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PROTECTIVE EFFICACY OF THE ETHANOLIC EXTRACT OF *ROSMARINUS OFFICINALIS* (Linn) AGAINST BRADYKININ INDUCED INFLAMMATION IN RATS

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The present study investigates the anti-inflammatory activity of ethanolic extracts of Rosmarinus officinalis Linn. The dried leaves of Rosmarinus officinalis were extracted with ethanol using soxhlet apparatus. The effect was observed on bradykinin induced paw edema in female SD rats. Inflammation was induced by subcutaneous injection of freshly prepared 0.1ml bradykinin (1mg/ml) in the right hind paw. The two treatment group rats received 250mg/kg b.wt and 500mg/kg b.wt respectively orally, one hour prior to bradykinin induction. The increase in the paw thickness in the rats after induction with bradykinin was significantly lowered on treatment with the plant extract. The ethanolic extract was found to improve the antioxidant status in the animals in a dose dependent manner. The effect of the extract was compared with the effect of the standard drug indomethacin administration. Histopathological analysis of the hindpaw tissue supports the protective effect of the ethanolic extract of R. officinalis.

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Inflammation is inherent to the pathogenesis of a variety of diseases. An inflammatory response implicates macrophages and neutrophils, which secrete a number of mediators (eicosinoids, oxidants, cytokine and lytic enzymes) responsible for the initiation, progression and persistence of the acute or chronic state of inflammation (Lefkowitz *et al*., 1999). Currently, non-steroidal anti-inflammatory drugs (NSAIDs) supplemented with steroid hormone remains the major recommended strategy for its treatment (Scott *et al*., 1998). While these drugs transiently suppress inflammation and ameliorate symptoms, they do not significantly improve the long-term disease outcome. Furthermore, long-term treatment with NSAIDs may result in serious side effects, such as gastrointestinal ulcergenicity and renal morbidity (Pincus *et al*., 1992).

Much attention has been directed towards the characterization of the antioxidant properties of the plant extracts, their fraction and identification of the constituents responsible for inflammation (Valentao et al., 2001; Haraguchi et al., 1996). The anti-inflammatory agents of present use exert their effect through a spectrum of different modes of action possessing well known side and toxic effects. It is therefore essential to introduce new medicinal plants to develop cheaper drugs.

Rosmarinus officinalis, a member of the family Lamiaceae is a flowering plant that grows in Mediterranean countries, Southern Europe and in the Littoral region through minor Asia wildly (Derwich et. al, 2011). Previous studies have shown that rosemary essential oil had antimicrobial, antioxidant, anti-carcinogenic, cognition improving and certain glucose level lowering properties, which make it useful as a natural animal feed additive. Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes (Omabola olunranti okoh, 2010).

MATERIALS AND METHODS

2.1 Chemicals

Bradykinin for induction was purchased from Sigma Aldrich, Bangalore, India. All the chemicals used for the study were of Analytical grade.

2.2 Preparation of Plant extract

The leaves of *Rosmarinus officinalis* were bought from the local market and shade dried. The dried leaves was powdered and extracted with Ethanol, Chloroform, Petroleum ether, Ethyl acetate, Acetone and Benzene solvents using soxhlet apparatus. The extract obtained was rotary evaporated and the powder was preserved in an air tight container and stored at 4ºC for further use.

2.3 Phytochemical screening of the extract

The phytochemicals were analysed with the dried extract according to the published standard methods.
The amount of phenols present was analyzed by Singleton and Rossi, 1965, flavonoids by Lamaison and Carnat, 1990, flavonols by Nakamura *et al*., 2003 and tannins by Robert *et al.*, 1971 procedures.

2.4 Bradykinin induced acute inflammation model

There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of inflammation (Di and Willoughby, 1971). Enzymes in a local area of injury are activated to free bradykinin. The main function of bradykinin is to increase the sensation of pain. A secondary function of bradykinin is to promote the production of histamine and is that of increasing blood flow into the involved area by dilation of arteries and increasing capillary vessel permeability.
Bradykinin (BD) induced paw edema model is a widely used model to evaluate the anti-inflammatory potency of plant extracts.

2.5 Experimental design

Female Sprague Dawley rats weighing approximately 180-200g obtained from small animal breeding station, Thrissur, Kerala, India were used for the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12h light/dark). The animals were divided into five groups of six animals each. Inflammation was induced by sub plantar injection of bradykinin (Mahat and Patil, 2007).
Group I: Control
Group II: Inflammation was induced by subcutaneous injection of freshly prepared 0.1ml BD in saline into the right hind paw.
Group III: Treated with 250mg/kg body weight of ethanolic extract of *R.officinalis* (ROEtOH) orally, 1 hour prior to BD induction
Group IV: Treated with 500mg/kg body weight of ethanolic extract of *R.officinalis* (ROEtOH) orally, 1 hour prior to BD induction Group V: Treated with 10mg/kg body weight of indomethacin orally, 1 hour prior to BD induction.
The edema was measured after 1, 2, 3 and 4 h. After 4h, the rats were killed by cervical dislocation and then the whole liver, spleen, hind paw were removed and washed with ice-cold saline. The tissues were homogenized using 0.1M Tris-HCl buffer (pH7.4) to give 10% homogenate.

2.6 Measurement of edema

Paw thickness was measured using Vernier caliper before and after BD challenge in each group. Increase in paw thickness was calculated using the formula Pt-Po, where Po is the initial paw thickness at time to and Pt is the thickness at time t (3h). Percent inhibition was calculated by the formula, (1-pt/pc)*100, where pt is the increase in paw thickness of treated and pc is that of BD induced control.

2.7 Biochemical Estimations

Serum Nitric oxide (NO) was determined by method described by Green *et al*, (1982). Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions that is estimated spectrophotometrically at 540nm. The detection of CRP is more

sensitive and reliable indicator of inflammatory process. This test is based on the immunological reaction between CRP as an antigen and latex particles as described by Tillet *et al*, (1930). The amount of protein present in the sample were estimated using standard method of Lowry *et al*, (1957).The activity of the enzyme, Superoxide dismutase (SOD) was assessed by the method of Das *et al*.,(2000).The method involves generation of superoxide radical of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride. The enzymic activity of Catalase (CAT) was determined by the method described by Sinha, (1972).The Glutathione(GSH) content of the hind paw tissue was determined by using Ellman's reagent as described by Moron *et al*., (1979) Vitamin C is a very effective free radical scavenger. It is estimated spectrophotometrically at 520nm by method described by Omaye *et al* (1975) The extent of lipid peroxidation was measured through malondialdehyde reactivity with thiobarbituric acid in acidic condition to generate a pink coloured chromophore which were read at 535nm as described by Niehius and Samuelsson, 1968.

2.8 Histopathological analysis

Rats were sacrificed and the hindpaw tissues were collected and preserved in 10% formalin immediately after removal from animal.

2.9 Statistical analysis

All the values obtained from animals are expressed as mean ±SD. Statistical comparision was done at significance level, $P<0.05$ using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

3. RESULTS AND DISCUSSION

In Indian system of medicine, certain herbs are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. The present study was designed to investigate the anti-inflammatory effects of the medicnal plant, Rosemary (*Rosmarinus officinalis*). Studies have shown that phenolic compounds such as catechin and quercetin were very efficient in stabilising phospholipid bilayers against peroxidation induced by reactive oxygen species (ROS) (Gülçin *et al*., 2010). Flavonoids are a class of phenolics that exhibit powerful antioxidant effects in biological systems, including free radical scavenging and metal ion sequestering, but their effectiveness greatly depends on particular chemical features. However, it is widely accepted that phenols have complex pro- and antioxidant effects in vitro, depending on their structure and the assay system used, and it is often hard to predict their actual action (Rice-Evans and Miller, 1996; Rice-Evans *et al*., 1995).

The results of the qualitative and quantitative analysis of phytochemical screening are given in the table 1 and table 2 respectively.

Table 1 Phytochemical qualitative analysis of *Rosmarinus officinalis*

Phytochemical		Solvent					
		Ethanol	Chloroform	Petroleum ether	Ethylacetate	Acetone	Benzene
Alkaloids	Dragendroff test	+	+	+	+	+	+

	Wagner's test	+	-	+	+	+	-
	Meyer's test	+	-	-	-	-	-
Flavonoids		+	+	+	+	+	+
Saponins		-	-	-	-	-	-
Carbohydrates	**Fehling's reaction**	+	+	+	-	-	+
	Benedicts test	+	+	+	+	+	+
	Molisch's reaction	+	+	-	-	-	+
Protein	**Millon's reaction**	-	-	-	-	-	-
	Biuret test	+	-	-	-	+	+
Phenols	**Ferric chloride test**	+	+	+	+	+	+
	Lead acetate test	+	+	-	-	-	-
	Libermann reaction	+	+	-	-	-	+
Steroids	**Libermann -Burchards**	-	-	-	-	-	-
	Salkowski reaction	+	+	-	-	-	+
Glycosides		+	+	-	-	-	-
Resins		+	+	-	-	-	+
Tannins	**Ferric chloride**	+	+	+	+	+	+

	Lead acetate	+	+	+	+	+	+
Thiols		-	-	-	-	-	-

In the qualitative analysis of phytochemicals, it was found that the ethanol extract gave better results and hence the ethanolic extract of *Rosmarinus officinalis* was chosen to carry out the further study.

Table 2Phytochemical constituents in the ethanolic extract of *Rosmarinus officinalis*

Total phenols		**Flavanoids**		**Flavonols**	**Tannins**	**Condensed Tannins**
mg /g (GAE)	mg/g (CE)	mg /g (QE)	mg /g (RE)	mg/g (CAE)	mg/g (CAE)	mg/g (CAE)
61.34 ± 2.37	55.50 ± 3.12	6.22± 0.23	10.40 ± 0.61	43.3 ± 3.05	18.9 ± 1.21	1.53 ± 0.12

The paw thickness of the experimental animals of different groups was analyzed upon induction with BD at a concentration of 1mg/ml. The initial paw thickness of all the experimental animals was measured. The paw thickness of group I animals that served as normal control, was found to be 0.58±0.004cm. The group II animals that served as BD control group, there was observed a significant raise in the paw thickness to 0.90±0.014cm. This increase could be due to the inflammation induced by BD. Upon treatment with the ethanolic extract of *R.officinalis* there was observed a significant reduction in the paw thickness in the group III and IV animals to 0.81±0.005 cm and to 0.75 ±0.007cm respectively. The results obtained were similar to those evidenced in the group V animals that were treated with indomethacin. Thus it could be said that our extract was able to reduce inflammation in comparison to the standard drug. The values are shown in table 3.

Table 3 Effect of ethanolic extract of *Rosmarinus officinalis* on paw thickness of the control and experimental animals

Groups	Initial paw thickness (cm)	Paw thickness after 4 h (cm)	Increase in paw thickness (cm)
Bradykinin Control	0.58 ± 0.004	0.90 ± 0.014	0.32 ± 0.002
ROEtOH (250 mg/kg b wt)+BD	0.57 ± 0.007	0.81± 0.005 [a]	0.24 ± 0.006
ROEtOH (500 mg/kg b wt)+BD	0.58 ± 0.006	0.75 ± 0.007 [a]	0.17 ± 0.008 [a]

Indomethacin(10mg/kg b wt)+ BD	0.58 ± 0.006	0.73 ± 0.007 [a]	0.15 ± 0.010 [a]

Values are expressed as mean ± SD (n=6)
Group comparison and statistical significance at $p<0.05$: [a]: Group II vs. II, III, IV, V

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Hagerman *et al*., 1998)
Serum NO was found to increase significantly at $p<0.05$ from group I animals. After treatment with the plant extract, the serum NO decreased from group I animals. The 500mg/kg bwt dose of ROEtOH was found to be more effective when compared to 250mg/kg b.wt dose of ROEtOH.
Both nitric oxide (NO) and prostaglandin E_2 (PGE_2) are pleiotropic inflammatory mediators that are overproduced and involved in the pathogenesis of chronic inflammations and infections. NO is synthesized from L-arginine by nitric oxide synthase (NOS) (Kohno *et.al.*, 2008).CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages
NO along with superoxide ($O_2^{\bullet-}$) and the products of their interaction, also initiates a wide range of toxic oxidative reactions causing tissue injury (Hogg, 1998). Likewise, the neutrophils too produce oxidants and release granular constituents comprising of lytic enzymes performing important role in inflammatory injury (Yoshikawa and Naito, 2000). Inhibition in the release of these mediators is a potential strategy to control inflammation and is implicated in mechanism of action of a number of anti inflammatory drugs including the representative ones like dexamethasone (Bourke and Moynagh, 1999).
Thus by reducing the levels of NO in the serum, the extract reduces BD induced inflammation. The results of the study was found to be in accordance with Yoke Keong *et al* .(2011) who reported the protective effect of *Bixa orellana* leaves. The *Bixa orellana* extract was found to inhibit bradykinin-induced inflammation through suppression of nitric oxide production.The hydroalcoholic extract of *Coronopus didymus* was found to reduce NO levels in the serum of the rats that were induced with BD(Busnardo *et al*., 2010).
The CRP level in serum of induced group was very high when compared to group I and other groups. Induced group showed a hike to 25.75±0.23 µg/ml. Compared to group III, extract administration to group IV has more effect in treating inflammation which reduced the CRP level to 9.66±0.11µg/ml. The values are shown on table 4.

Table 4 Effect of ethanolic extract of *Rosmarinus officinalis* on the levels of serum nitric oxide and CRP and in control and experimental animals

Parameters	Control	Bradykinin (1mg/ml)	ROEtOH (250mg/kg b wt) + BD	ROEtOH (500 mg/kg b wt) + BD	Indomethacin (10 mg/kg) + BD
Nitric	7.05 ± 0.21	10.20 ±	8.28 ± 0.21 [a b]	7.25 ± 0.32 [b]	7.08 ± 0.31 [b]

oxide	[b]	0.20 [a]			
CRP	5.76 ± 0.17 [b]	25.75 ± 0.23 [a]	14.54 ± 0.33 [ab]	9.66 ± 0.11 [b]	6.71 ± 0.28 [b]

Values are expressed as mean ± SD (n=6)

Units: Nitric oxide - mg/dl; CRP-µg/ml

Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V

[b:] Group II vs. I, III, IV, V

The generation of reactive oxygen species (ROS) by phagocytic leukocytes (neutrophils, monocytes, macrophages, and eosinophils) is one of the most important hallmarks in the inflammatory process. The ROS are mediators of cellular injury and are involved in the onset of cellular damage during endotoxemia (Ginn-Pease and Whisler, 1998; Forman and Torres, 2001). ROS are involved in a variety of cellular stress mechanisms. Several lines of evidence indicate that the redox status of cells participates in modulating NK and B activation (D'Acquisto *et al*., 2002). A number of reports have shown that a broad range of antioxidants abolish NF-_B activation (Bai *et al*., 2005)

In accordance with the present state of scientific knowledge, there is enough evidence that the excessive production of free radicals in the organism, and the imbalance between generation of reactive oxygen species and antioxidant defenses is related to processes such as aging and several diseases (Kasapoglu and Ozben, 2001; Mattson *et al*., 2001).

SOD may play an important role in protecting cells against ROS .SOD is the first enzyme of the anti-oxidant process (Beyer *et al*., 1991). SOD catalyzes the breakdown of $O_2^{\cdot -}$ and H_2O_2, removes singlet oxygen as well as $O_2^{\cdot -}$, prevents formation of $OH^{\cdot}$ (Fridovich, *1973*), and has been implicated as an essential defense against the potential toxicity of oxygen.

The H_2O_2 formed by SOD and other processes is scavenged by catalase that catalyzes the dismutation of H_2O_2 into water and molecular oxygen. (Sumanth and Rana, 2006)

Enzymic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT) play important roles in scavenging of reactive oxygen species. The activities of SOD and Catalase was analysed in spleen, thymus and hindpaw samples to check the antioxidant status in experimental animals.

The results are presented in Table 5. The activities of SOD and CAT were observed to be significantly lowered ($p<0.05$) in the BD control group animals as compared with group I normal control animals.. The prior treatment with ROEtOH at 250mg/kg bwt and 500mg/kg bwt was found to significantly improve the antioxidant status in a dose dependent manner. The results were comparable with that of the standard drug, indomethacin. A decrease in the activity of SOD and CAT may be due to enormous production of free radicals. Akira *et al*., (1988) reported that increase in the production of free radicals namely superoxide anions and hydroxy radicals could suppress the activity of SOD and CAT in inflammatory conditions. Superoxide anions are thought to be involved in inflammatory reactions since they are produced by phagocytic cells (Babior *et al*., 1973). These cells are reported to produce hydroxy radical (Salin and McCord, 1975) and singlet oxygen (Allen *et al*., 1972).

Table 5 Effect of ethanolic extract of *Rosmarinus officinalis* on the activities of enzymic antioxidants in spleen, thymus and hind paw of experimental animals

Parameters	Control	Bradykinin (1mg/ml)	ROEtOH (250mg/kg b wt) + BD	ROEtOH (500 mg/kg b wt) + BD	Indomethacin (10 mg/kg) + BD
Spleen					
SOD	4.12 ± 0.03^{b}	0.52 ± 0.02^{a}	1.82 ± 0.04^{ab}	3.38 ± 0.12^{b}	3.95 ± 0.23^{b}
CAT	11.31 ± 0.09^{b}	3.96 ± 0.05^{a}	7.07 ± 0.06^{ab}	10.19 ± 0.05^{b}	11.25 ± 0.05^{b}
Thymus					
SOD	5.91 ± 0.09^{b}	3.04 ± 0.03^{a}	4.28 ± 0.04^{b}	5.50 ± 0.03^{ab}	5.65 ± 0.03^{ab}
CAT	12.45 ± 0.11^{b}	4.13 ± 0.22^{a}	9.12 ± 0.56^{b}	12.09 ± 0.91^{b}	11.95 ± 0.58^{b}
Hind paw					
SOD	5.48 ± 0.41^{b}	3.97 ± 0.06^{a}	4.62 ± 0.26^{ab}	5.20 ± 0.17^{b}	5.26 ± 0.15^{b}
CAT	8.15 ± 0.23^{b}	3.37 ± 0.28^{a}	5.85 ± 0.31^{ab}	7.56 ± 0.52^{b}	7.61 ± 0.48^{b}

Values are expressed as mean ± SD (n=6) Units:

SOD- inhibition of 50% nitrite formation/min; CAT-μmole of H_2O_2 consumed/min;

Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V

[b:] Group II vs. I, III, IV, V

GSH acts directly as a free radical scavenger by neutralizing OH^-, restores damaged molecules by hydrogen donation, reduces peroxides, and maintains protein thiols in the reduced state (Sies, 1986).
Vitamin C is an outstanding antioxidant in biological systems and powerful reducing agent, also involved as cofactor in numerous metabolic processes. Vitamin C, a chain-breaking antioxidant, protects biological membranes against reactive oxygen species (ROS) (Frei, 1989). Ascorbic acid protects cells against oxidative damage to essential molecules. In addition vitamin C may reduce carcinogenesis through stimulation of the immune system.
The levels of Total Reduced Glutathione and Vitamin C were analysed in spleen, thymus and hindpaw samples. The decreased levels of GSH and Vit C in the group II animals , was found to be improved significantly ($p<0.05$) on treatment with ROEtOH at both the doses. The 500mg/kg bwt dose was found to be more effective than the 250mg/kg bwt dosage. The values are shown in table 6.

Table 6 Effect of ethanolic extract of *Rosmarinus officinalis* on GSH and Vitamin C in the spleen, thymus and hind paw of experimental animals

Groups	Control	Bradykinin (1mg/ml)	ROEtOH (250mg/kg b wt) + BD	ROEtOH (500 mg/kg b wt) + BD	Indomethacin (10 mg/kg) + BD
Spleen					
GSH	1.81 ± 0.16 [b]	1.43 ± 0.05 [a]	1.64 0.09 [ab]	1.69 ± 0.06 [b]	1.72 ± 0.06 [b]
Vit C	1.13 ± 0.05 [b]	0.83 ± 0.04 [a]	0.95 ± 0.03 [ab]	1.04 ± 0.05 [b]	1.08 ± 0.02 [b]
Thymus					
GSH	2.11 ± 0.09 [b]	1.22 ± 0.05 [a]	1.77 0.09 [ab]	2.09 ± 0.12 [b]	1.98 ± 0.08 [b]
Vit C	3.55 ± 0.05 [b]	1.43 ± 0.11 [a]	2.90 ± 0.19 [ab]	3.33 ± 0.23 [b]	3.28 ± 0.21 [b]
Hind paw					
GSH	1.14± 0.10 [b]	1.07 ± 0.0	1.34 ± 0.07 [ab]	1.43 ± 0.05 [ab]	1.5 ± 0.05 [ab]
Vit C	1.22 ± 0.07 [b]	0.73 ± 0.04 [a]	0.85 ± 0.05 [ab]	0.97 ± 0.08 [ab]	1.10 ± 0.05 [ab]

Values are expressed as mean ± SD (n=6)
Units: GSH, Vitamin C - µg/ml or mg protein;

Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V

[b:] Group II vs. I, III, IV, V

Lipid peroxidation can inactivate cellular components and plays an important role in oxidative stress.
Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids found primarily in cell membranes. An increase in the level of lipid peroxides in tissues, therefore, reflects membrane damage (Kawamura *et al*., 1992).
The levels of lipid peroxides in serum, spleen, thymus and hindpaw was found to be significantly ($p<0.05$) elevated in the BD induced group and the levels were restored in the treatment groups (Group III and IV) indicating the effect of the extract. The values are shown in table 7.

Table 7 Effect of ethanolic extract of *Rosmarinus officinalis* on lipid peroxide levels in serum, spleen, thymus and hindpaw of experimental animals

Groups	Control	Bradykinin (1mg/ml)	ROEtOH (250mg/kg b wt) + BD	ROEtOH (500 mg/kg b wt) + BD	Indomethacin (10 mg/kg) + BD
Serum					
LPO	8.35 ±	18.39 ±	14.21 ± 1.35[ab]	9.68 ± 0.65[b]	8.84 ± 0.36[b]

	0.20[b]	1.29[a]			
Spleen					
LPO	1.07 ± 0.06[b]	1.82 ± 0.04[a]	1.18 ± 0.04[ab]	1.11 ± 0.05[b]	1.10 ± 0.08[b]
Thymus					
LPO	1.07 ± 0.06[b]	2.12 ± 0.04[a]	1.58 ± 0.08[ab]	1.09 ± 0.06[b]	0 .97 ± 0.08[b]
Hind paw					
LPO	1.16 ± 0.03 [b]	2.36 ± 0.10[a]	2.14 ± 0.08[b]	1.84 ± 0.07 [b]	1.73 ± 0.10[a]

Values are expressed as mean ± SD (n=6)

Units: LPO - nmoles of MDA formed/min/ml or mg protein; Group comparison and statistical significance at $p<0.05$: [a]: Group I vs. II, III, IV, V [b:] Group II vs. I, III, IV, V

The hind paw tissue of the experimental animals was analysed for histological changes. The results are presented in slide.

The various architectural changes in the tissue were depicted as follows.

Slide 1 : Shows the section of hind paw tissue of Group I animals. The architecture reveals no obvious abnoramilty

Slide 2 : Shows the section of hind paw tissue of Group II (BD induced) animals. The architecture indicates severe inflammation with infilteration of neutrophils

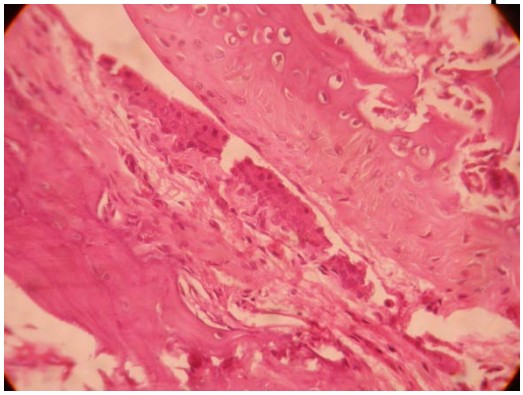
Slide 3 : Shows the section of hind paw tissue of Group III (BD + *R. officinalis* 250mg/kg b wt) .The architecture shows mild inflammation

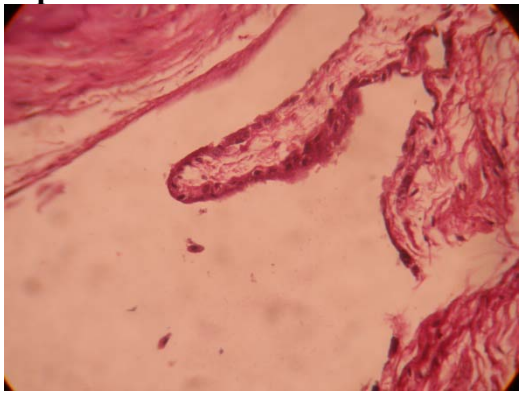
Slide 4 : Shows the section of hind paw tissue of Group IV (BD + *R. officinalis* 500mg/kg b wt) .The architecture indicates mild inflammation

Slide 5: Shows the section of hind paw tissue of Group V (BD + indomethacin 10mg/ kg b wt) .The architecture of the muscle fibres and synovium show no obvious abnormality.

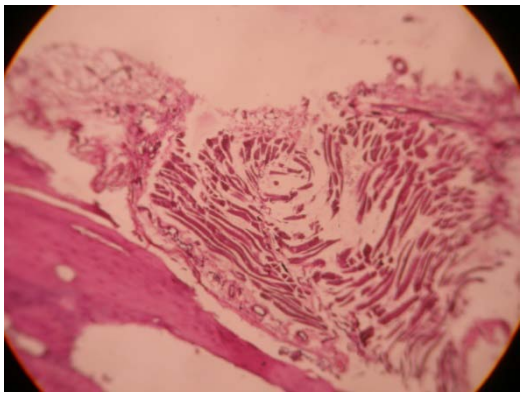
The histopathological investigation revealed the protective nature of the ethanolic extract of *R officinalis* against bradykinin induction.

Effect of the ethanolic extract of *Rosmarinus officinalis* on the histology of the hind paw tissue of experimental animals

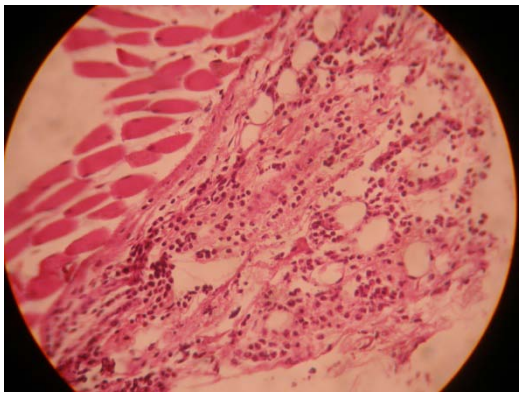




Slide 1: Normal Control.
The architecture reveals no obvious abnormality



Slide 2: Bradykinin induced
The architecture reveals inflammation

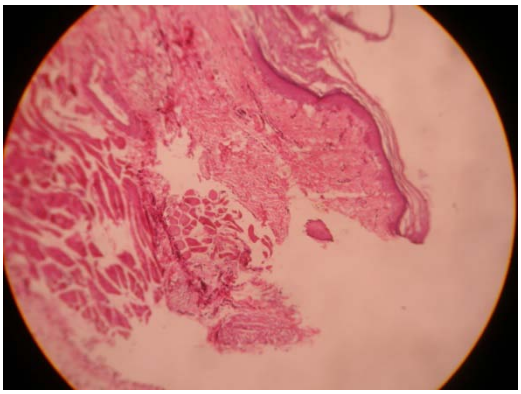


Slide 3: Bradykinin induced + *R. officinalis* (250mg/kg b wt)

The architecture shows mild inflammation

Slide 4: Bradykinin induced + *R. officinalis* (500 mg/kg b wt)

The architecture shows no obvious abnormality



Slide 5: Bradykinin+ indomethacin (10mg/ kg b wt)
The architecture of the muscle fibres and synovium show no obvious abnormality

Thus from the results of the biochemical analysis and histopathological investigation, it could be said that the ethanolic extract of *Rosmarinus officinalis* has potent anti-inflammatory action. This could be due to the various phytochemical constituents present.

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