EFFECT OF THE EMU OIL IN THE TRETMENT OF ULCERATIVE COLITIS

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ABSTRACT
Colitis is an inflammation of colon which is often used in medical context to describe an inflammation of the large intestine (colon, caecum and rectum). The term colitis can be used broadly to define Crohn's disease if the aetiology of the inflammation in the colon is undetermined or to Ulcerative colitis if the context is clear and disease is diagnosed. Emu oil comes from a thick pad of fat on the back of the bird that was initially provided by nature to protect the animal from the extreme temperatures of its Australian homeland. Approximately 70 % of the fatty acids in emu fat are unsaturated – Omega 3, 6 and 9. In this current study effect of the EMU oil in the treatment of Dextran sulphate sodium-induced colitis were evaluated.

KEYWORDS
EMU oil, Ulcerative colitis, Omega 3 fatty acids and Crohn's disease.

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INTRODUCTION[1]
Colitis is an inflammation of colon which is often used in medical context to describe an inflammation of the large intestine (colon, caecum and rectum). The term colitis can be used broadly to define Crohn's disease if the aetiology of the inflammation in the colon is undetermined or to Ulcerative colitis if the context is clear and disease is diagnosed. Colitis may be acute and self-limited or chronic, i.e. persistent, and broadly fits into the category of digestive diseases.

Ulcerative colitis[1,2]

Ulcerative colitis (Colitis ulcerosa, UC) is a relapsing non-transmural inflammatory disease restricted to the mucosa and superficial submucosa of the colon which is a form of inflammatory bowel disease (IBD). (DiPiro *et al*., 2002).

Emu oil comes from a thick pad of fat on the back of the bird that was initially provided by nature to protect the animal from the extreme temperatures of its Australian homeland.

Approximately 70 % of the fatty acids in emu fat are unsaturated – Omega 3, 6 and 9. This composition is consistent with current recommendations for a "heart healthy" diet. Studies and testimonial have also shown these Omega Fatty Acids to lower bad cholesterol and triglyceride levels while increasing good cholesterol; reduce inflammation in body tissue and joints; improve the immune system and assist the body with many functions.

MATERIALS AND METHODS[3,4]

In the present study Dextran sulfate sodium (DSS) induced colitis model in rats was used to evaluate the effect of *Emu oil* as an anti-coloitic agent.

Treatment schedule[5,6,7]

Thirty animals were taken and were divided into five groups of six animals each (n=6). Experimental colitis was induced in rats by oral administration of 3% dextran sulfate sodium (DSS) for seven consecutive days.

Group-I: Normal control

Group-II: Colitic control (dextran sulphate sodium (DSS) induced rats)

Group-III: Standard drug treated group (DSS + Mesalazine100mg/kg)

Group-IV: Test drug treated group (DSS + *Emu oil* 0.2ml/lit)

Group-V: Test drug treated group (DSS + *Emu oil* 0.5ml/lit)

All animals received treatments for seven consecutive days and were sacrificed after 24 hours of their last dose by cervical dislocation. Abdomen was opened and colons were exposed. Distal 8 cm of colon was excised and opened by a longitudinal incision. The mucosa, intestinal contents and debris were washed out by using normal saline's.

Estimation of C-reactive protein levels in blood[8]

Principle

C-reactive protein (CRP) is a serum protein, which is synthesized in the liver. Its rate of synthesis increases within hours of an acute injury or the onset of inflammation and may reach as high as 20 times the normal levels. Elevated serum concentration of CRP is an unequivocal evidence of an active tissue damage process and CRP measurement provides a simple screening test for organic disorders. Agglutination is the main principle behind the estimation of CRP through a slide test. The serum of test sample is mixed with CRP latex reagent which is a suspension of polystyrene latex particles in glycine-saline buffer, pH 8.4±0.2. Latex particles are coated with monospecific anti CRP antibodies which react with test serum CRP to produce agglutination. If CRP concentration is greater than 0.6 mg/dl a visible agglutination is observed. If CRP concentration is less than 0.6 mg/dl, then no agglutination is observed.

Procedure

After collection of the blood sample, it was allowed to clot, then the serum was separated by centrifugation at 3,000 rpm, for or 10 min. One drop of serum was added to the slide with dark background circle and to it one drop of CRP latex reagent is added. Using a mixing stick the test specimen and CRP latex reagent are mixed uniformly over the entire circle and observed for visual agglutination for 2 minutes. Samples with positive results are again observed for agglutination by using a semi quantitative method in which an isotonic saline preparations of serial dilutions of the serum 1:2, 1:4, 1:8,1:16, 1:32, 1:64 and so on are prepared and CRP latex reagent was added to the dilution 177.
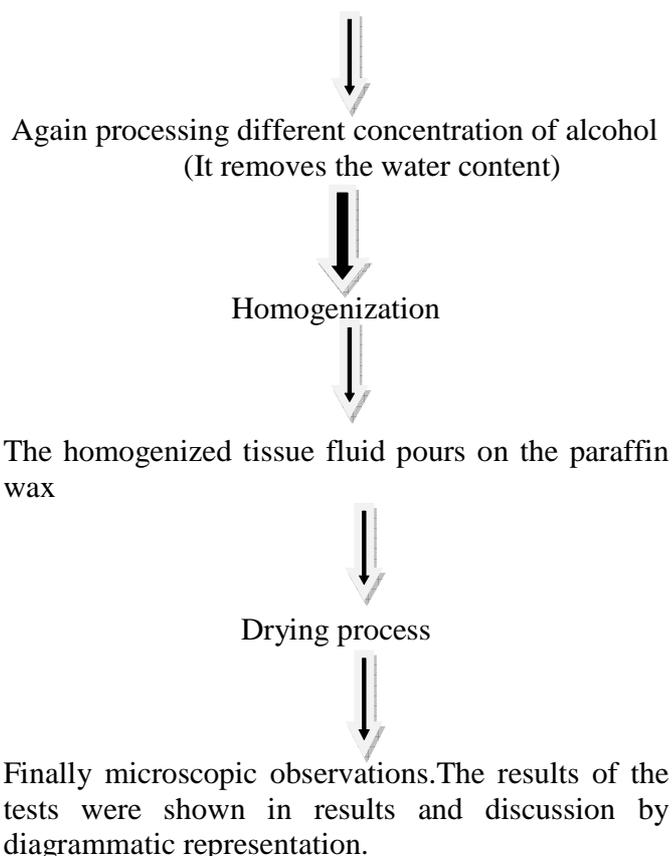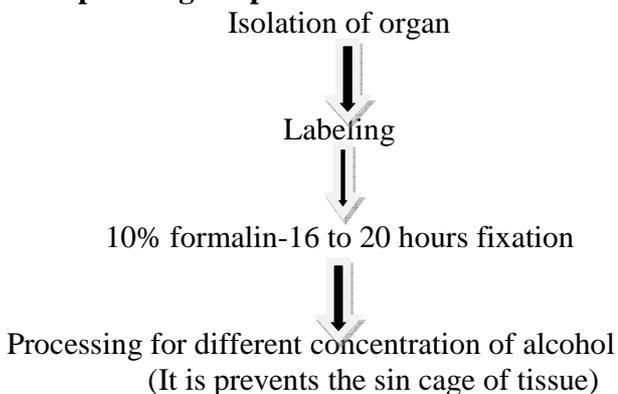
Colon weight/length ratio (mg/cm)

Animals were killed by cervical dislocation and the entire colon was removed, gently flushed with saline, placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper to dry. Each colon was weighed and its length was measured and

ratio was determined. The results should shown in tables.

Histopathological analysis

Colon of rats were excised, washed with saline and then fixed in 10% natural buffered formalin solution, embedded in paraffin, cut into tissue sections, and stained with haematoxylin and eosin (H and E). The stained sections were examined by light microscope for evidence of colitis using the following criteria: presence of inflammatory cell infiltration, presence of crypt abscesses, crypt distortion, and regenerative changes in the form of nuclear enlargement and increased mitotic activity, cases treated with drugs were examined for histological signs of resolution[9].

Histopathological procedure

Isolation of organ

↓

Labeling

↓

10% formalin-16 to 20 hours fixation

↓

Processing for different concentration of alcohol
(It is prevents the sin cage of tissue)

↓

Again processing different concentration of alcohol
(It removes the water content)

↓

Homogenization

↓

The homogenized tissue fluid pours on the paraffin wax

↓

Drying process

↓

Finally microscopic observations.The results of the tests were shown in results and discussion by diagrammatic representation.

RESULTS AND DISCUSSION

The results from the various tests are listed below I the tables (From Table No.1 to 11).

Table No.1: Hematology for control Group

S.No	Hematology	Findings	Normal range
1	Hem globulin(HB)	70%,10.2gm	13.0-18.0gm/dl
2	Total White blood cells	4100 cells/cumm	5000-11000cells/cumm
3	Total Red blood cells	4.5miln/cumm	3.5-5.5milin/cumm
4	DC		
5	Neutrophis	60%	50%-70%
6	Lymphocytes	38%	20%-40%
7	Eosinophiles	2%	1%-6%
8	Monocytes	0.1%	1.5%
9	Basophils	0.1%	0-0.5%
10	Erythrocyte sedimentation Rate	2mm	1-15mm/Hour

Table No.2: Hematology for DSS Induced group

S.No	Hematology	Findings	Normal range
1	Hem globulin(HB)	62%,8.6gm	13.0-18.0gm/dl
2	Total White blood cells	5900 cells /cum	5000-11000cells/cumm
3	Total Red blood cells	4.7milian/cumm	3.5-5.5milin/cumm
4	Neutrophis	50%	50%-70%
5	Lymphocytes	48%	20%-40%
6	Eosinophiles	2%	1%-6%
7	Monocytes	0%	1.5%
8	Basophils	0%	0-0.5%
9	Erythrocyte sedimentation Rate	1mm	1-15mm/Hour

Table No.3: Hematology for Standard Group

S.No	Hematology	Findings	Normal range
1	Hemoglobulin(HB)	52%,7.5gm	13.0-18.0gm/dl
2	Total White blood cells	7600cell/cum	5000-11000cells/cumm
3	Total Red blood cells	3.8milian/cumm	3.5-5.5milin/cumm
4	Neutrophis	60%	50%-70%
5	Lymphocytes	39%	20%-40%
6	Eosinophiles	1%	1%-6%
7	Monocytes	0%	1.5%
8	Basophils	0%	0-0.5%
9	Erythrocyte sedimentation Rate	5mm	1-15mm/Hour

Table No.4: Hematology for Emuoil

S.No	Hematology	Findings	Normal range
1	Hem globulin(HB)	68%, 9.8gm	13.0-18.0gm/dl
2	Total White blood cells	3100 cells /cum	5000-11000cells/cumm
3	Total Red blood cells	3millin/cumm	3.5-5.5milin/cumm
4	Neutrophis	52%	50%-70%
5	Lymphocytes	46%	20%-40%
6	Eosinophiles	2%	1%-6%
7	Monocytes	0%	1.5%
8	Basophils	0%	0-0.5%
9	Erythrocyte sedimentation Rate	1mm	1-15mm/Hour

Table No.5: Biochemical parameters for control group

S.No	Bio-chemistry	Findings	Normal range
1	Blood sugar	80mg/dl	70-100mg/dl
2	Blood urea	20mg/dl	10-40mg/dl
3	Serum creatinine	0.7mg/dl	0.5-1.4mg/dl
4	Serum uric acid	3.0mg/dl	2.8-7.0mg/dl
5	Serum cholesterol	222mg/dl	140-230mg/dl
6	Serum sodium	125mmo/l	135-145mmo/l
7	Serum potassium	4.5mmo/l	3.5-5.5mmo/l
8	Serum calcium	9mmo/l	8.8-11.0mmo/l

Table No.6: Biochemical parameters for DSS Induced group

S.No	Bio-chemistry	Findings	Normal range
1	Blood sugar	78mg/dl	70-100mg/dl
2	Blood urea	25mg/dl	10-40mg/dl
3	Serum creatinine	0.8mg/dl	0.5-1.4mg/dl
4	Serum uric acid	3.5mg/dl	2.8-7.0mg/dl
5	Serum cholesterol	213mg/dl	140-230mg/dl
6	Serum sodium	125mmo/l	135-145mmo/l
7	Serum potassium	4.6mmo/l	3.5-5.5mmo/l
8	Serum calcium	9.2mmo/l	8.8-11.0mmo/l

Table No.7: Biochemical parameters for Standard group

S.No	Bio-chemistry	Findings	Normal range
1	Blood sugar	75mg/dl	70-100mg/dl
2	Blood urea	25mg/dl	10-40mg/dl
3	Serum creatinine	1.0mg/dl	0.5-1.4mg/dl
4	Serum uric acid	4.1mg/dl	2.8-7.0mg/dl
5	Serum cholesterol	235mg/dl	140-230mg/dl
6	Serum sodium	147mmo/l	135-145mmo/l
7	Serum potassium	4.1mmo/l	3.5-5.5mmo/l
8	Serum calcium	8.4mmo/l	8.8-11.0mmo/l

Table No.8: Biochemical parameters for Emuoil

S.No	Bio-chemistry	Findings	Normal range
1	Blood sugar	65mg/dl	70-100mg/dl
2	Blood urea	22mg/dl	10-40mg/dl
3	Serum creatinine	1.0mg/dl	0.5-1.4mg/dl
4	Serum uric acid	3.9mg/dl	2.8-7.0mg/dl
5	Serum cholesterol	190mg/dl	140-230mg/dl
6	Serum sodium	130mmo/l	135-145mmo/l
7	Serum potassium	3.8mmo/l	3.5-5.5mmo/l
8	Serum calcium	8.2mmo/l	8.8-11.0mmo/l

Table No.9: Histo-toxicological studies

S.No	Organ	Control	Rat No.1	Rat No.2	Rat No.3
1	Brain	Edematous Mild gliosis	Nil-remarkable	Endothelial proliferation	Endothelial proliferation
2	Heart	Nil-remarkable	Nil-remarkable	No specific change	No specific change
3	Lung	Nil-remarkable	Congestion Peribronchiolar inflammation	No specific change	No specific change
4	Stomach	Nil-remarkable	Nil-remarkable	Edematous and Congested mucosa	Edematous mucosa
5	Liver	Nil-remarkable	Hepatic congestion Centralvein congestion Portal tried congestion	Perilobular congestion Central vein congestion	Portal vein congestion
6	Intestine	Nil-remarkable	Nil-remarkable	Nil-remarkable	Nil-remarkable
7	Bone	Nil-remarkable	Nil-remarkable	Nil-remarkable	Nil-remarkable
8	Skin	Nil-remarkable	Nil-remarkable	Nil-remarkable	Nil-remarkable
9	Kidney	Nil-remarkable	Nil-remarkable	Mild congestion in the collecting tubules	Congestion at the collecting tubules MildHaemarrages at the collecting tubules
10	Spleen	Nil-remarkable	Nil-remarkable	Congestion	Mild congestion

Table No.10: Histopathological Analysis Estimation of C-reactive protein levels in blood

S.No	Rat	CRP value
1	Normal	0.7 mg/dl
2	Test (D.S.S)	2.1 mg/dl
3	Emu oil low dose	1.1 mg/dl
4	Emu oil high dose	1.5 mg/dl
5	Standard (Mesalamine)	0.9 mg/dl

Table No.11: Colon weight/length ratio (mg/cm)

S.No	Rat	Colon weight	Colon length
1	Normal	2gm	3.5 cm
2	Test (D.S.S)	4gm	2.6cm
3	Emu oil low dose	3gm	3cm
4	Emu oil high dose	3gm	4cm
5	Standard (Mesalamine)	1.8gm	2.5cm
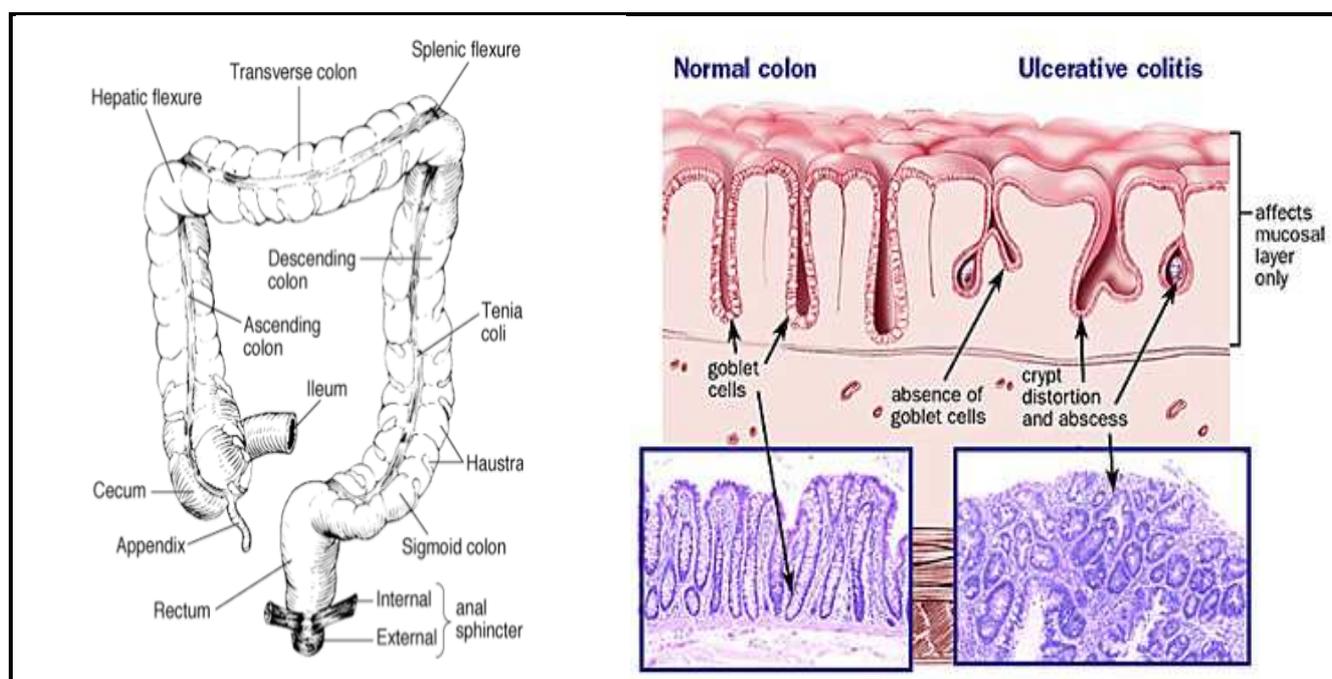
Figure No.1: Ulcerative colitis Intestine
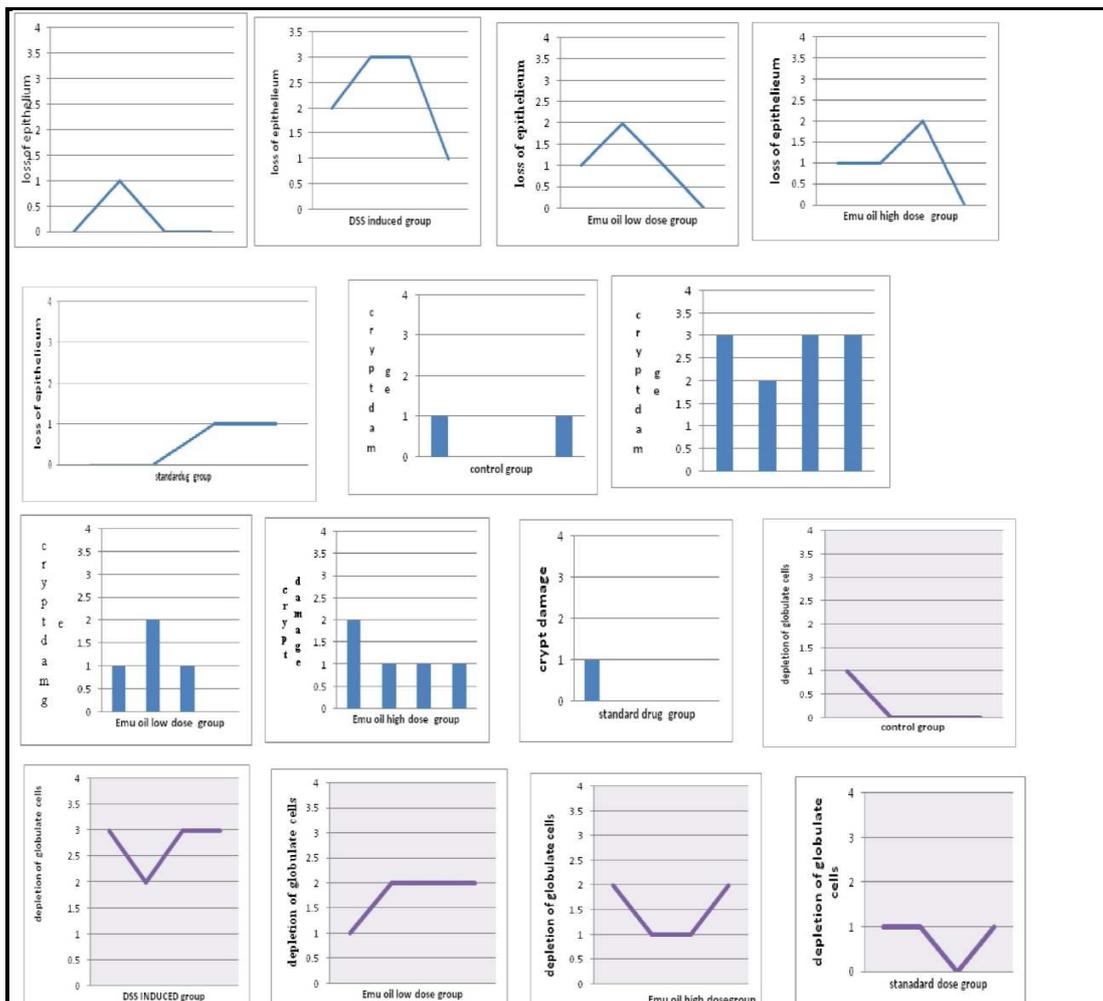
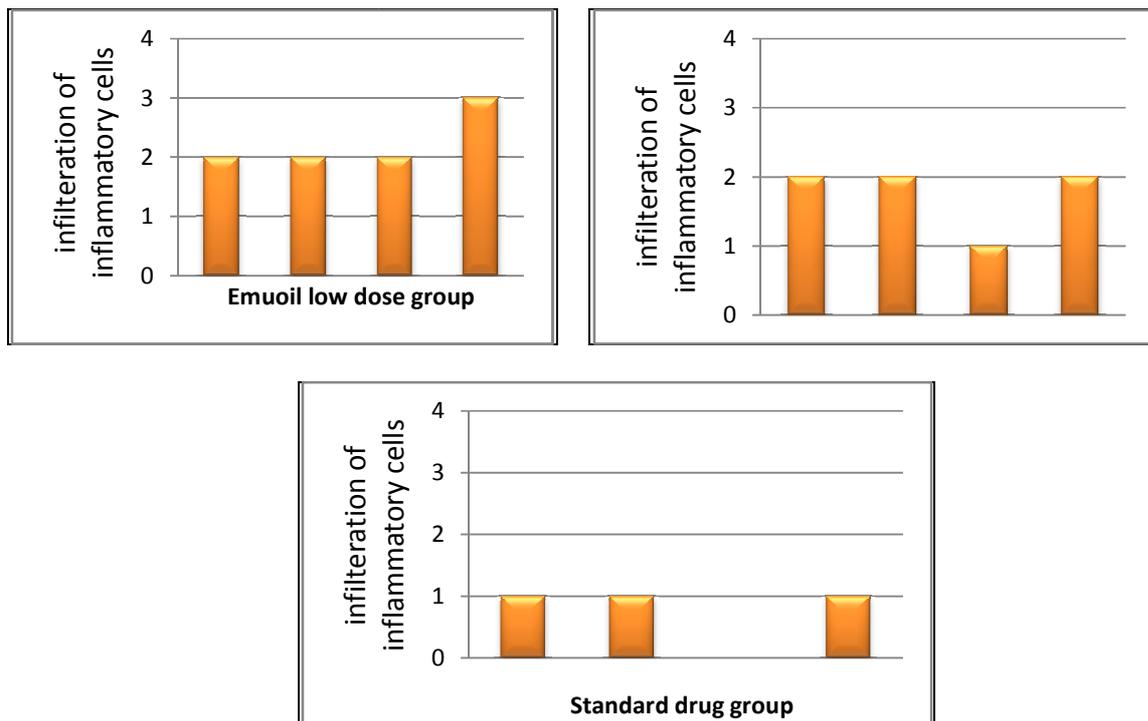**Figure No.2: Results of different tests**
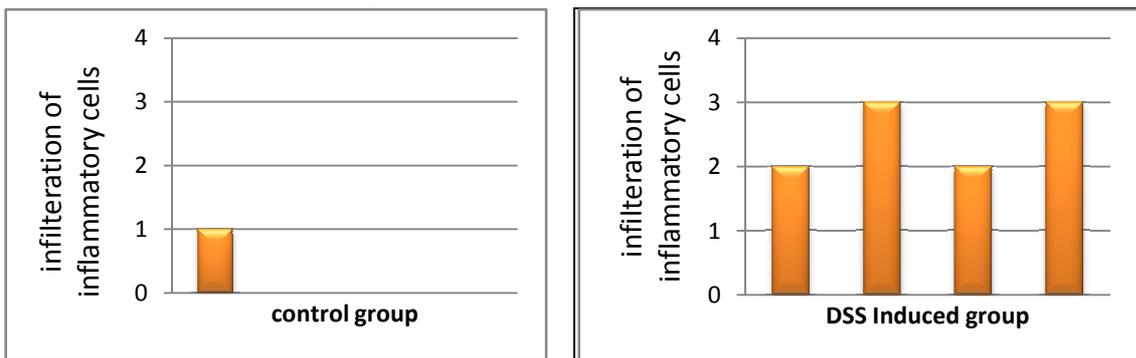
Figure No.3: Results of various tests

Test rat colon

Microscopic structure of test (dss) rat colon

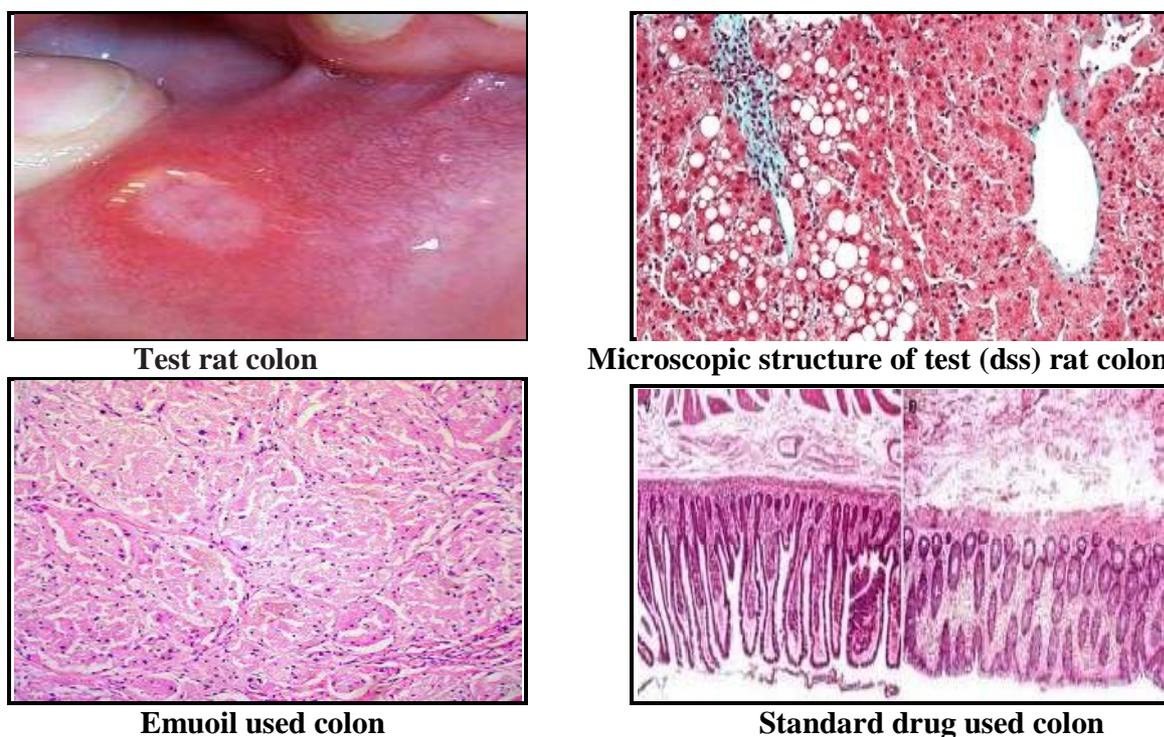Emuoil used colon

Standard drug used colon

Figure No.4: Histopathology Figures

CONCLUSION

The Dextran sulphate sodium- induced colitis is an effective model which resembles the human ulcerative colitis. The findings from this study suggest that *Emu oil* formulation has an anti-oxidant and anti-inflammatory action in reducing colitis in rats. These activities are due to the presence of oleic acid. The dose of Emu oil (0.5ml/lit) has an effect comparable to that of the standard drug (Mesalazine).

ACKNOWLEDGEMENT

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