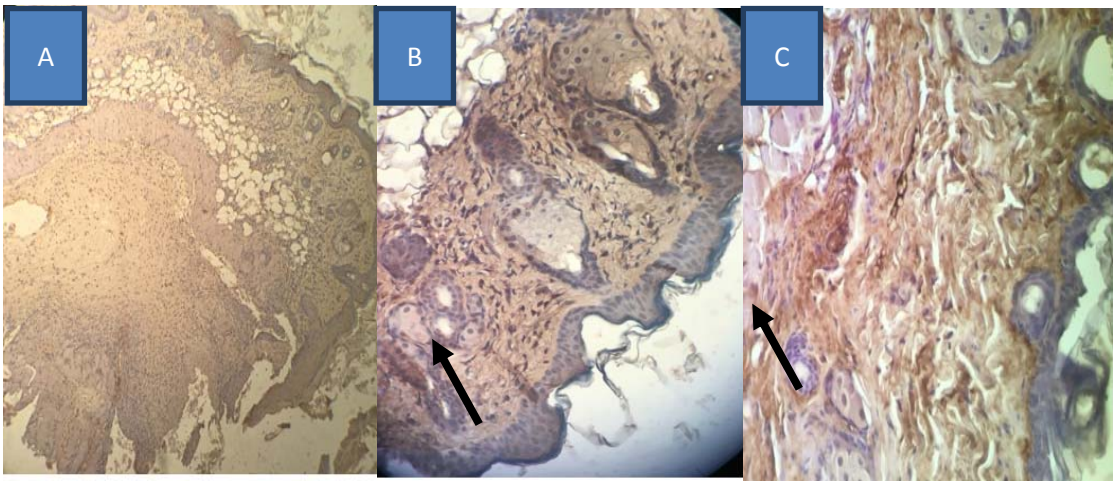

Figure 5: Extracellular immunohistochemical expression of collagen III within the dermis (black arrow). (A) 10X; (b) and (C) 40X.

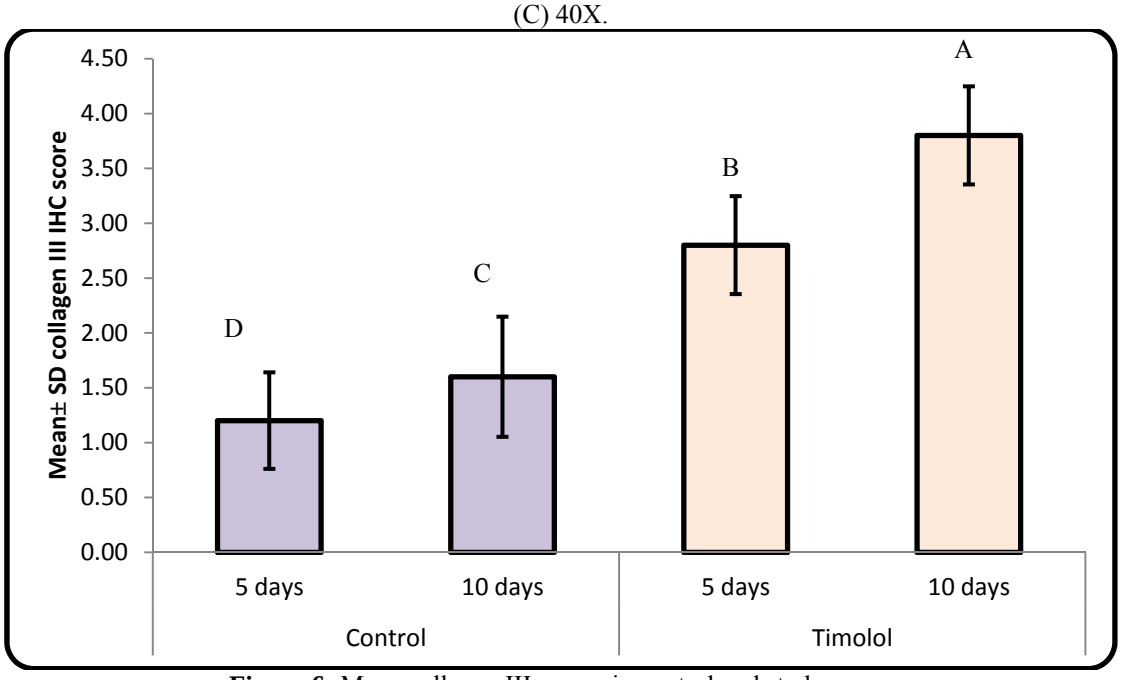

Figure 6: Mean collagen III scores in control and study group

Capital letters indicate the level of significance at (P≤0.05); different letters indicate significant variation; (A) indicates the highest value.

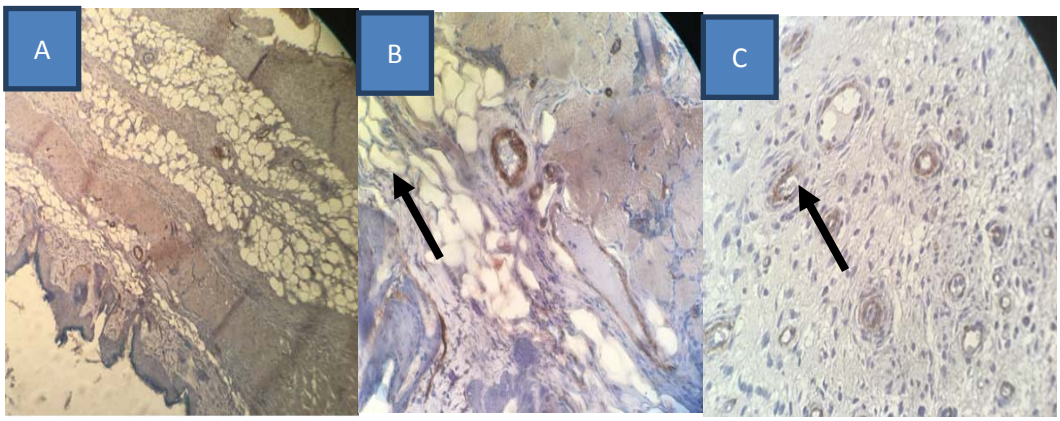

Figure 7: Cytoplasmic immunohistochemical expression of SMA in the wall of blood vessels (black arrow). (A) 10X; (b) and (C) 40X.

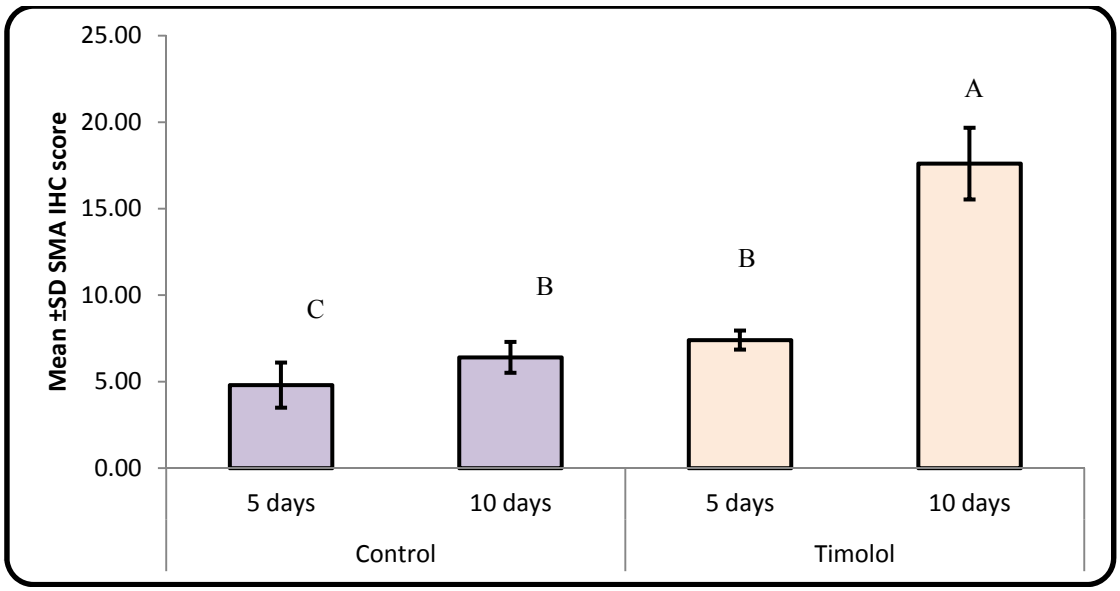

Figure 8: Mean SMA score in control and study groups
Capital letters indicate the level of significance at (P≤0.05); different letters indicate significant variation; (A) indicates the highest value.

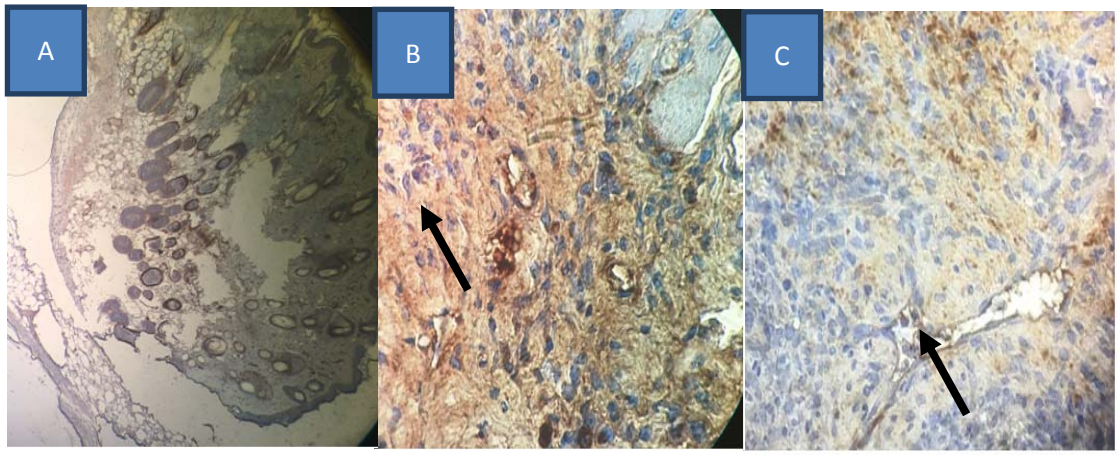

Figure 9: Cytoplasmic immunohistochemical expression of CD31 by vascular endothelial cells (black arrow) . (A) 10X; (b) and (C) 100X.

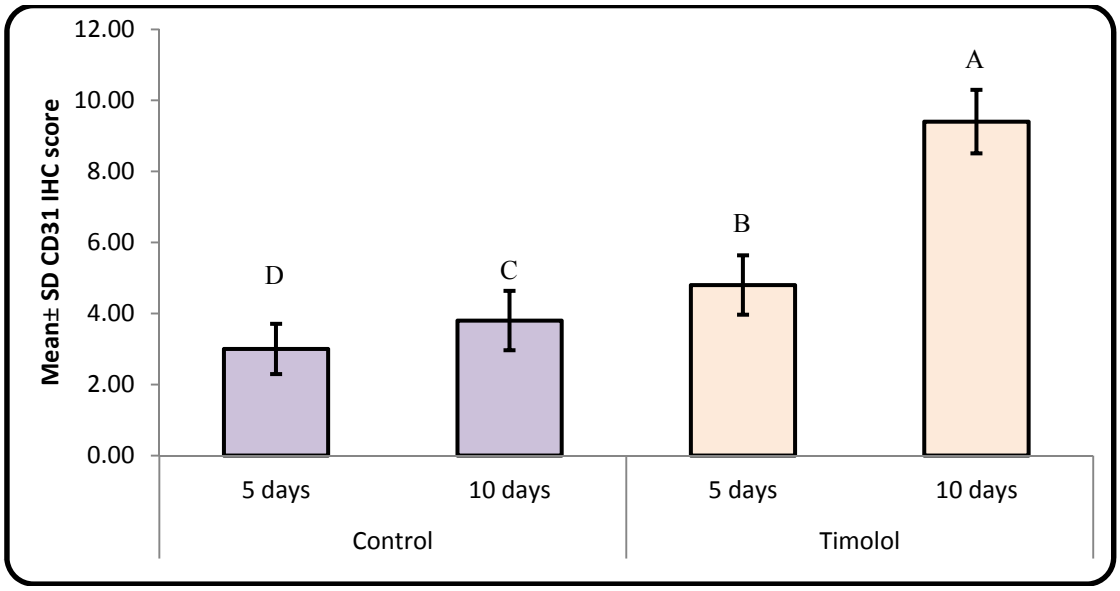

Figure 10: Mean CD31IHC score in control and study groups
Capital letters indicate the level of significance at (P≤0.05); different letters indicate significant variation; (A) indicates the highest value.

DISCUSSION

Current study showed that the rate of wound healing was highly significant faster in timolol group than in control group. Romana-Souza *et al*., 2014 mentioned that the wound area appeared to be smaller in the adrenoceptor(AR) knockout (KO) mice than in the wild-type mice 7 and 10 days after wounding [13]. The observation of increased rate of wound healing in the present study and also in previous study [13] following treatment with β2AR antagonist and in AR KO mice, may be attributed to the fact that angiogenesis process is accelerated and hence there will be increase in nutrient and oxygen supply and also increased rate of removal of metabolic waste products and also to the ability of β2AR antagonist in increasing keratinocyte migration speed[22]. Natural wound healing proceeds involve an inflammatory response and associated cellular migration, proliferation, matrix deposition, and tissue remodeling[24,25]. It has been shown that increased proinflammatory cellular infiltrates composed largely of neutrophils and macrophages contribute to delayed healing in chronic ulcers [26]. Pullar *et al*., in 2012 studied the role of β2AR on inflammatory process involved in wound healing and concluded that topical β2AR antagonist treatment had no effect on the number of polymorphonuclear cells or macrophages recruited to the wound site either 3 or 5 days post wounding [22]and this supports the finding of the present study in that β2AR antagonist have no significant effect on inflammatory response accompanying wound healing in mice. In this study it was found that inflammation severity on day 10 was significantly less than that on day 5and this may be due to the natural process of wound healing in which inflammation severity becomes less toward the end of the healing process and being replaced by the formation of granulation tissue (angiogenesis and fibroblast proliferation).

The current study showed that using the β_2AR antagonist (timolol) resulted in significant increase in collagen deposition in comparison with control group, which is agreed with the findings of Pullar *et al*. (2012) who stated that administration of β_2AR antagonist resulted in a significant increase in collagen III depositions in wounds after being followed for 5 days [22]. Pullar and Isseroff in 2005 studied the β2AR antagonist effects on fibroblast activity and collagen synthesis and deposition in Fibroblast-seeded collagen gels (an in vitro media) and found that it increased fibroblast collagen formation [27]. This finding supports the results of the present study. while Raut *et al*., in 2012 studied the effect of two β_2AR antagonist agents (propranolol and metoprolol) on collagen deposition in wound healing and found that these two β_2AR antagonists significantly reduced collagen deposition[28], a result which is in contrary to the finding of the present study ,this controversy may be attributed to different approaches in assessing collagen deposition; Raut *et al*. assessed collagen deposition in wound healing in rats after examination of formalin fixed paraffin embedded tissue sections that have been routinely stained with H and E stain, where as in the present study, immunohistochemistry using collagen III type specific primary antibodies were used to assess collagen III status; it is well known that during healing process the first type of collagen deposited is collagen III then it is later on replaced by collagen I. and routine H and E stained section permit a difficult chance to differentiate between them. Also, the present study showed a marked increase in collagen III in relation to duration so that longer duration (10 days) was associated with significantly more deposition of collagen. This phenomenon may be due to the fact that early in wound healing inflammation is more marked than fibroblast proliferation and activation; however, when the time elapsed fibroblast proliferation, action and collagen deposition predominates[29]. In the present study, the density of myofibroblast was assessed by measuring the immunohistochemical expression of SMA because it is a reliable marker for myofibroblast differentiation and its expression is a directly correlated with myofibroblast density in tissues [30,31]. The result showed that adding timolol resulted in highly significant increased SMA immunohistochemical score in 5 and 10 days, in agreement with the findings of the present study, Pullar *et al*. (2012) stated that administration of β_2AR antagonist caused a significant increase in SMA immunohistochemical expression in wounds after being followed for 5 and 10 days. Romana-Souza *et al*., (2008) found that propranolol significantly increased SMA expression [14]. This result again is in accordance with the result of the present study. Raut *et al*., in 2012 studied the effect (propranolol and metoprolol) on myofibroblast density in wound healing and found a significantly reduced in it, a result which is in contrary to the finding of the present study. this controversy also may be due to different approaches in assessing myofibroblast density, It is obvious that routine H and E stained section which was used by Raut permit a difficult chance to differentiate between ordinary fibroblast and those exhibiting myofibroblast differentiation. The increase in SMA expression is an indirect marker of myofibroblast density in examined skin tissue. The addition of β_2AR antagonist causes an increase in myofibroblast density and hence promotes significant wound contraction. Myofibroblast-mediated contraction is the major mechanism of wound contraction; the interaction between myofibroblasts and the surrounding extracellular matrix (ECM) plays an important role in this phenomenon; myofibroblast differentiation, collagen fiber deposition and myofibroblast–ECM interaction is the most important determinant of wound contraction[28,32,33]. It should be mentioned here that SMA is also a marker of smooth muscles within the wall of newly formed blood vessels and may indirectly speculate the degree of angiogenesis in wound healing. β_2AR antagonist (timolol) has been found to increase SMA expression in wound healing in the present study and so by this way they are pro-angiogenic agents. The current study also showed immunohistochemical SMA expression increases significantly with time. This phenomenon may be due to the fact that early in wound healing inflammation is more marked than fibroblast and myofibroblast proliferation; however when the time elapsed fibroblast and myofibroblast proliferation predominates[29].

The immunohistochemical CD31 expression is a reliable marker of endothelial cells lining newly formed blood vessels and hence predicting the degree of angiogenesis in wound healing [34,35]. For that reason, it was used in the present study as a marker of angiogenesis. The current study showed that adding timolol (β_2AR antagonist) resulted in a highly significant increased CD31 immunohistochemical score in 5 and 10 days. In agreement with the findings of the present study, Pullar *et al*. (2012) stated that administration of β_2AR antagonist caused a significant increase in CD31 immunohistochemical expression in wounds after being followed for 5 and 10 days. The mechanism by which β_2AR inhibition modulates angiogenesis has been fully discussed previously above. The present study showed that in 10 days the mean immunohistochemical CD31 expression was significantly duration of wound healing. This phenomenon may be due to the fact that early in wound healing inflammation is more marked than endothelial cell proliferation and migration; however when the time elapsed endothelial cell proliferation and migration predominate[29].

CONCLUSION

In conclusion, the current study shown that the administration of β_2 adrenergic receptor antagonist (timolol) promotes wound healing through increased angiogenesis, collagen III deposition and myofibroblast density.

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