

Figure 3. Status of malonaldehyde (MDA) and antioxidant enzymes in hemorrhoids and piles cure treated groups. All data are mean ±SD of ten animals each group. One way analysis of variance (ANNOVA) followed by Turkey's t-test multiple comparison was performed for statistical significance between control group (GI) vs. hemorrhoids induced group (GII) and hemorrhoids induced group vs. piles cure treated group
 ***p<0.0001: Highly significant; **P<0.001: Significant; *P<0.05: Less significant and Ns P>0.05: Insignificant

Effect on antioxidant parameters

A significant (p<0.0001; p<0.001) reduction of antioxidant enzymes viz. catalase, glutathione peroxidase, glutathione reductase and reduced glutathione enzyme activities were found in hemorrhoid induced group as compared with control group. After treatment with piles cure drug in hemorrhoid plus piles cure treated group, these enzymes activities were significantly (p<0.0001; p<0.001) increased when compared with hemorrhoids induced group. These enzyme activities were normalized and come back to control group (Figures 2 and 3).

Histopathological examination

The histological examination of recto-anal tissue showed that partly skin and anal mucosa with normal cytoarchitecture of the recto-anal region found in control group (Figure 4A) whereas group II treated with croton oil application developed the polypoid mass of smooth muscle layer by papillary mucosa and significantly found higher inflammatory cells in recto-anal region (Figure 4B). After treatment with piles cure drug in treated group, the section showed that the inflammatory cells were seen less and tissue of rectum lined found by mucosa forming luminal folds (Figure 4C).

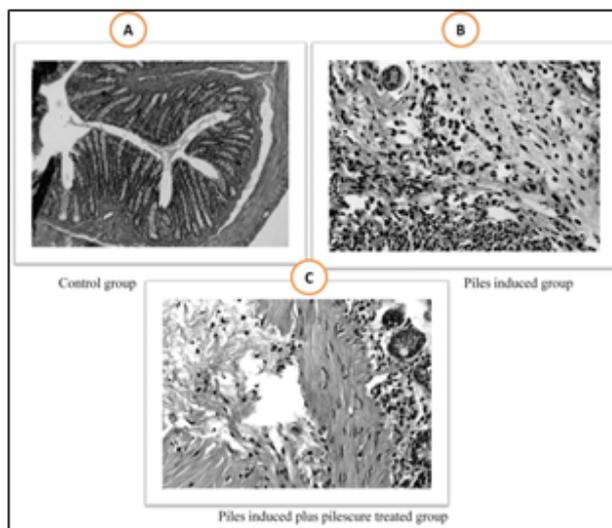


Figure 4. Histopathological examination of recto-anal tissue in control, hemorrhoid and drug treated groups: Slide A shows normal cytoarchitecture of the recto anal region in control group (4A), whereas, slide B (hemorrhoid induced) showed that after application of croton oil in recto-anus area, a developed the polypoid mass of smooth muscle layer by papillary mucosa and found higher inflammatory cells (4B). After treatment with piles cure drug for three weeks, the inflammatory cells were less seen and section showed the normal tissue of rectum lined by mucosa forming luminal folds (4C).

DISCUSSION

Hemorrhoids are a pathological state that is characterized by a severe vasodilatation at the region of recto-anal area that causes the inflammation surrounding tissues, thus further leading to secondary complications such as extravasation of fluid into fluid interstitial space mainly due to increased vascular permeability and migration of large amount of inflammatory cells [8]. So in this study croton oil used as inducer for induction of hemorrhoid model in animals. Croton oil causes inflammation due to release of soluble factors inflammatory lipid metabolites such as prostaglandins [26], leukotrienes, nitric oxide (NO) [27] and cytokines (TNF- α , IL-6 and IL- β) [28,29], etc. The present finding suggested that a significant improvement in stool frequency on the basis of stool weight examination in pilesure treated group when compared with hemorrhoid induced group. The result clear indicated that drug suppresses the constipation symptom which is one of the causative factors for hemorrhoid disease. Such action was seemed due to presence of active ingredients *Terminalia chebula* in a drug which act as laxative and vata detoxification and digestive balancing. Recto-anal weight and recto-anal coefficients were found significantly decreased along with increased the hematological parameters after treatment with drug for 21 days in pilesure treated group as compared with hemorrhoid induced group. After treatment with drug these parameters were come back to control group. These findings clear suggesting that the active ingredients of drug shows potent anti-inflammatory effect and improve the hematological parameter due to homeostatic balance and its main action on the blood capillaries, due to its KASHAYA RAS (Astringent) and SHEET VIRYA (cool nature). The results also showed that there were significantly increased inflammatory parameters C-reactive protein, neutrophil, cytokines (TNF- α , IL-6 and IL- β) and nitric oxide in hemorrhoid induced group when compared with control group. These results are indicating that croton oil leads to inflammation due to release of soluble factors involving inflammatory lipid metabolites, kinins (chemokines) and cytokines, etc. These factors alone or and/or in combination, regulate the activation of resident cells (fibroblast, microphages, endothelial cells and mast cells) and newly recruited inflammatory cells (lymphocytes, basophil and neutrophil) causes the systemic response to inflammation [30]. After treatment with drug for 21 days, these inflammatory parameters were significantly decreased in pilesure treated group as compared with hemorrhoid induced group and come near to control group. The inflammatory parameters were reduced in pilesure treated group due to presence of anti-inflammatory property of *Terminalia chebula*, *Mesua ferrea* and *Ricinus communis* herbs present in the drug. Various studies have been reported that these herbs shows as anti-inflammatory effect [31-34].

Oxidative damage to biological compounds, especially lipids through lipid peroxidation has been shown to play a

significant role in various pathophysiological states. Superoxide dismutase (SOD) is a specific antioxidant enzyme that repairs the cells and reduced the damage caused by superoxide anions ($O_2^{\cdot-}$), which is most common free radical in the body. Free radical can lead to lipid peroxidation in organisms. Malonaldehyde (MDA) is end product of poly unsaturated fatty acids (PUFAs) in cells, and an increase in free radical causes overproduction of MDA. MDA is biomarker of oxidative stress in various diseases [35]. So authors have measured the malonaldehyde level as a biomarker of oxidative stress. In normal cell, there is proper balance between oxidative stress and antioxidant enzyme levels, any imbalance between these two ratios that lead to various pathophysiological states. In our study, we found that there were statistically ($p < 0.0001$) significant decreased antioxidant enzymes activities (Catalase, glutathione peroxidase, glutathione reductase and reduced glutathione) along with increased MDA level in hemorrhoid induced group as compared with control group. After treatment with pilesure drug for 21 days, these enzymatic activities were significant improved along with lowered the MDA level in pilesure treated group when compared with hemorrhoid induced group. These results clear demonstrated that the drug consists of polyherbal has prominent antioxidant effect. The similar result has been published by other investigator in croton oil hemorrhoid induced animals [10]. Based on above findings it is clear indicating that croton oil application causes severe inflammation that altered recto-anal physiology, hematological, biochemical and cytokines parameters along with enhancement of oxidative stress that leads to hemorrhoid. After treatment with pilesure drug the above parameters were improved in hemorrhoid condition. Base on above finding it is clear indicating that the poly herbal drug pilesure is most therapeutic effects for reduction of recto-anal pain, inflammation and inhibit the free radical generation and enhance the antioxidant enzymes level. These anti-inflammatory and antioxidant properties are present in a drug due to presence of active constituents of tannin, alkaloids, and polyphenol ingredients in polyherbal ayurvedic formulation.

CONCLUSION

So conclusion these finding suggesting that pilesure is nontoxic with LD₅₀ more than 2000 mg/kg body weight and it is most effective ayurvedic polyherbal drug which reduces inflammation, oxidative stress and antioxidants properties along with improvement of haematological parameters during hemorrhoid condition. The drug can improve the healthy and quality of life people who are suffering from hemorrhoid problem.

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COMPETING INTERESTS

None

SOURCE OF SUPPORT

None

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