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Histological changes in small intestine and vastus lateralis of male swiss albino mice after fenoterol administration

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The protein anabolic and hypertrophic effects of fenoterol in skeletal and smooth muscles have been confirmed from an increase in tissue mass following drug administration. Based upon anabolic properties, β-agonists have been proposed as valuable adjunct to the treatment of muscle wasting conditions. The aim of the study was to ascertain the effect of fenoterol on vastus lateralis and small intestine morphology. Equimolar dose of fenoterol was administred to mice for 28 days to see its effects on vastus lateralis and small intestine in order to test the hypothesis that fenoterol would produce powerful anabolic and ergogenic effects.

Key words : Beta agonist, Fenoterol, Vastuslateralis, Small intertine

INTRODUCTION

A severe loss of muscle mass is a risk factor for mortality in a number of conditions and diseased states. β- agonists cause increase in body mass and dilation of tissues.The drug fenoterol exerts its effect on skeletal muscle via $\beta_1$and β_2 adrenoceptors.It affects smooth muscle by stimulatory or inhibitory effects on structures remote from the muscle tissue and by local action on smooth muscle cell (Bourne, 1960). Most muscle tissue contain primary β_1 and β_2 receptors which when activated cause specific muscular function. Fenoterol treatment exerts its effects on skeletal and smooth muscles and stimulate muscle growth (Reeds *et al*., 1986). The drug acts as a stimulator of growth and increases mass and protein content with decrease in fat content. After drug treatment, there is significant change in the dimensions of villi during mucosal atrophy and hypertrophy. It is known that villous amplification vary with intestinal location as well as during development, experimental treatment and disease (Fisher and Parson, 1950; Boyne *et al*.,1966, Diamond *et al*., 1984). An unique observation in the histopathological preparation is myonecrosis and is characterized by peculiar anatomical changes in the nuclear morphology. Apoptosis has been found in small intestine showing loss of surface contact with neighbouring cells. It may be triggered by cellular injuries due to hyperthermia (Barry *et al*., 1990, Lennon *et al*., 1999). Damage to the muscular coat of intestine is seen after drug treatment.

MATERIALS AND METHODS

The present investigation has been carried out on skeletal muscle (Vastus lateralis) and 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 a 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 (1.4 mg/ kg body wt.) for 28 days. Vastus lateralis and small intestine were 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% to30% (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. A dip was done 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.

RESULTS AND DISCUSSION

Microscopic examination of mice vastus lateralis muscle revealed circular, oval or polygonal cells with subsarcolemmal disposition of nuclei (Fig. 1). The muscle cells were stained pink while nuclei were sained purple. Haematoxylin–eosin staining of muscle sections demonstrated innumerable interfibrillar nuclei in tightly packed muscle fibers at 14 days stage (Fig. 2). The fibers were round with subsarcolemmal nuclei at 28 days stage in vastus lateralis (Fig. 3). Constituent muscle fibers displayed variable shapes and sizes and hence point towards a heterogeneous population of cells. Some of these fibers were large and others were relatively smaller in their cross- sectional dimensions.

Mice vastus lateralis muscle exhibited significant changes when treated with fenoterol orally. Size of muscle cell increased at 7 days stage and splitting of fibers lead to large interfibrillar and interfascicular spaces (Fig. 4) at 14 days stage. Complete fibrolysis along with interfibrillar space was noticed (Fig. 5). Variable shapes like triangular, elongated (Fig. 6) and merged fibers by loosing their boundaries and increasing collageneous material (Fig. 7) were noticed. Fiber degeneration resulted in large interfascicular spaces. Muscle hypertrophy with initiation of degeneration of muscle fibers was also visualized. In some muscle sections, atrophy is also seen. Complete merging lead to the extrusion of purple coloured nuclei to

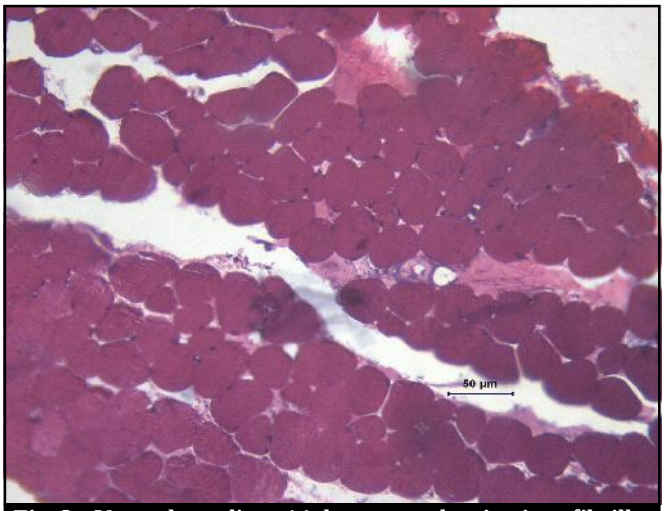

Fig. 2 : Vastus lateralis at 14 days stage showing interfibriller nuclei

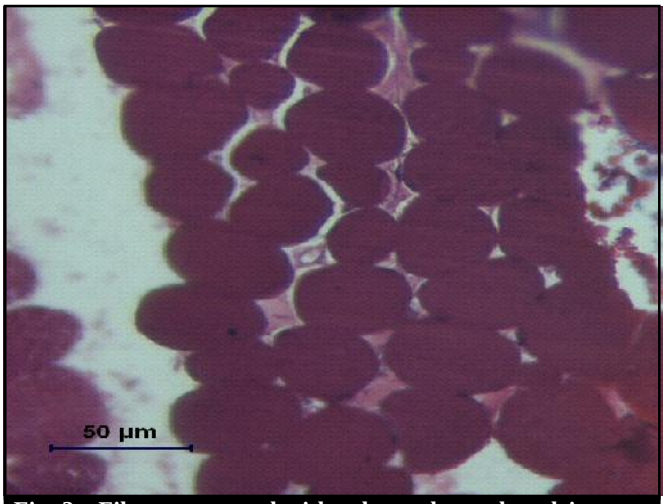

Fig. 3 : Fibers are round with subsarcolemmal nuclei

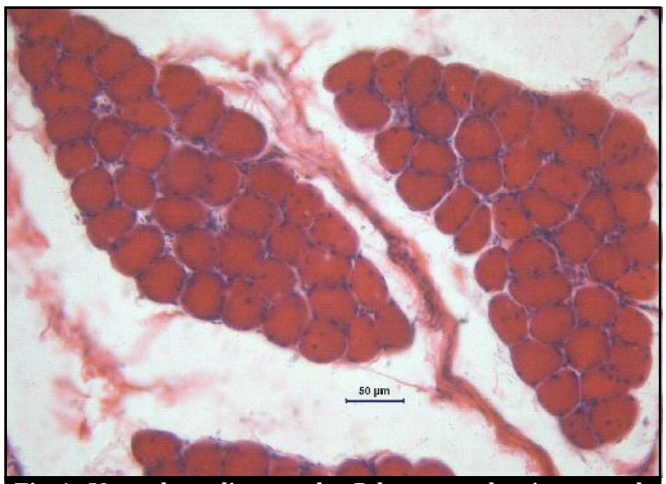

Fig. 1 : Vastus lateralis normal at 7 days stage showing normal rounded fibers

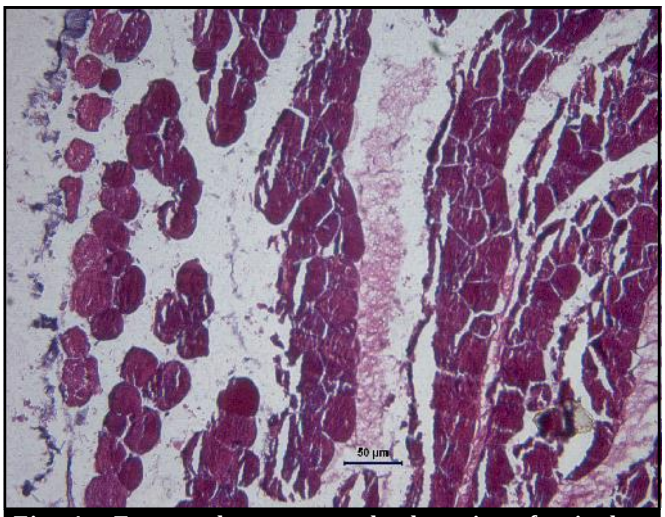

Fig. 4 : Fenoterol treatment leads to interfascicular interfibriller area at 7 days stage

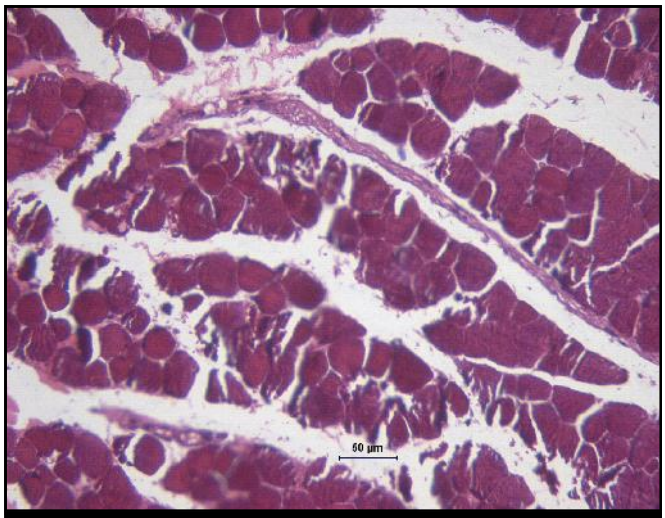

Fig. 5 : Complete fibrolysis leading to interfibriller space after fenoterol treatment at 14 days stage

the interfibriller spaces at 28 days stage Splitting of fibers and clumping of nuclei which form long chains in interfibrillar spaces (Fig. 8) were seen at 28 days stage. The small intestine is the largest component of digestive tract and the major site of digestion and absorption. The small intestine was divided in to three parts – duodenum, jejunum, ileum. The mucosa of the small intestine is highly modified. The microscopic examination of normal mice duodenum at 7 days stage showed luminal surface which was covered by a leaf like projections called villi serosa (Fig. 9). The villus is an extension of laminapropria. Opening on to the luminal surface at the bases of the villi are simple tubular structures called intestinal glands or Crypts of Liberkuhn. Brunner's gland were penetrating the muscularis mucosa to enter the lamina propria (Fig. 10). At 28 days stage, the mice duodenum section showed

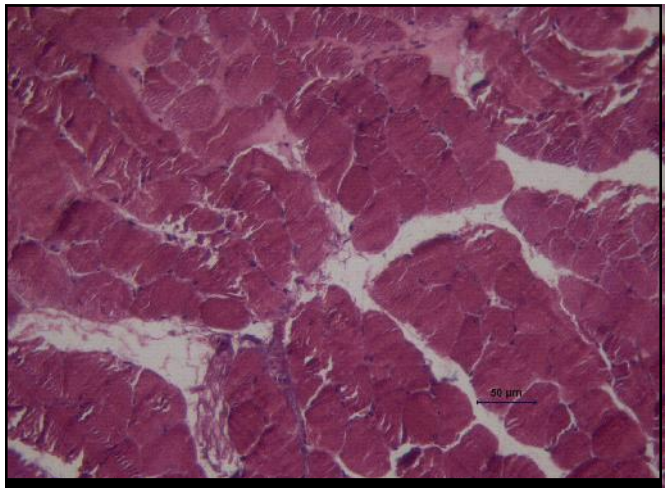

Fig. 6 : Various shapes of the fibers elongated, triangular after drug treatment

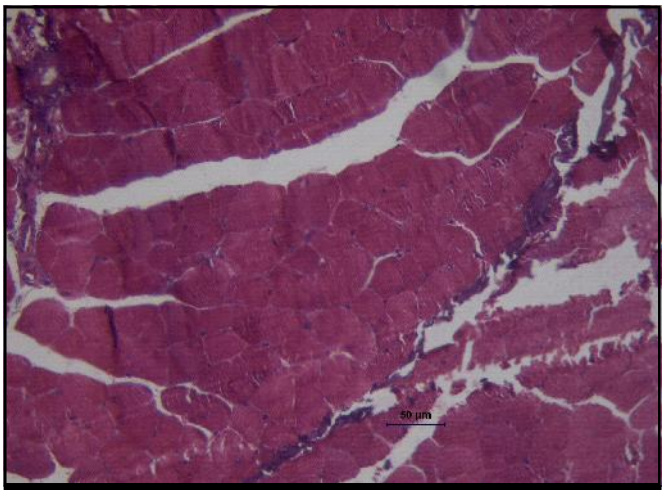

Fig. 8 : Degeneration of the fiber and clumping of nuclei which form long chain

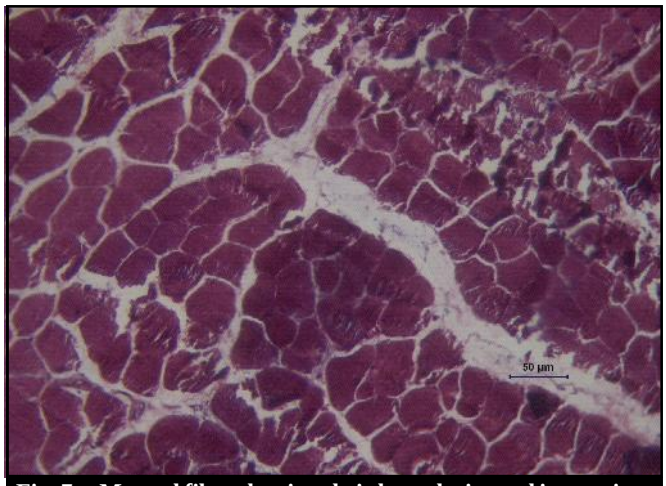

Fig. 7 : Merged fibers loosing their boundaries and increasing collageneous area at 21days

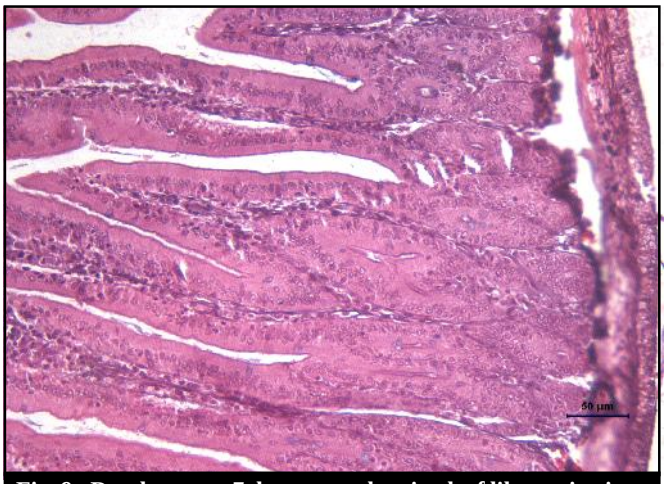

Fig. 9 : Duodenum at 7 days stage showing leaf like projection called villi serosa

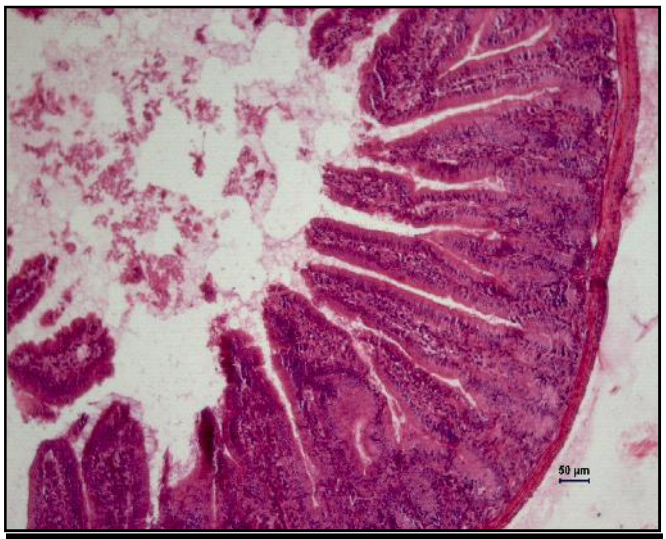

Fig. 10 : Brunner's glands penetrating the muscularis mucosa

a large fold in the submucosa that raised the overlying mucosa with its villi and crypts with numerous caveolae can be seen (Fig. 11).

Duodenum of mice after 7 days of fenoterol administration demonstrated broad spaces between the villi (Fig. 12). Brunner's gland got expanded, with ruptured villi (Fig. 13). The plicae circularis could be seen clearly with scattered caveolae at 28 days stage (Fig. 14). The serosa is thick, mucosa and submucosa part are well formed of a continuous band. In some places splitting of cells takes place in submucosal part.

The epithelium is several layers thick. A few goblet cells can nevertheless can be distinguished. However, both the central lacteal and the smooth muscle strand accompanying it, were present among the lymphocytes

Fig. 11 : At 28 days stage large number of caveole are seen

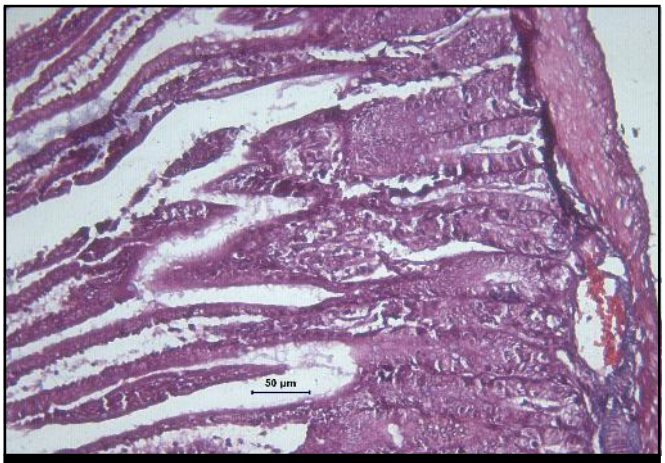

Fig. 12 : After drug treatment at 7days stage broad space between villi occurs

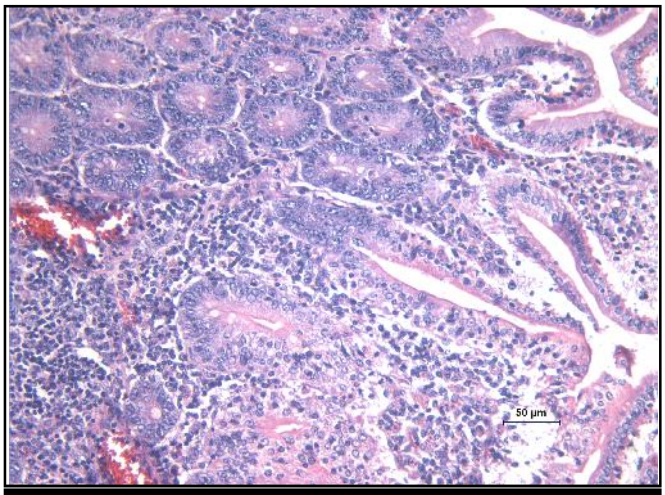

Fig. 13 : After drug treatment Brunner's glands get expanded with Ruptured villi

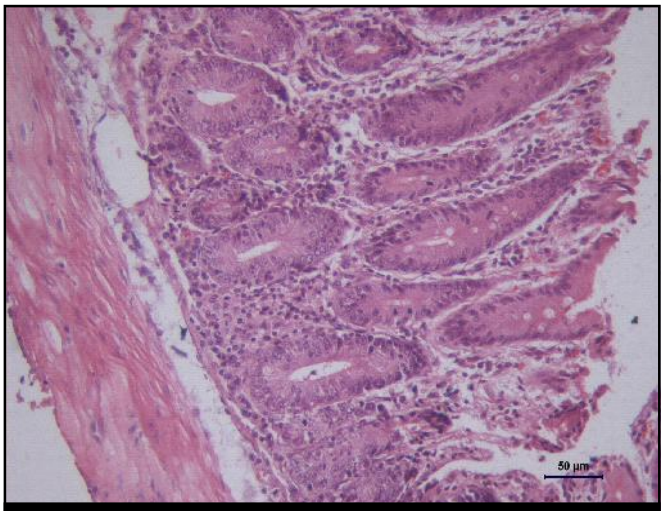

Fig. 14 : Thick serosa mucosa and sub-mucosa are well formed in a continuous band

in laminapropria. The transverse section of normal mice jejunum at 7 days staged revealed mucosa, sub mucosa and serosa with complete absence of Brunner's gland in submucosa part (Fig. 15). Goblet cells were clearly seen. Large intercellular space was seen in between the villi. Fenoterol treated mice jejunum showed thin serosa and villi got ruptured (Fig. 16).After drug treatment villi got bursted and crypts got destructed. In normal mice ileum, Brunner's glands are completely absent. Peyer's patches in submucosa are present (Fig. 17). The villi were small and were arranged in in the form of ridge. The ileum is characterized by large aggregates of lymph nodules, called peyer's patches, in the submucosa. After the drug treatment, serosa became thick, and villi got ruptured, Crypts becames very small (Fig. 18). There was no gap or space between the villi after drug administration.

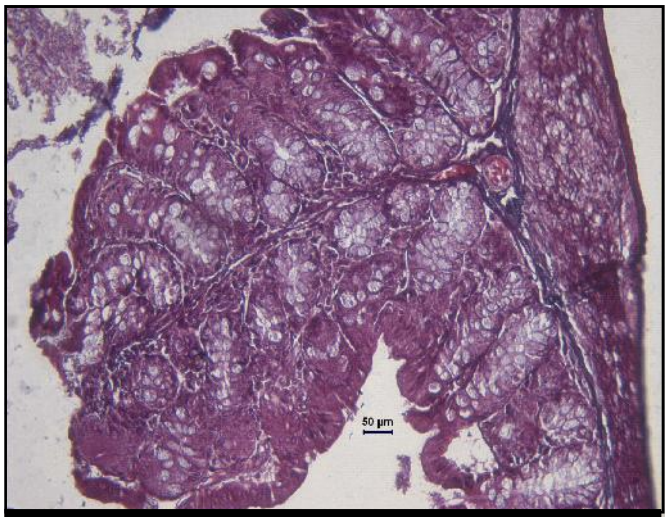

Fig. 15 : Brunner's glands are absent in submucosa

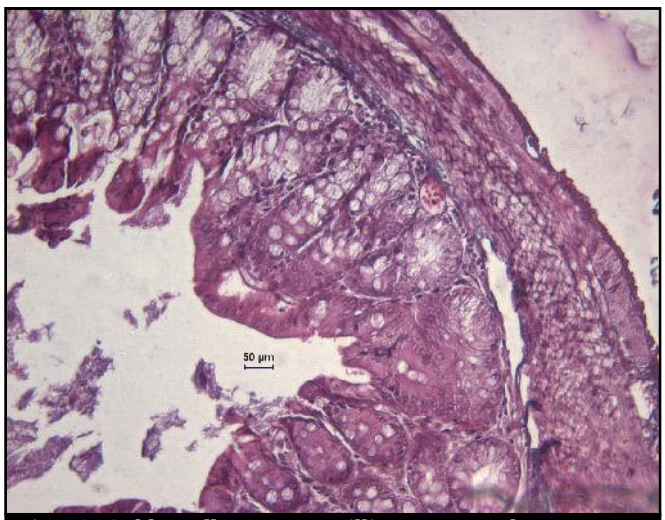

Fig. 16. Goblet cells are seen, villi get ruptured

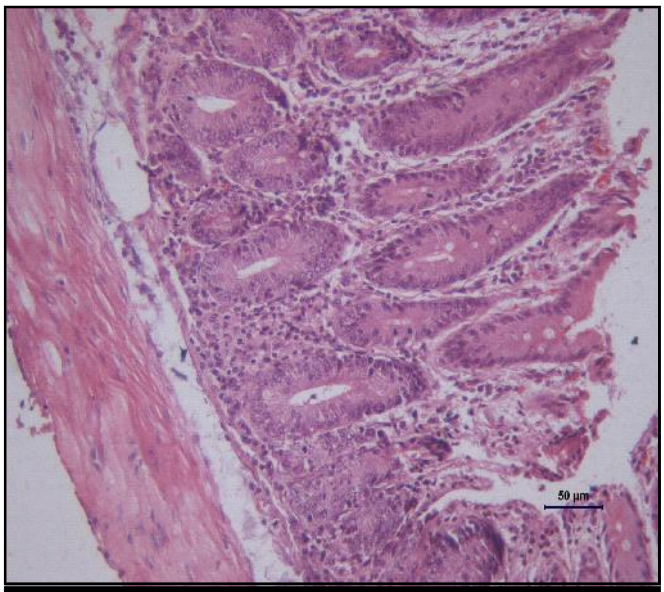

Fig. 17 : Bursted villi and crypts get disrupted, peyer's patches are also seen

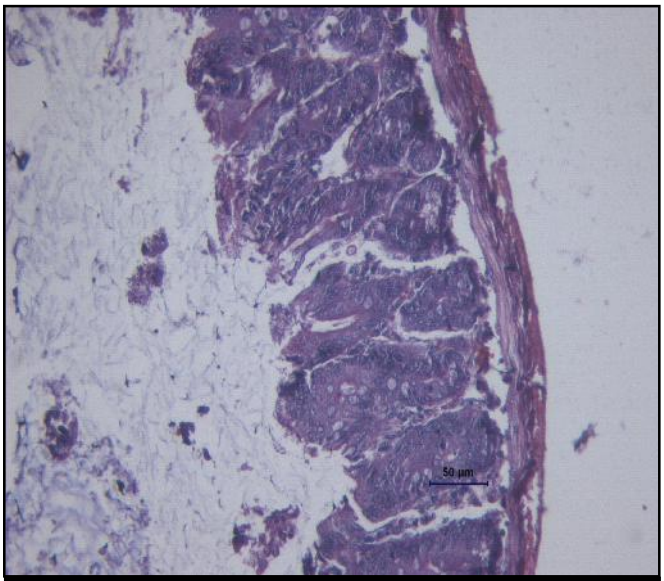

Fig. 18 : At 28 days stage serosa becomes thick, villi get ruptured and crypts becomes very small after drug treatment

The histological investigation has established that fenoterol has more or less muscle specific effects. It was more effective in smooth muscle tissues than in skeletal muscle,in terms of its anabolic influences or physiological modulations. An unique observation in the histopathological preparation is myonecrosis and progressive insufficiency of regeneration of affected muscle fibers. It was thought to be responsible for replacement of muscle tissue with connective tissue and

also fat in later stages of diseases. 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). Normal mice when fed on diet containing fenoterol exhibited hypertrophy of skeletal as well as smooth muscle fibers. Apoptosis has been found in the small intestine showing loss of surface contact with neighbouring cells. Recently, it has been indicated that apoptosis is triggered by mild cellular injuries due to hyperthermia (Barry *et al*., 1990), hypoxia (Korr and Harmon, 1991) and direct acting agents including anticancer drugs (Barry *et al*., 1990). Damage to the muscular coat of the intestine was seen after drug treatment. Similar results were reported earlier in the rat small intestine by a number of workers (Park *et al*., 1990; Wagner *et al*., 1979). Crypts showed greater depth, muscle layer was thick in jejunum and ileum at 14 days stage. This is in accordance with the results reported earlier (Holt *et al*., 1984; Brown *et al*., 1979) in the rat jejunum and ileum fed with pectin. Fenoterol helps the smooth muscle to relax. Gu *et al*. (1995) have suggested that there are at least three receptors, galalin 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 *et al*. (1992 ; 1995) and suggests that fenoterol relaxed 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 myofilament Ca^{2+} sensitivity of all smooth muscles (Kitazawa *et al*.,1989; Moreland *et al*., 1992). Release of Ca^{2+} to the extracellular spaces does not take place and thus prevents the phosphorylation of MLC (Myosin Light Chain). During present investigation, use of beta agonist caused an increase in muscle function capacity and muscle fiber size. It might be achieved at lower concentration, with only minimal concomitant cardiac hypertrophy. If a lower dose of fenoterol is combined with selective β_1–antagonist then it may be possible to eliminate the unwanted side effects associated with cardiac hypertrophy yet maintain a physiologically significant effect on skeletal muscle function. Only thereafter, the full therapeutic potential of fenoterol treatment will be realized.

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