Results

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Post surgical Follow-Up

The consequences of hind limb ischemia were more profound in the old animals. Severe atrophic necrosis of the distal part of the ischemic limb was noted in the five old ischemic untreated rabbits studied .In comparison, none of the five young rabbits studied developed limb necrosis.

Angiography

The number of angiograpically visible collateral vessels (angiographic score) was markedly reduced in old rabbits when compared to young rabbits (Figure 4). At day 40, angiography disclosed significantly fewer collateral vessels in the medial thigh area of old compared with young NZW rabbits (angioscore = 0. 48 ±0.05 versus. 0.70 ±0.05). Treatment with rh VEGF165 protein resulted in a significant and similar increase in the number of angiographically visible collaterals in both young and old rabbits (Figure 4). The angioscore for young animals treated with rh VEGF165 (0. 91 ± 0.08) was significantly higher than the corresponding value (0.70 ± 0.05) obtained in untreated animals. In old treated rabbits, the angioscore (0.69 ± 0.04) was also significantly higher than that recorded for the untreated group (0. 48 ± 0.05).

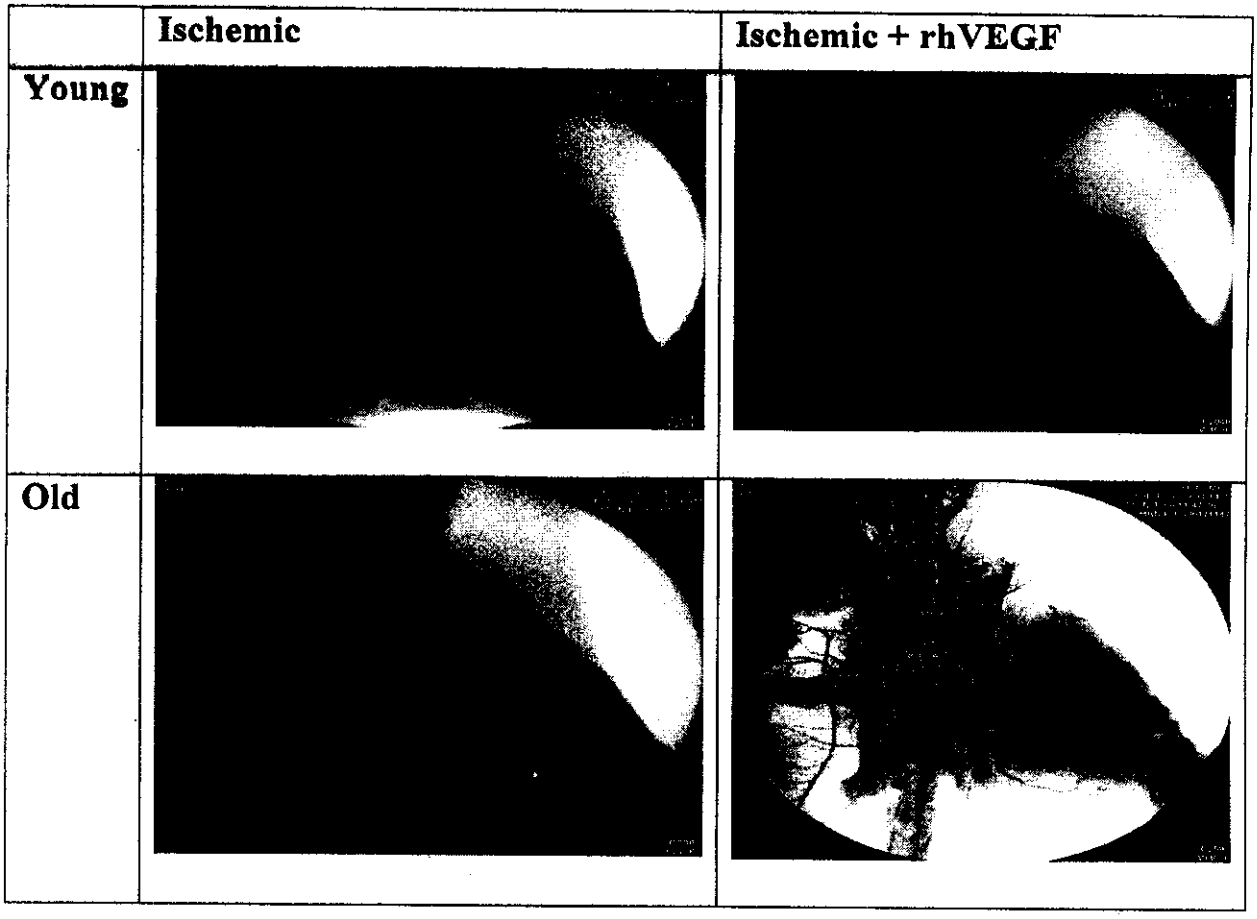

Figure (4) Angiography

Immunohistochemical results:

(I) Capillary density:

Capillary density assessed by CD31 immunostaning was increased in old and young ischemic rabbits compared to normal rabbits of the control group although it is still lower in old ischemic animals compared to young ones 40 days after surgery. Figures 5,6,7,8.

Treatment with rhVEGF165 induced a significant increase in capillary density in both young & old treated rabbits compared to ischemic untreated rabbits figures (9,10). However, the ultimate level of capillary density achieved in the hind limbs of VEGF treated animals was still lower in the old rabbits than in young rabbits.

Figure (5): Immunostaining of a section in the skeletal muscle of a young control rabbit showing no angiogenic blood vessels.
Immuno peroxidase staining micro wave technique for CD_{31}.
X 400.

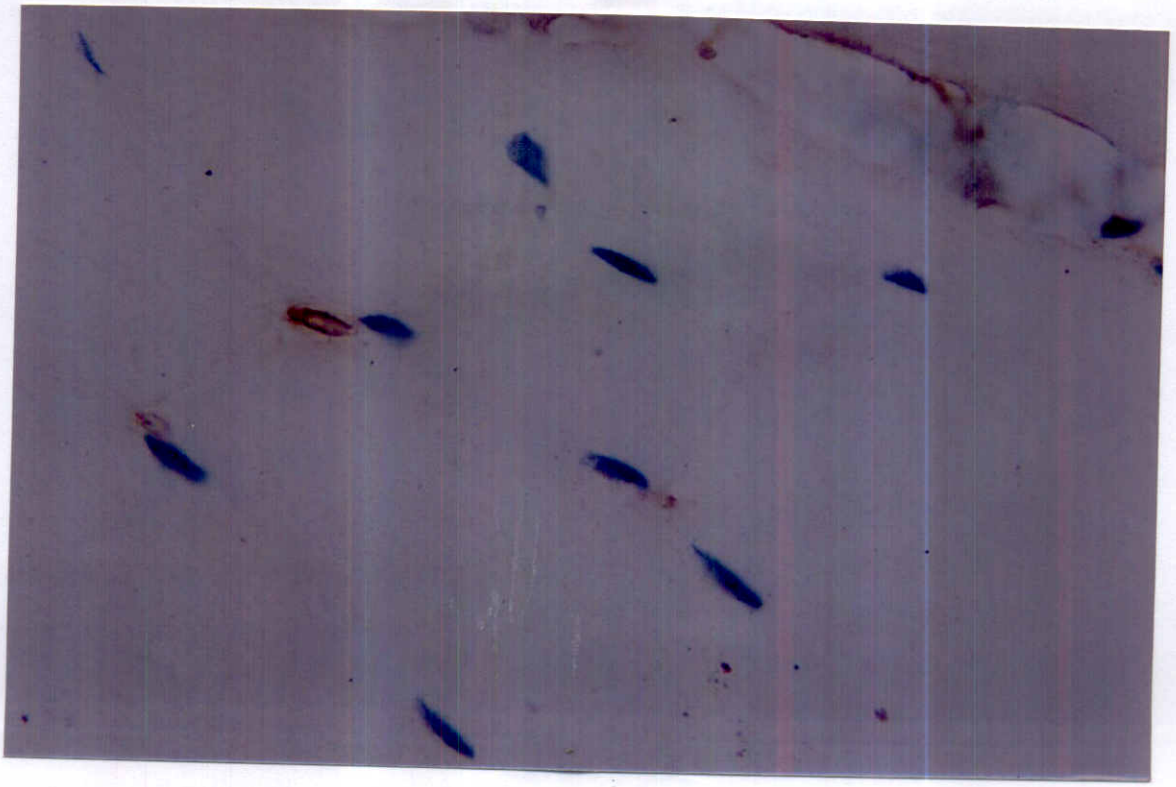

Figure (6): Immunostaining of a section in the skeletal muscle of an old control rabbit showing no angiogenic blood vessels.

Immuno peroxidase staining micro wave technique for CD_{31}.X 400.

Figure (7): Immunostaining of a section in the skeletal muscle of a young ischemic rabbit showing sites of angiogenic blood vessels (arrows).

Immuno peroxidase staining micro wave technique for CD_{31}. X 400.

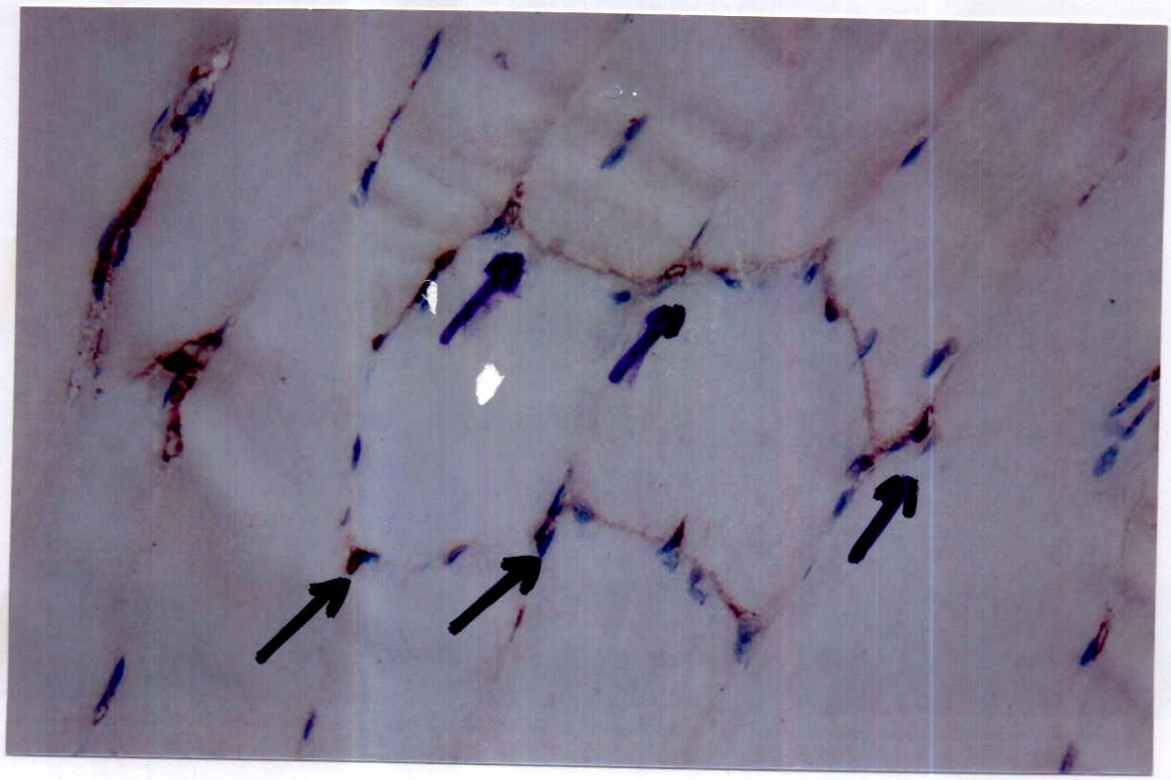

Figure (8): Immunostaining of a section in the skeletal muscle of an old ischemic rabbit showing sites of angiogenic blood vessels (arrows).

Immuno peroxidase staining micro wave technique for CD_{31}. X 400.

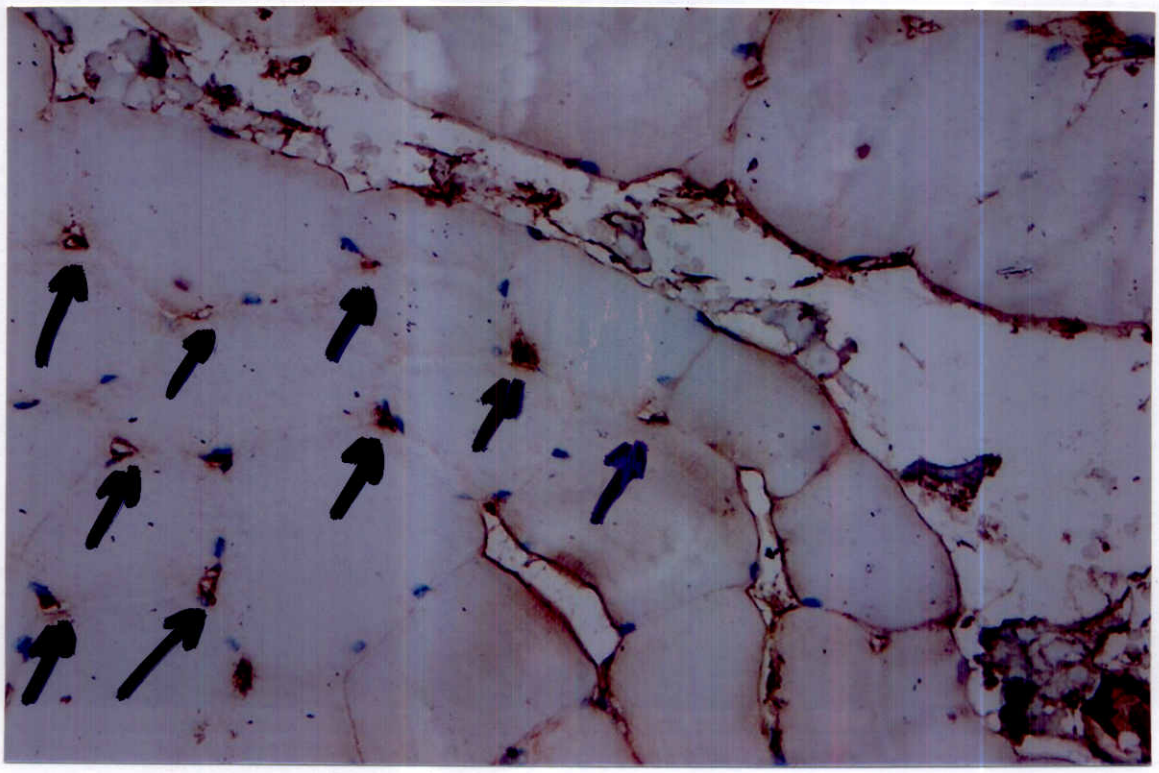

Figure (9): Immunostaining of a section in the skeletal muscle of a young treated rabbit (injected with rh $VEGF_{165}$) showing increased angiogenic blood vessels (arrows).

Immuno peroxidase staining micro wave technique for CD_{31}. X 400.

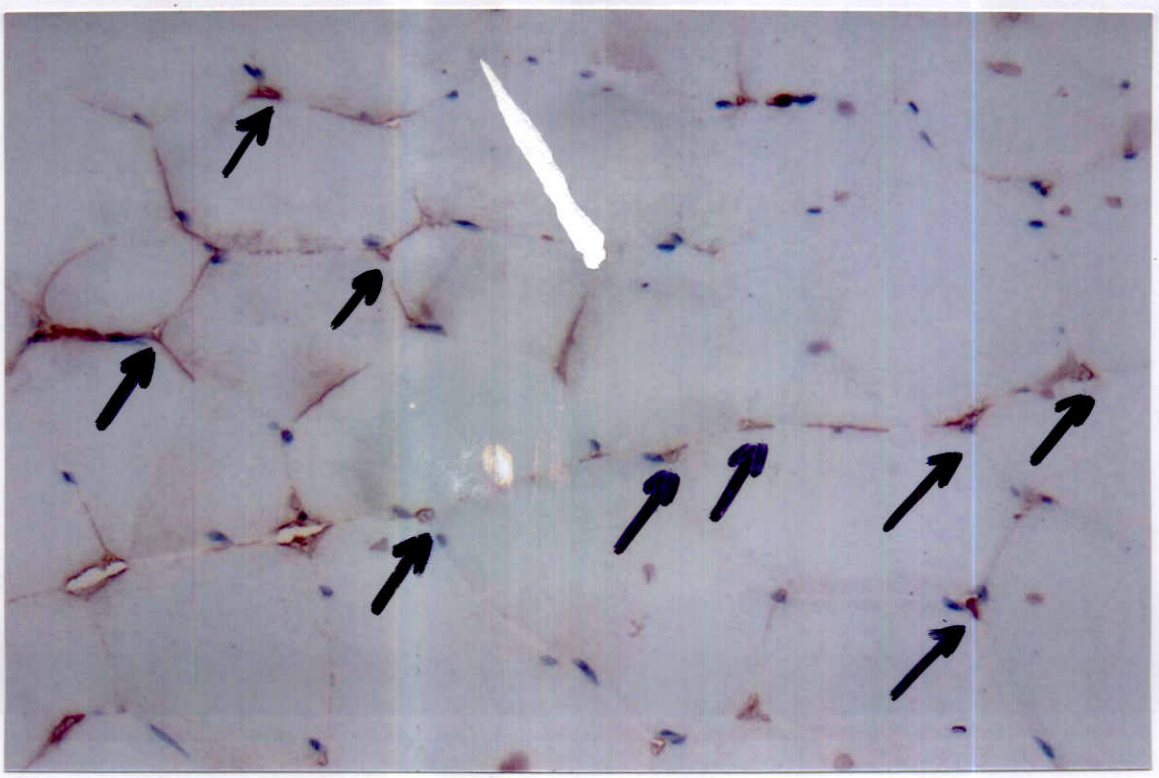

Figure (10): Immunostaining of a section in the skeletal muscle of an old treated rabbit (injected with rh $VEGF_{165}$) showing increased angiogenic blood vessels (arrows).

Immuno peroxidase staining micro wave technique for CD_{31}. X 400.

(II) T-lymphocytes:

Immunostaining for CD3 revealed no presence of T-cells in normal tissue of control animal group .figures 11,12.

Lower number of infiltrating T-cells in old ischemic rabbits than in young ischemic rabbits figures 13,14.

Treatment with rhVEGF165 induced a significant increase in T-cells infiltrating tissues of both old & young treated rabbits . figures (15,16) however , the ultimate level of infiltrating T-cells achieved in the hind limbs of VEGF treated animals was still lower in the old rabbits than in young rabbits .

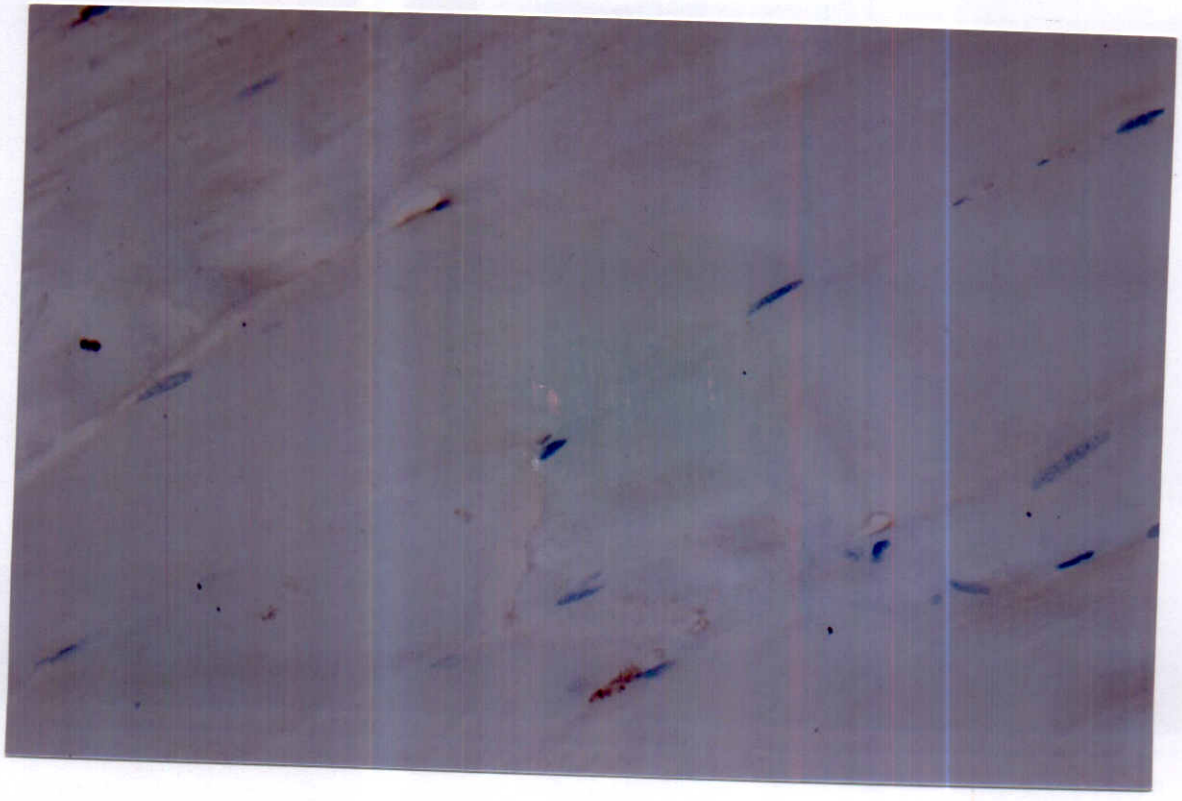

Figure (11): Immunostaining of a section in the skeletal muscle of a young control rabbit showing no T- Lymphocytes.
Immuno peroxidase staining micro wave technique for CD_3.
X 400.

Figure (12): Immunostaining of a section in the skeletal muscle of on old young control rabbit showing no T- Lymphocytes.

Immuno peroxidase staining micro wave technique for CD_3.

X 400.

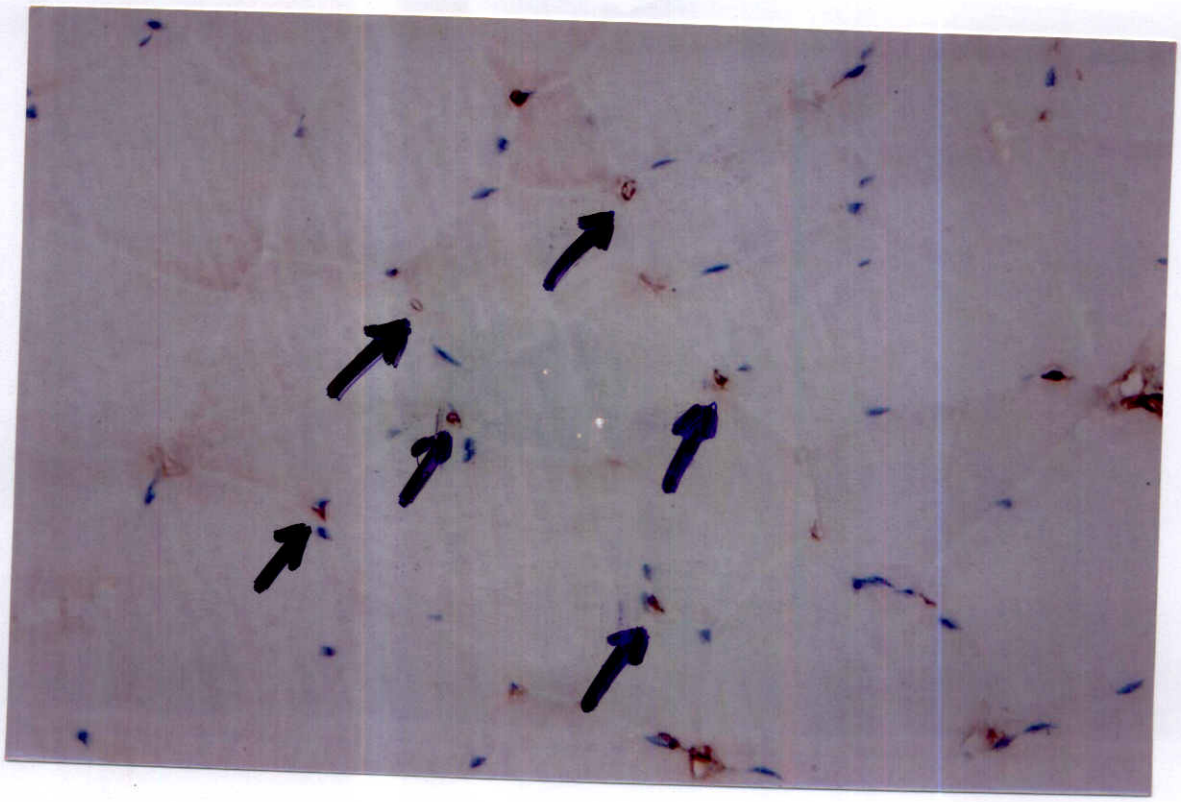

Figure (13): Immunostaining of a section in the skeletal muscle of a young ischemic rabbit showing presence of T-Lymphocytes (arrows).

Immuno peroxidase staining micro wave technique for CD_3. X 400.

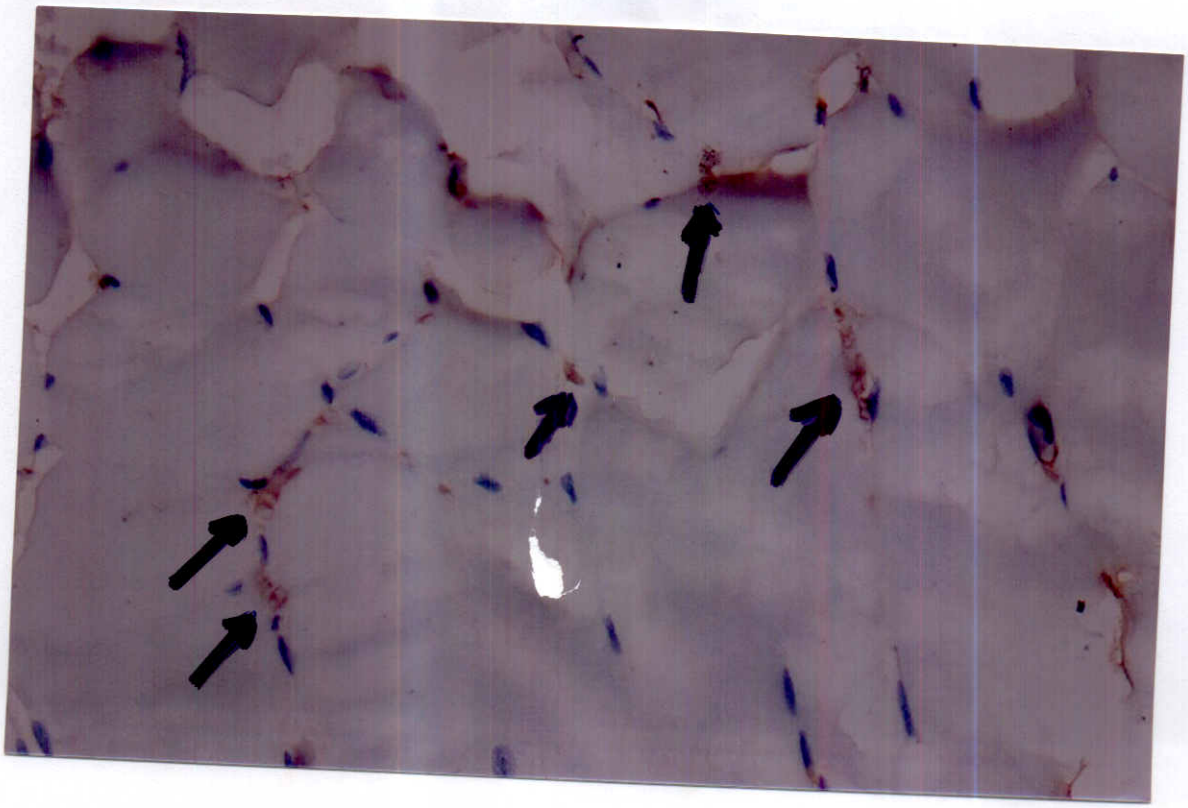

Figure (14): Immunostaining of a section in the skeletal muscle of an old ischemic rabbit showing presence of T-Lymphocytes (arrows).

Immuno peroxidase staining micro wave technique for CD_3. X 400.

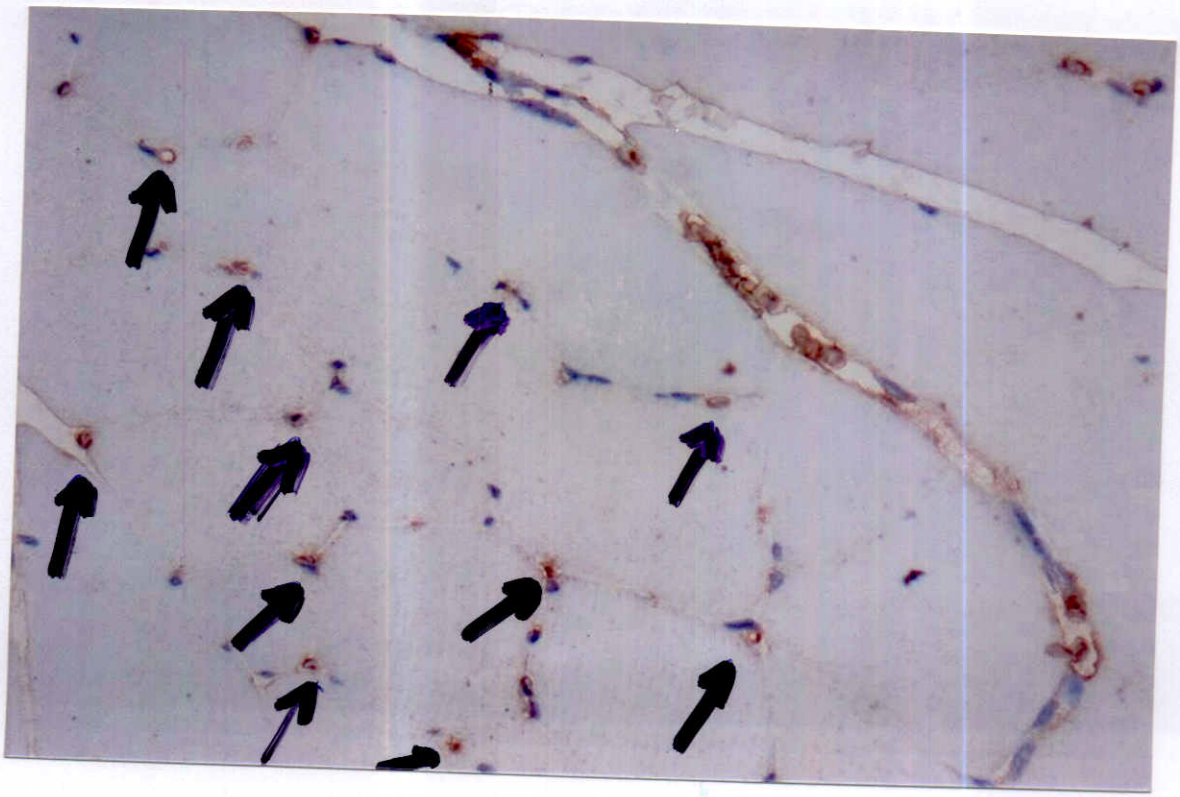

Figure (15): Immunostaining of a section in the skeletal muscle of a young treated rabbit (injected with rh $VEGF_{165}$) showing increased number of T- Lymphocytes (arrows).

Immuno peroxidase staining micro wave technique for CD_3. X 400.

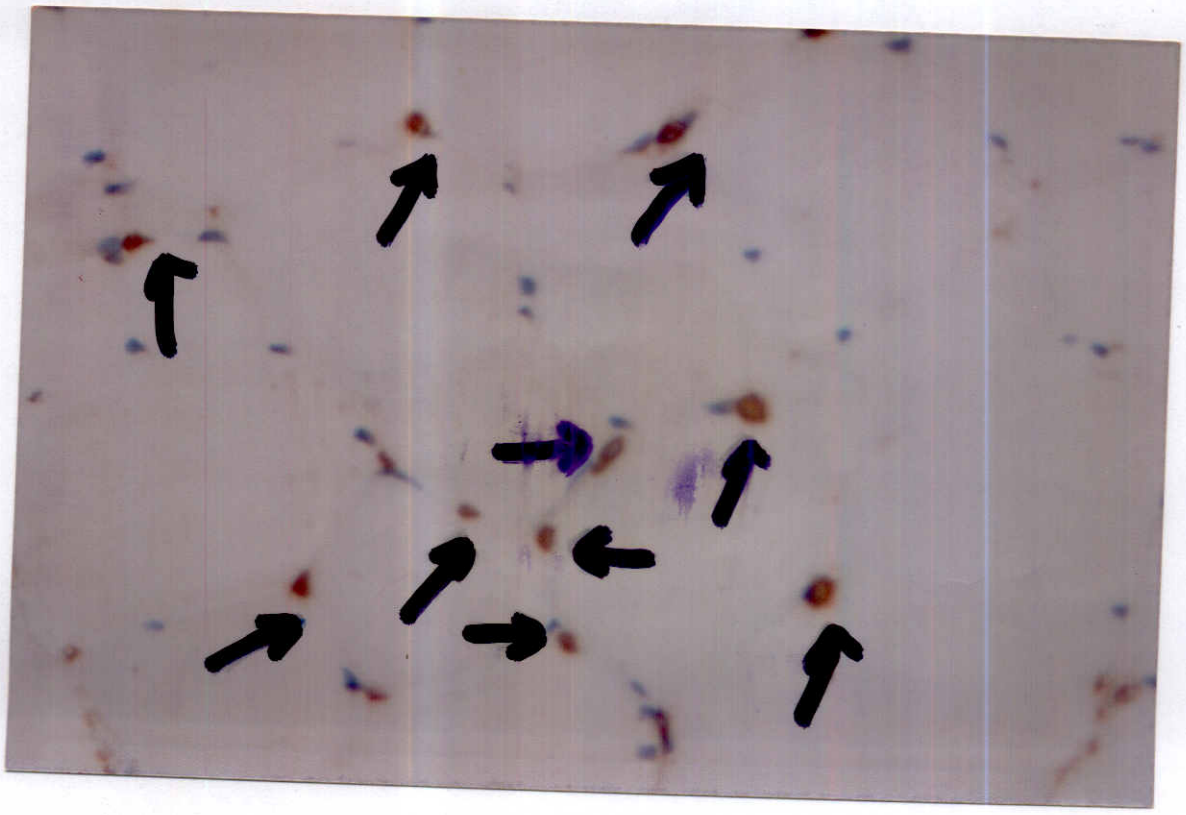

Figure (16): Immunostaining of a section in the skeletal muscle of an old treated rabbit (injected with rh $VEGF_{165}$) showing increased number of T-Lymphocytes (arrows).

Immuno peroxidase staining micro wave technique for CD_3. X 400.

Histochemical results:

After an indoxyl- tetrazolium method for alkaline phosphatase:

Alkaline phosphatase was significantly increased in young and old ischemic rabbits compared to normal rabbits. The old ischemic rabbits showed more increase than young ischemic animals .figures 17,18,19,20.

Treatment with rhVEGF165 induced a significant decrease in sites of alkaline phosphatase staining in tissues of both young & old rabbits. figures 21,22.

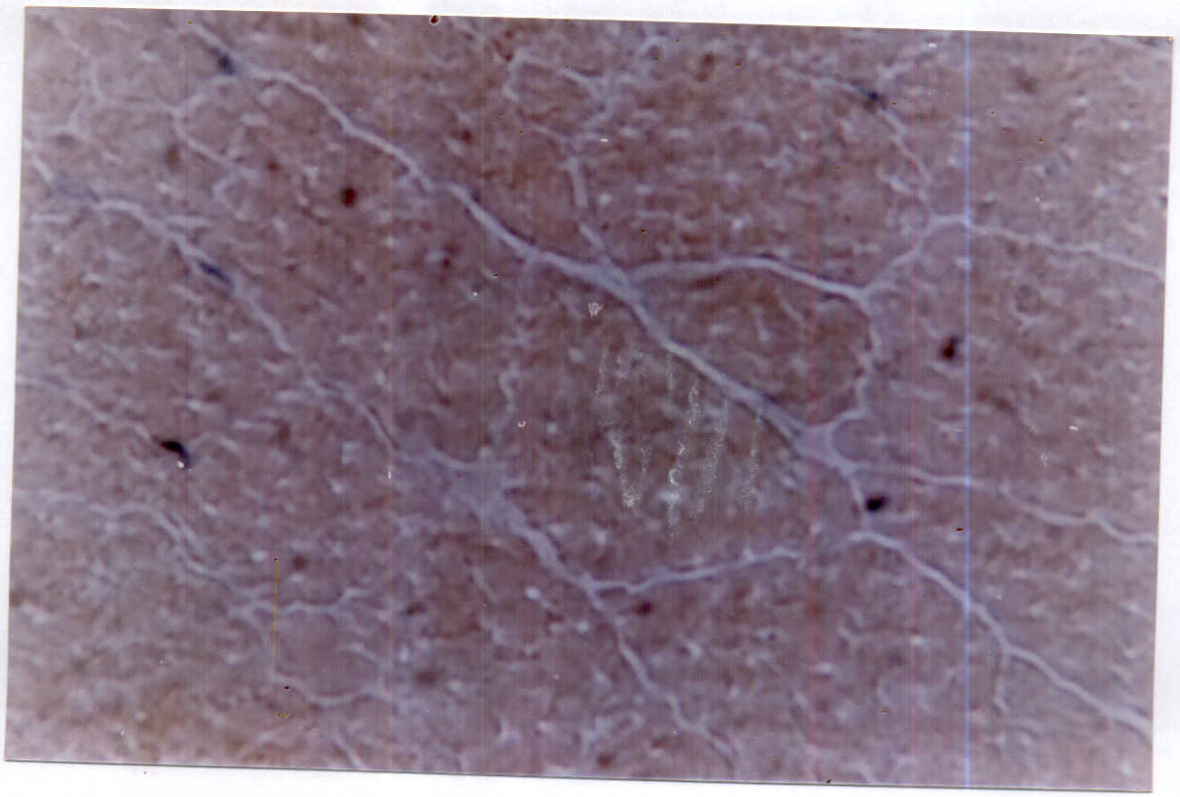

Figure (17): A photomicrograph of a section in the skeletal muscle of a control young rabbit showing no sites of alkaline phosphates staining.

Indoxyl tetrazolium method for alkaline phosphatase staining. X 400.

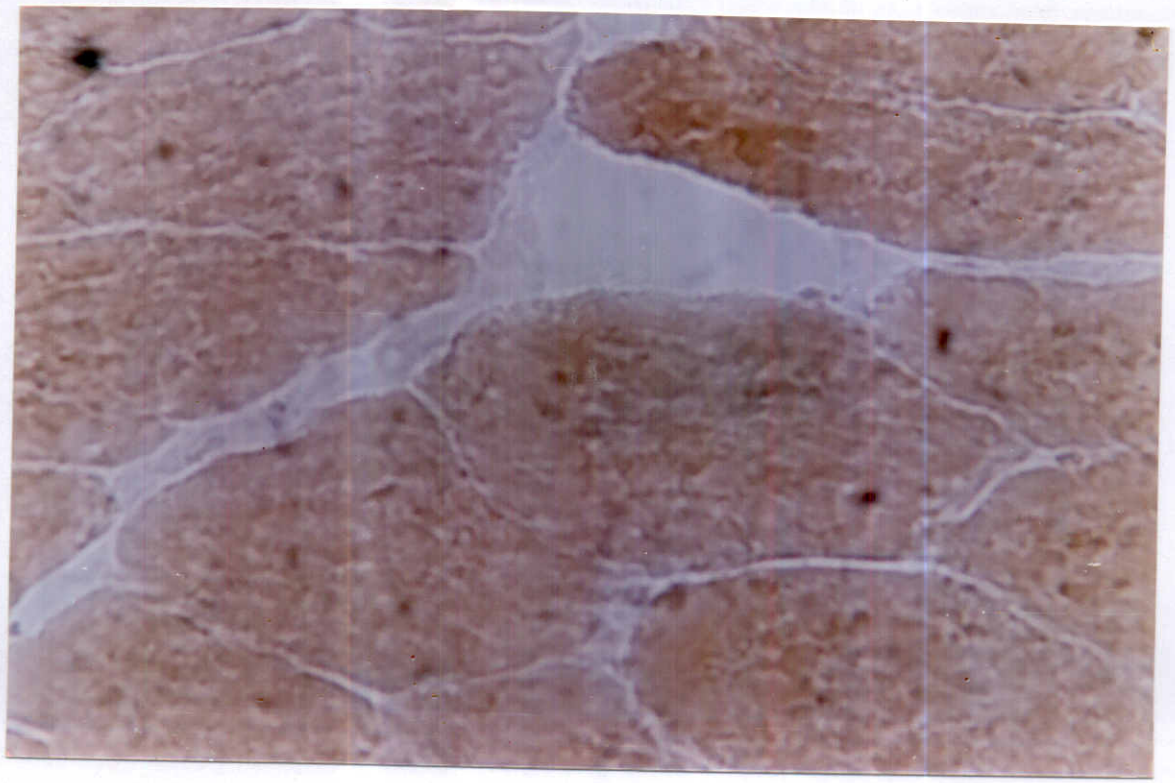

Figure (18): A photomicrograph of a section in the skeletal muscle of a control old rabbit showing no sites of alkaline phosphates staining.

Indoxyl tetrazolium method for alkaline phosphatase staining. X 400.

Figure (19): A photomicrograph of a section in the skeletal muscle of an ischemic young rabbit showing sites of alkaline phosphates activity (arrows).

Indoxyl tetrazolium method for alkaline phosphatase staining. X 400.

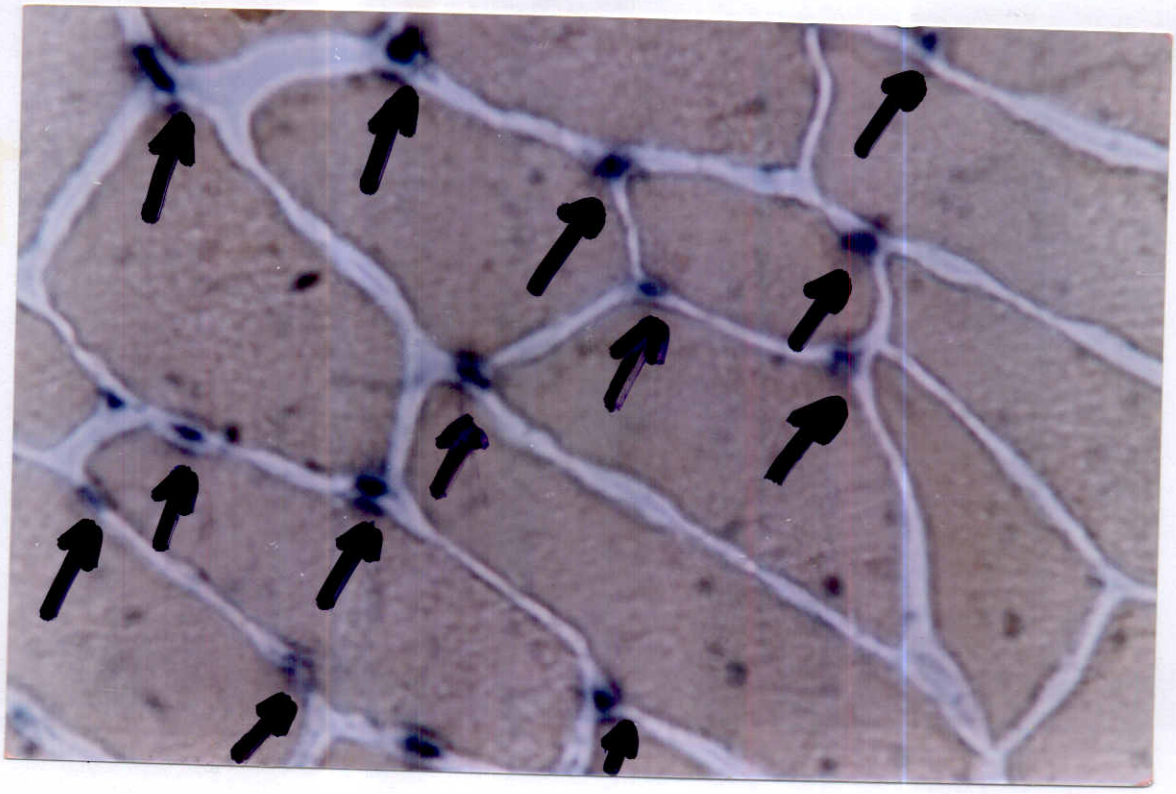

Figure (20): A photomicrograph of a section in the skeletal muscle of an ischemic old rabbit showing sites of alkaline phosphates activity (arrows).

Indoxyl tetrazolium method for alkaline phosphatase staining. X 400.

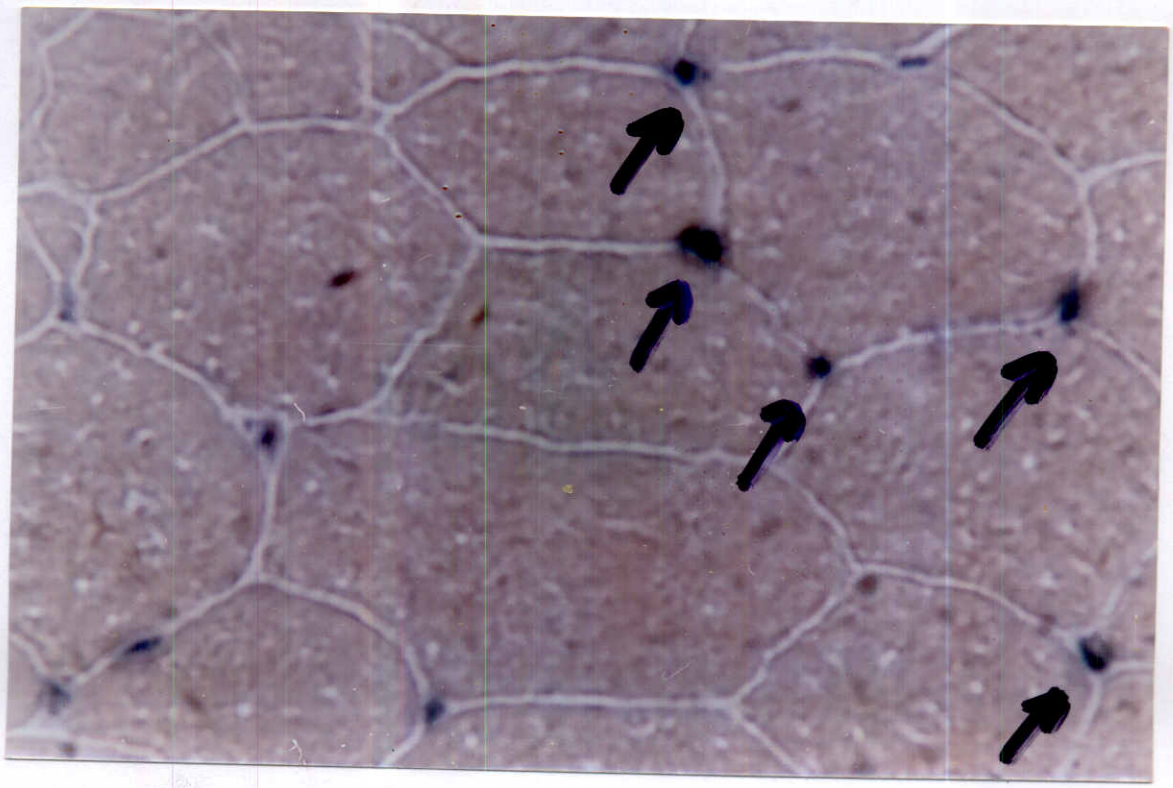

Figure (22): A photomicrograph of a section in the skeletal muscle of a treated old rabbit (injected with rh $VEGF_{165}$) showing a decrease in sites of alkaline phosphates activity (arrows). Indoxyl tetrazolium method for alkaline phosphatase staining. X 400.

Statistical results

Capillary density:

Capillary density assessed by CD31 immunostaining.

- Ischemic groups showed an increase in capillary density.
  - Young control group was (30 ± 9.0).
  - Old control group was (13 ± 2.2).
  - Young ischemic group was (171 ± 9.5).
  - Old ischemic group was (130 ± 5.8).

$P < 0.01$

- Treated groups showed on increase in capillary density.
  - Young treated group was (282 ± 5.0).
  - Old treated group was (191 ± 7.8).
  - Young ischemic group was (171 ± 9.5).
  - Old ischemic group was (130 ± 5.8).

$P < 0.001$

Figure 23.

T-lymphocytes:

Immunostaining for CD3.

- Ischemic groups showed an increase number of infiltrating T-lymphocytes compared to control groups:
  - Young control group was (1 ± 1.1).
  - Old control group was (0 ± 0.0).
  - Young ischemic group was (11 ± 1.8).
  - Old ischemic group was (3 ± 0.2).

  $P < 0.05$.

- Treated groups showed an increase number of infiltrating T-lymphocytes compared to ischemic groups:
  - Young treated group was (17 ± 0.5).
  - Old treated group was (13 ± 1.5).
  - Young ischemic group was (11 ± 1.8).
  - Old ischemic group was (3 ± 0.2).

  $P < 0.05$

  Figure 24.

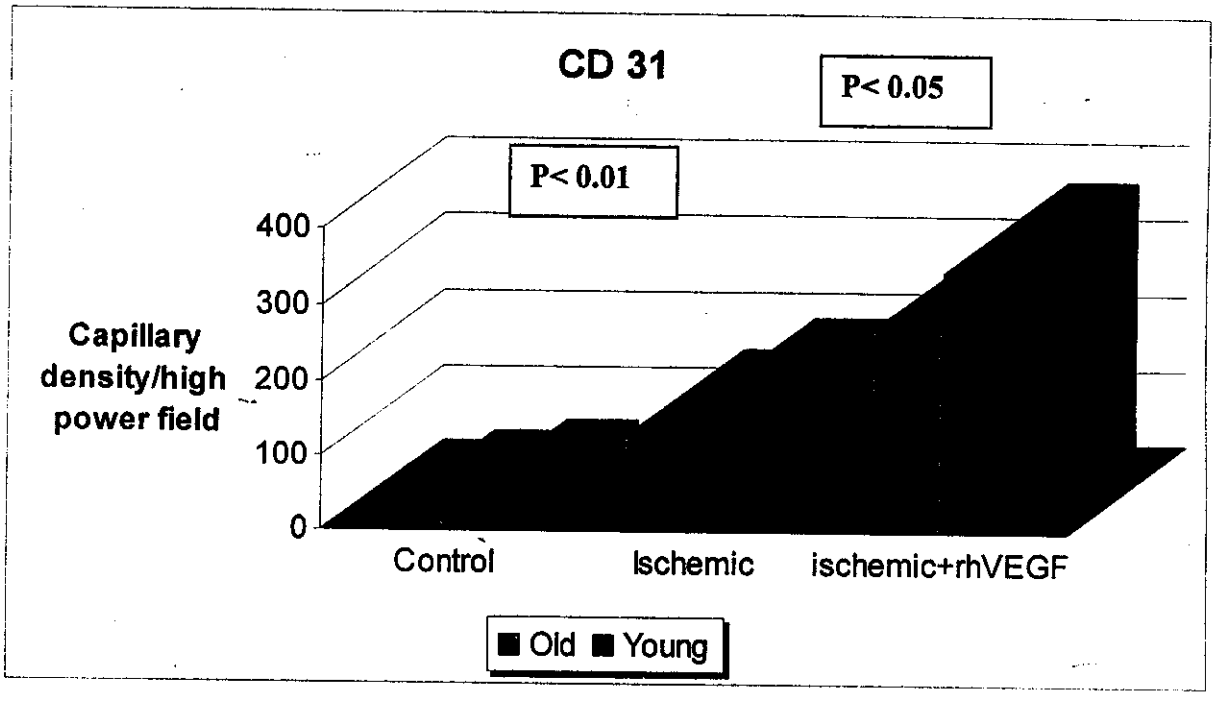
CD 31
P< 0.05
P< 0.01
Capillary density/high power field
400
300
200
100
0
Control
Ischemic
ischemic+rhVEGF
Old
Young

Figure : 23

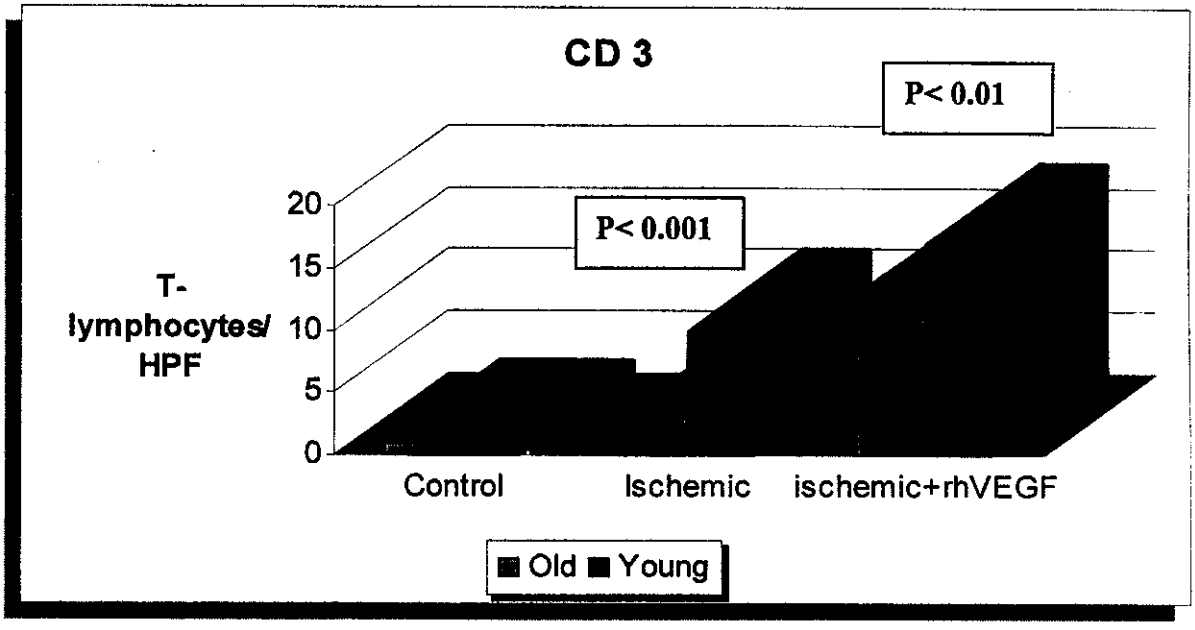
CD 3
P< 0.01
P< 0.001
T-lymphocytes/ HPF
20
15
10
5
0
Control
Ischemic
ischemic+rhVEGF
Old
Young

Figure : 24