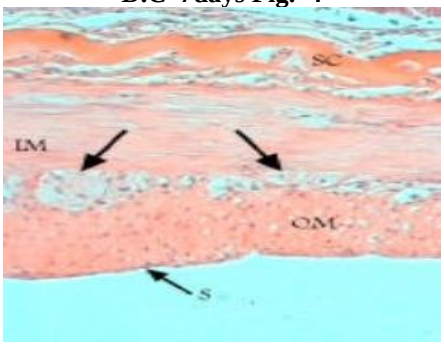


D.C 7 days Fig. 2

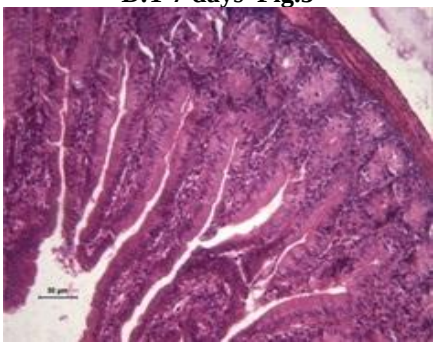


D.C 7 days Fig. 3

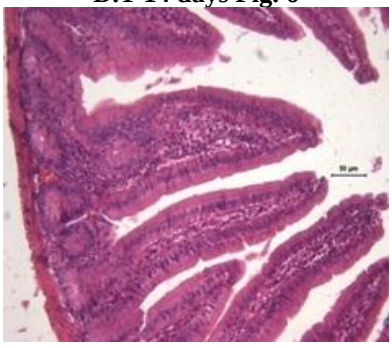
D.C 7days Fig. 4



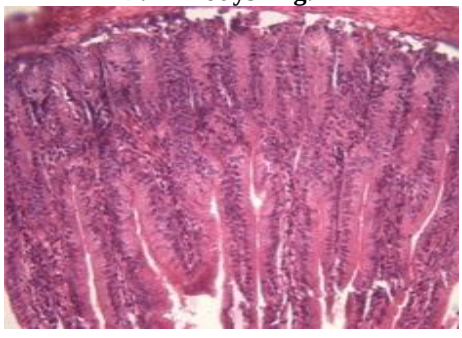
D.T 7 days Fig.5



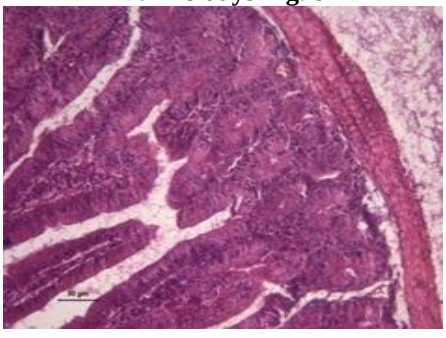
D.T 14 days Fig. 6



D.T 21 days Fig.7



D.T 28 days Fig. 8



D.T 28 days Fig. 9J.C 7 days

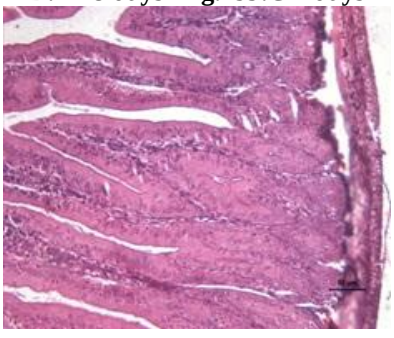
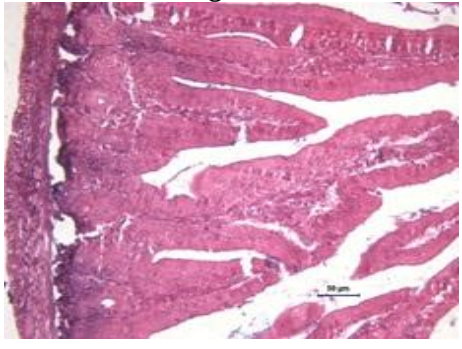
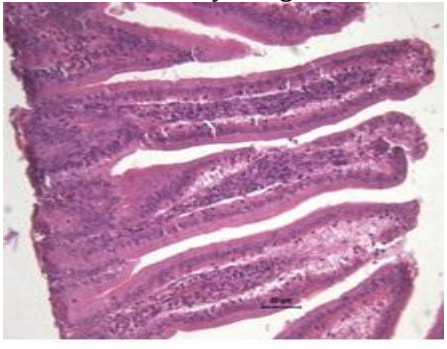


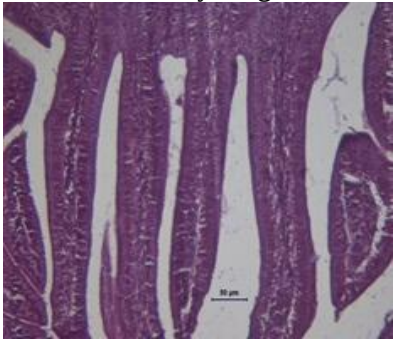
Fig. 10



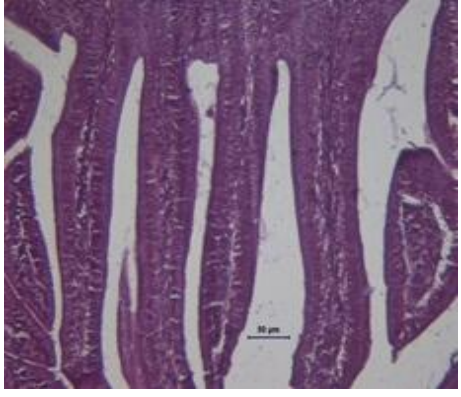
J.T 7 days Fig.11



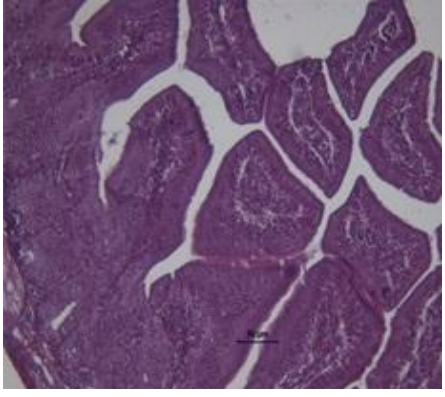
J.T 14 days Fig.12



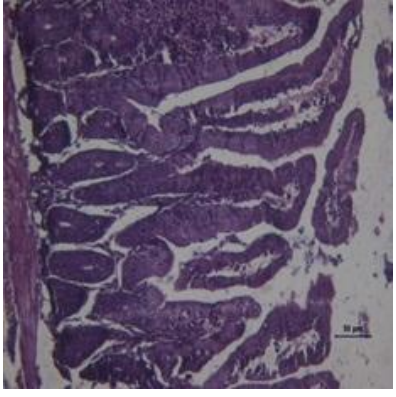
J.T 21days Fig. 13



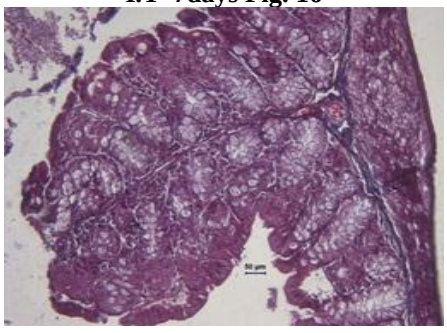
J.T 28 days Fig.14



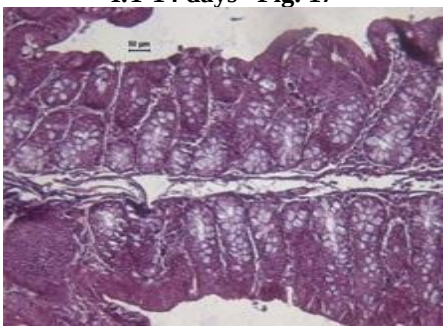
I.C 7days Fig.15



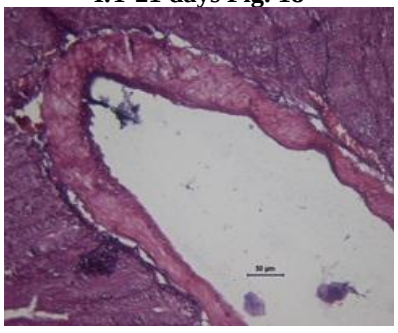
I.T 7days Fig. 16



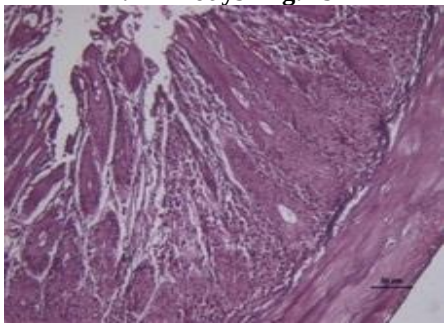
I.T 14 days Fig. 17



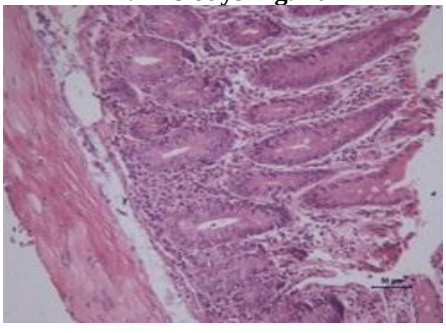
I.T 21 days Fig. 18



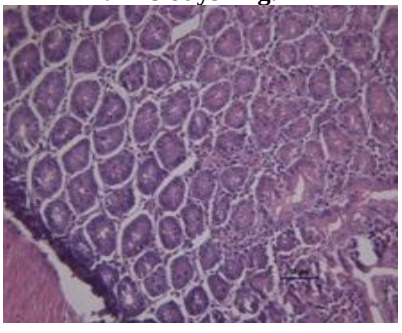
I.T 21 days Fig. 19



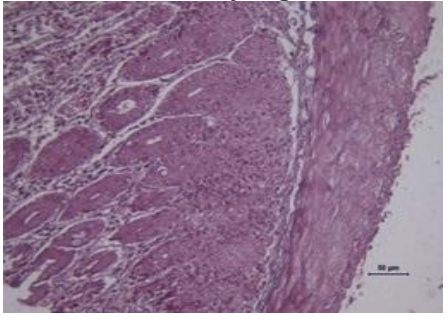
I.T 28 days Fig. 20



I.T 28 days Fig.21



I.T 28 daysFig.22



DISCUSSION

Smooth muscle relaxation is thought to occur following stimulation of β_2– receptor in the cell membrane causing conversion of ATP to cAMP, which then activates protein kinase.This leads to phosphorylation of proteins and binds intracellular calcium thereby reducing its availability for actin – myosin cross linkage and therefore relaxation of the muscle. β_2 – agonists also have mild anti-inflammatory activity because they have been shown to enhance mucociliary clearance (Tandon, 1991) and have metabolic effects such as raising free fatty acids, glucose and insulin concentrations. Hypokalaemia also occurs commonly, especially following intravenous administrations, and thought to be related to linkage of β_2-receptors to Na^+/K^+- ATP ase (Cheong *et al*., 1988). Drugs may affect smooth muscle by a stimulatory or inhibitory effect on structures remote from the muscle tissue (Bourne, 1960).

The extreme variation in the functions of smooth muscle tissue emerges as a consequence of an inherent structural and functional plasticity which enable the tissue to undergo adaptive responses, whenever the changed conditions are imposed on them (Dilley*et al*., 1987; Owens, 1989; Schwartz *et al*., 1990; Thyberg*et al*., 1990). The adaptive responses comprise essentially of variation in the smooth muscle tissue mass which may include myointimal hyperplasia, myointimal hypertrophy, a change in wall thickness, alteration in wall to lumen ratio and also changes in the dedifferentiation state of constituent muscle cells (Dilley*et al*., 1987 ; Schwartz *et*

al., 1990; Thyberg*et al*., 1990). Agonist of β_2 – adrenoceptors have been shown to oppose weakness, loss of muscle mass in a variety of conditions associated with neuromuscular diseases including spinal cord injury, steroid myopathy, muscular dystrophies (Maltin *et al*., 1986 ; Zeman*et al*., 1987). The drug act as a stimulator of growth and increases mass and protein content with decrease in fat contents (Carter*et al*., 1991).

β_2 –agonists have some side effects which include muscle cramps, anxiety and headache. Both tremor and palpitations are more commonly seen with oral dose. The increase in maximum force production in fenoterol treated mice was due to increase in muscle mass, fiber cross-sectional area, and protein content. These finding suggest that a physiological role of β_2- adrenoceptor mediated mechanisms in muscle regeneration show clearly that fenoterol hasten recovery after injury, indicating its potential therapeutic application. Fenoterol treatment can promote recovery of muscle function in conditions where normal muscle regeneration is impaired. Treatment with powerful β_2 – agonist like fenoterol may prove useful in promoting functional recovery of muscle after severe trauma, and for combating muscle wasting and weakness associated with ageing, neuromuscular disorder, prolonged sepsis, aquried immunodeficiency syndrome, burn, injury, and cancer cachexia (Lynch, 2002).

The small intestine is the largest component of digestive tract and the major site of digestion and absorption. The small intestine is divided in to three parts – duodenu, jejunum, ileum. The mucosa of the small intestine is highly modified. The intestinal villi are extremely susceptible to ischemic damage, and their necrosis is one of the earliest histological changes that occur during intestinal ischemia (Williams, 1971, 1988). Recently, it has been indicated that apoptosis is triggered by mild cellular injuries due to hyperthermia (Barry *etal*., 1990), hypoxia (Korr *et al*., 1991) and direct acting agents including anticancer drugs (Barry *et al*., 1990). Damage to the muscular coat of the intestine is seen after drug treatment. Similar results are reported earlier in the rat small intestine by a number of workers (Park *et al*., 1990; Wagner *et al*., 1979). In the rat jejunum and ileum fed with pectin various researchers (Holt *et al*., 1984 have reported earlier that crypts becomes more deeper.

Fenoterol helps the smooth muscle to relax. Gu *et al*., (1995) have suggested that there are at least three receptors, gelatin antagonists, M 15 and M 35, demonstrated that M35 is more potent in inhibiting contractile responses, whereas M15 is more potent in inhibiting relaxation. Therefore, present study under investigation is with regard to works of Botella*etal*., 1992; 1995, and suggests that fenoterol relax the small intestine by binding to M 35 sensitive receptor coupled to G- protein. It has been known for several years that receptor and G – protein stimulation increases the myofilamentCa^{2+} sensitivity of all smooth muscles (Kitazawa *etal*.,1989; Moreland *etal*., 1992). Release of Ca^{2+} to the extracellular spaces does not take place and thus prevents the phosphorylation of myosin light chains. The concentration of fenoterolchoosen for the experiments was based on the optimal dose of clenbuterol which was used previously to produce significant changes in muscle structure and function. An increase in muscle function might be achieved at lower concentrations of fenoterol, with minimal concomitant cardiac hypertrophy. Intestinal hyperplasia was reported in 1 and 3 week old pigs (Adeola and King, 2006). It is known that villous amplification vary with intestinal location as well as during development ,experimental treatment and disease (Boyne *et al*.,1966, Diamond *etal*,1984). Apoptosis in small intestine may be triggered by cellular injuries due to hyperthermia (Barry *et al*., 1990, Lennon *et al*., 1999).

The villi are the most obvious feature of the mucosa which house a dynamic, self-renewing population of epithelial cells that include secretary cells which take up nutrients from the lumen and transport them in to blood, fulfilling the basic function of the digestive system. The administration of fenoterol has been associated with the relaxation of the small intestine with broader and longer villi, thick mucosa, expanded Brunner's gland. The severity of intestinal morphological changes appears to increase with dose and time. The morphology of gut of normal animals differs from that of treated one. The longer villi, expanded glands , splitting and degeneration of fibers account for differences in gastrointestinal structure and function of the normal from that of treated counterpart. Fenoterol , on the other hand, may directly affect the epithelial cells counteracting any villus alterations or degeneration of fibers. It results in hypertrophy of skeletal and smooth muscles. . Based upon anabolic properties, ß-agonists have been proposed as valuable adjunct to the treatment of muscle wasting conditions. The potential clinical benefit of treatment by this agonist is to reduce loss of mass and forces in atrophied muscles. In conclusion, fenoterol treatment causes a time and concentration dependent development of constitutive β_2-adrenoceptor activity , which can be reversed by various inverse agonists. β-agonist induced changes could represent a novel regulation mechanism of β_2 – adrenoceptor activity.

REFERENCES

Adeola O, King DE. Developmental changes in morphometryof small intestine and jejunalsucrase activity during the first nine weeks of postnatal growth in pigs. *JAnim. Sci*, 84, 2006, 112-118.

Barry MA, Behnke CA and Eastman A. Activation of programmed cell death Apoptosis.(Eds. Brown J, Kellecher MS and Losowsky. The effect of pectin on the structure and function of rat small intestine. *Br. J. Nut,* 42(3), 1979, 357- 365.

Botella A, Delvaux M, Fioramonti J,Frexinos J and Bueno L. Galanin contracts and relaxes guinea pig and canine intestinal smooth muscle cells through distinct receptors. *Gasroenterol*.108, 1995, 3-1.

Bourne GH. Structure and function of muscle. *Berkely square House, London WI*. 1960, 110.

Boyne R, Fell BF, and Robb I. The surface area of intestinal mucosa in the lactating rat. *J Physiol,* 183, 1966, 570 – 575.

Carter WJ, Dang AQ, Faas FH and Lynch ME. Effects of clenbuterol on skeletal muscle mass body composition and recovery from surgical stress in senescent rats. *Metabolism*, 40, 1991, 855.

Cheong B, Reynolds SR, Rajan G and Ward MJ. Intravenous -agonist in severe acute asthma1988; *British Med,* 297, 1988, 448-50.

Diamond JM, KarasovWH, Cary C, Enders D and Yung R. Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine *in vitro. J Physio*,349, 1984, 419-440.

Dilley RJ, McGeachie JK, and Prendergast FJ . A review of the proliferative behaviour, Morphology and phenotypes of vascular smooth muscle. *Atherosclerosis.* 63, 1987, 99- 107.

Domeneghini C, Arrighi S, Radaelli G, Bosi G and Mascarello F. Morpholgicaland histochemical peculiarities of the gut in the white sturgeon, *Acipenser Transmontanus. Eur. J. Histochem*. 43, 1999, 135-145.

Gu ZF, Pradhan TK, Coy DH, and Jensen RT. Interaction of galanin fragments with galaninreceptors on isolated smooth muscle cells from guinea pig stomach: Identification of a novel galanin receptor subtype. *J. Pharmacol. Exp. Ther,* 272, 1995, 371 – 378.

Holt PR, Pascal RR and Koller DP. Effects of aging upon small intestinal structure in the fisher rat. *J Gerontol*, 39(6), 1984, 642 – 647.

King DE, Asem EK and Adeola O. Ontogenetic development of intestinal digestive functions in White Pekin ducks. *J. Nutr*, 130, 2000, 57–62.

Kitazawa T, Kobayashi TS, Horiuti K, Somlyo AV, and Somlyo AP Receptor coupled ,permeabilized smooth muscle. Role of phosphatidylinositol cascade, G – protein and modulation of the contractile response to Ca^{2+}. *J Biol. Chem*, 264, 1989, 5339 –5342.

Korr JFR and Harmon BV. Defination and incidence of apoptosis: an histological perspective. The molecular basis of cell death. (Eds. L.D Tomeri and F.O Cope) Cold Spring Harbor , NY . *Cold Spring Harbor Laboratory Press*, 1991, 5 – 29.

Lennon SV, Martin SJ and Cotter TG. Dose dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell prolif*, 24, 1999, 203 – 214.

Lynch GS .β_2-agonist. In: Performance enhancing substances in sport and Exercise, (Eds. MS Bahrke and C.E. Yesalis) Champaign IL. *Human Kinetics*, 2002, 47-64

Moreland S, Nishimura J, Van Breemen C, Ahn HY, and Moreland RS. Transient myosin phosphorylation at constant Ca 2+ during agonist activation of permeabilized arteries. *Am .J Physiol*, 263, 1992, 540 – 544.

Park PO and Haglund U. The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion. *Surgery*, 107, 1990, 574 – 580.

Schwartz Foy L, Bowen- pope DF and Ross R. Derivation and properties of platelet-derived growth factor-independent rat smooth muscle cells. *Am. J. Pathol*, 136, 1990, 1417-1428

Thyberg J, Hedin U, Sjolund M, Palmberg L and Bottger BA. Regulation of differentiated properties and proliferation of arterial smooth muscle cells. *Arteriosclerosis*, 10, 1990, 966-990.

Williams LF .Vascular insufficiency of the intestine. *Gastroenterol*, 61, 1971, 555-757.

Zbar AP, Simopoulos C, Karayiannakis A. Cadherins J. An integral role in inflammatory bowel disease and mucosal restitution 2004. *J Gastroenterol*, 39, 2004, 413-421.

Zeman RJ, Ludemann R, Eastoni TG and Etliner JD. Slow to fast alterations in skeletal muscle fibers caused by clenbuterol, a β_2- receptor agonist. *Am. J. Physiol. Endocrinol. Metab*, 254, 1988, E 726 – E 732.

Zeman RJ, LudemannR and Etlinger JD. Clenbuterol, β-agonist retards atrophy in denervated muscles. *Am. J. Physiol*, 252(15), 1987, E152- 155.