International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

FULL Length Research Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

Anti-Inflammatory and Analgesic activity of D_l-Alpha-Tocopheryl Acetate and its interaction with aspirin in Wistar rats.

Suneel. I. Majagi*, T. N. Bhosle[1], P. A. Patil[2]
*Associate Professor, Dept.of Pharmacology, J.N. Medical College, Nehru Nagar, Belgaum- 590010, Karnataka.
[1]K.L.E College of Pharmacy, Nehru Nagar, Belgaum- 590010, Karnataka, India
[2]Professor, Dept. of Pharmacology, J.N. Medical College, Nehru Nagar, Belgaum- 590010, Karnataka, India.

Abstract

In the present study anti inflammatory activity of different doses of d_l-alpha-tocopheryl acetate viz., 9,18,36 and 72 mg/kg is investigated in both acute and sub-acute models of inflammation in rats. Similarly their analgesic activity and effect on gastric ulcer were also studied. The study also aimed to elicit the possible interactions of d_l-alpha-tocopheryl acetate with aspirin. Except 9 mg/kg, all the therapeutic equivalent doses of d_l-alpha-tocopheryl acetate in acute model and in the dose of 36 and 72 mg/kg in sub-acute model of inflammation exerted significant anti-inflammatory activity. Combination of sub anti-inflammatory (SAI) dose of d_l-alpha-tocopheryl acetate (9mg/kg) with that of aspirin (54mg/kg) showed significant anti-inflammatory activity in both the models of inflammation. D_l-alpha-tocopheryl acetate in the dose of 18, 36 and 72 mg/kg individually and its SAI dose when coadministered with that of aspirin showed significant analgesic activity at 1 and 3 hours. D_l-alpha-tocopheryl acetate in the dose of 36 and 72 mg/kg and combination treatment of SAI dose of d_l-alpha-tocopheryl acetate with that of aspirin found to be gastroprotective. These findings clearly indicate the anti-inflammatory and analgesic activity of d_l-alpha-tocopheryl acetate. In interaction studies, d_l-alpha-tocopheryl acetate acts synergistically with aspirin towards reducing inflammation and pain as well as the former reduced the gastric ulcerogenic potential of the latter. If these findings are extrapolated to human beings, Vitamin E can be used individually or else as an adjuvant to NSAIDs in the treatment of inflammatory conditions. Clinical studies in this regard are really worthwhile.

*Corresponding author, Mailing address:
Associate Professor, Department of Pharmacology,
J.N. Medical College, Nehru Nagar,
Belgaum- 590010.Karnataka- India.
E-mail: suneelmajagi@yahoo.co.in
Phone: 0831-24092082, Fax: 08312470759.

Key words:
Analgesia, Aspirin, D_l-alpha-tocopheryl acetate, Inflammation, Interaction.

How to Cite this Paper:
Suneel. I. Majagi*, T. N. Bhosle, P. A. Patil "Anti-Inflammatory and Analgesic activity of D_l-Alpha-Tocopheryl Acetate and its interaction with aspirin in Wistar rats.", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 86-93

Article History:----------
Date of Submission: 07-10-2010
Date of Acceptance: 21-11-2010
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

Inflammation, one of the most common clinical conditions has been subjected to intensive investigations in order to understand its pathogenesis, and several mediators of inflammation

have been recognised. Currently used anti-inflammatory agents produce their beneficial effects by suppressing the activity of one or the other inflammogen. Interestingly, in addition to NSAIDs several other drugs with diverse pharmacological actions viz., clonidine[1], epinephrine, norepinephrine[2], calcium channel blockers like verapamil [3], antimicrobial agents like dapsone[4] and sulfamethizole[5] have been shown to possess anti-inflammatory activity.

Role of free radicals in the pathogenesis of inflammation particularly in joint inflammation has been well documented [6,7]. Since recognition of oxygen free radicals and their role in pathogenesis of inflammation, several free radical scavengers and antioxidants have been investigated for their possible anti-inflammatory activity. The scavengers such as super oxide dismutase [8] and antioxidants such as ascorbic acid [9] have been reported to possess anti-inflammatory activity.

Tocopherols, which are known biological antioxidants have also been shown to scavenge singlet molecular oxygen[10] and to intercept lipid peroxyl radicals and their by terminating lipid peroxidation chain reactions [11].Morever vitamin E has been reported to inhibit biosynthesis of prostaglandins in chickens [12]. Therefore tocopherols by virtue of their free radical scavenging property and suppression of prostaglandin biosynthesis could be expected to possess anti-inflammatory activity.

Literature survey in this regard revealed that vitamin E possesses anti-inflammatory activity against dextran induced inflammation [13] and lens induced uveitis in rats[14]. However, in another study it failed to show any significant change in uveitis induced by bovine albumin in rabbits[15].Surprisingly local injection of vitamin E in rat foot has been shown to induce inflammation[16].

In view of these inconsistent reports regarding the influence of vitamin E on inflammation the present study was planned to explore the effect of dl-alpha-tocopheryl acetate (a Vitamin E preparation) using acute and sub-acute models of inflammation in Wistar rats.

Information regarding the interaction of vitamin E with NSAID's like aspirin on inflammation is not well documented, though they have been reported to act synergistically on platelets [17].However dl-alpha-tocopherol in high doses (83-250 mg/kg B.W) along with aspirin (250 mg/kg) has been reported to produce synergistic anti-inflammatory effect in adjuvant induced arthritis in rats[18].In the present study therefore an attempt has also been made to explore the possible interactions of dl-alpha-tocopheryl acetate in its clinical equivalent doses with aspirin in respect of their anti-inflammatory activity in Wistar rats.

Similarly their analgesic activity and effect on gastric ulcer were also studied.

MATERIALS AND METHODS

Animals: The experiments were carried out using healthy male, adult rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to normal laboratory conditions with 12-hr natural light-dark cycle and were maintained on standard laboratory diet with free access to water.

Drugs used and their doses: With the help of converting table, adult clinical doses of the drugs were converted into rat equivalent doses [19].The drugs (with their adult therapeutic daily dose in parenthesis) used were dl-alpha-tocopheryl acetate 9 mg/kg (100 mg), 18 mg/kg(200 mg), 36 mg/kg(400 mg), 72 mg/kg(800 mg) [I.P. grade powder, courtesy Merck India Pvt. Ltd, Mumbai], aspirin 200 mg/kg (2 g).[I.P. grade powder, courtesy Swastic pharmaceuticals, Mumbai].For interaction studies, sub anti-inflammatory (SAI) dose of dl-alpha-

tocopheryl acetate (vitamin E) 9 mg/kg was combined with SAI dose of aspirin 54 mg/kg[20].

In acute studies all the treatments were administered to different groups of animals (n=6 in each) in a single dose, thirty minutes prior to subplantar injection of carrageenan while in sub acute studies the treatment was started after implanting the sterile foreign bodies and continued every 24 hours for 10 days. Control animals received equivalent volume of gum acacia suspension. All the drugs were administered orally as a suspension with 1% gum acacia.

Acute inflammation: Animals which were fasted overnight but had free access to water, were subdivided in to a control and 6 treatment groups to receive the dose (mg/kg) of aspirin 200, vitamin E in four doses viz., 9,18,36 and 72.Remaining one group received vitamin E-9 with aspirin-54. Acute inflammation was produced by subplantar injection of 0.05 ml of 1% carrageenan (from Sigma Co. St Louis) in left hind paw. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw volume was measured with the help of plethysmograph by mercury displacement method at 0,1,3 and 6 hour. The difference between 0 hour and subsequent reading was taken as actual edema volume.

Subacute inflammation: With some modification in the method of D'Arcy et.al subacute inflammation was produced in the rats[21]. In overnight starved rats after clipping the hair in axillae and groin, under light halothane anaesthesia, two sterile cotton pellets weighing 10 mg and two sterile grass piths (25x 3 mm) were implanted subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the procedure. The animals were subdivided in to five groups(n=6 in each) to receive vehicle, or the dose (mg/kg) of aspirin-200, vitamin E-36,vitamin E-72, vitamin E-9 with aspirin-54.The treatments were started after implantation and were repeated every twenty four hours, regularly for ten days.

With an overdose of anaesthesia, the rats were sacrificed on eleventh day to remove foreign body granulation tissue and stomachs. The pellets, free from extraneous tissue were dried overnight at 60° C to note their dry weight. Net granulation tissue formation was calculated by substracting initial weights of cotton pellet (10mg) from the weights noted and expressed as mg/100 gm of body weight. The grass piths were preserved in 10% formalin for histopathological studies.

Histopathological studies: Histopathological studies were conducted on the tissue covering the grass piths. After removal, the tissue was fixed in 10% formalin and processed to prepare paraffin blocks. Sections were taken from these blocks and stained with haematoxylin and eosin. The slides were studied under microscope.

Analgesic activity: For assessing analgesic activity Janssen's caudal immersion test as described by Turner[22] was adapted. The test was carried out in animals subjected for carrageenan induced inflammation. The reaction time as indicated by complete withdrawal of tail, was noted in various treated groups at an interval of 1, 3 and 6 hour after drug administration to calculate the mean reaction time.

Ulcer index: In gastric ulcer studies, the stomachs were cut open along the greater curvature and gently washed with normal saline. Gastric mucosa was examined for the presence of erosions, haemorrhagic spots, ulcer and perforation if any, with the help of magnifying lens. To determine the severity of the ulcer, an arbitrary scoring system as described earlier [23] was followed. Ulcer index was calculated as mean score of ulcer severity in all the treated groups and was compared with that of control.

All the experiments were performed in accordance with the CPCSEA (Committee for the Purpose of

Control and Supervision on Experiments on Animals) guidelines under Ministry of Animal Welfare Division, Government of India and the study was approved by IAEC (Institutional Animal Ethics Committee).

Statistical Analysis: Data were expressed as Mean ± SEM and were analysed by ANOVA followed by Dunnet's test and p ≤0.05 was considered as significant.

RESULTS

Carrageenan induced acute inflammation:

Except in the dose of 9 mg/kg, all the other therapeutic equivalent doses of dl-alpha-tocopheryl acetate viz., 18 mg/kg, 36 mg/kg, 72 mg/kg and aspirin (200 mg/kg) individually as well as combination treatment of SAI dose of dl-alpha-tocopheryl acetate with that of aspirin significantly ($p<0.05$,$p<0.01$ and $p<0.001$) inhibited paw edema at 1,3 and 6 hours (Table I).

Sub acute inflammation :

Therapeutic equivalent doses of aspirin and dl-alpha-tocopheryl acetate (36 and 72 mg/kg) individually as well as combination treatment of SAI dose of dl-alpha-tocopheryl acetate with that of aspirin decreased mean granulation tissue dry weight significantly ($p<0.05$,$p<0.001$) when compared with that of control (Table II).

Histopathological studies:

The tissue over the grass piths of all the five groups were subjected to histopathological studies. Sections stained with haematoxylin and eosin when observed under light microscope (40 X) revealed a decrease in the thickness of granulation tissue, proliferation of capillaries, infiltration of leucocytes and in the fibroblast number in all the treated groups when compared with that of control group (Fig I).

Analgesic studies:

Therapeutic equivalent dose of aspirin significantly ($p<0.05$,$p<0.001$) increased the mean reaction time to thermal stimulus at 1,3 and 6 hours. Therapeutic equivalent doses of dl-alpha-tocopheryl acetate viz.,18 mg/kg,36 mg/kg and 72 mg/kg as well as combination treatment with SAI doses of dl-alpha-tocopheryl acetate and aspirin significantly ($p<0.05$,$p<0.01$ & $p<0.001$) increased the mean reaction time at 1 and 3 hour (Table I).

Ulcer Index:

All the treatment groups except aspirin 200 mg/kg, did not increase the ulcer index significantly when compared with that of control (Table II).

Table I: Effect of various treatments on carrageenan induced rat paw edema and thermal pain (caudal immersion test).

Groups (n=6)	Drugs and Dose (mg/kg)	Paw volume in ml (Mean ± S.E)			Mean value in seconds (Mean ± S.E)		
		1 Hr	3 Hr	6 Hr	1 Hr	3 Hr	6 Hr
1	Control	0.62 ±0.05	0.78 ±0.09	0.92 ±0.14	2.20 ±0.09	2.05 ±0.03	2.32 ±0.16
2	Aspirin – 200	0.14*** ±0.05	0.15 *** ±0.02	0.11*** ±0.03	2.53 * ±0.12	2.50 *** ±0.9	2.76*** ±0.06
3	Vitamin E – 9	0.52 ±0.48	0.62 ±0.05	0.55 ±0.08	2.50 ±0.11	2.17 ±0.07	2.05 ±0.03
4	Vitamin E - 18	0.20*** ±0.05	0.22*** ±0.02	0.10*** ±0.03	2.53* ±0.10	2.57*** ±0.08	2.35 ±0.09
5	Vitamin E - 36	0.15*** ±0.05	0.10*** ±0.04	0.15*** ±0.04	2.45* ±0.04	2.50* ±0.16	2.45 ±0.10
6	Vitamin E - 72	0.12*** ±0.05	0.13*** ±0.61	0.07*** ±0.30	2.52* ±0.13	2.63** ±0.14	2.55 ±0.13
7	Vitamin E - 9 with Aspirin - 54	0.15*** ±0.06	0.25 *** ±0.04	0.18*** ±0.05	2.86* ±0.09	3.05* ±0.14	2.73 ±0.01

ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

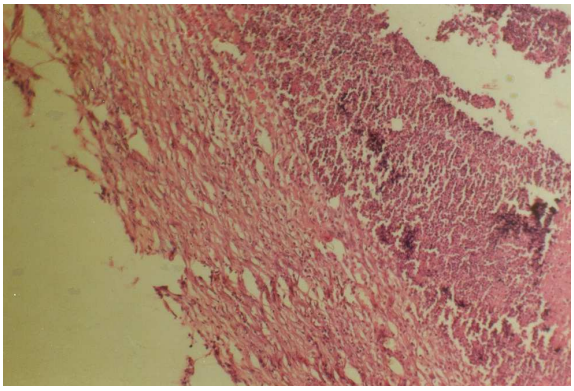
Table II: Effect of various treatments on foreign body induced granulation tissue and ulcer index.

Groups (n=6)	Drugs and Dose (mg/kg)	Granulation tissue dry weight (mg/100 g. B.W) (Mean± S.E)	Ulcer Index (Mean± S.E)
1	Control	58.37±3.58	30.00±6.32
2	Aspirin - 200	29.00±0.37***	40.00±0.01 **
3	Vitamin E - 36	49.80±0.98*	11.60±7.48
4	Vitamin E - 72	40.33±0.66***	11.66±7.40
5	Vitamin E - 9 with Aspirin - 54	33.50±0.34***	25.00±8.06

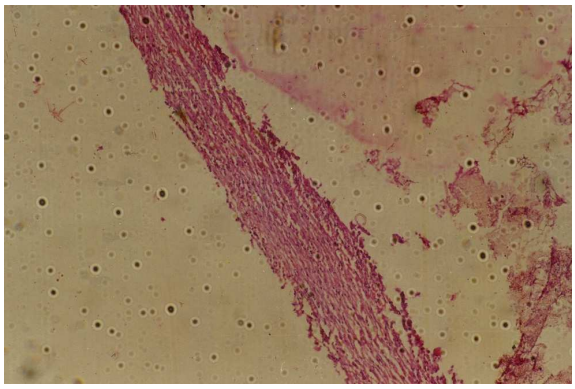
ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

Figure I: Microphotographs of granulation tissues stained with Haematoxylin & Eosin (H&E) (40 X)

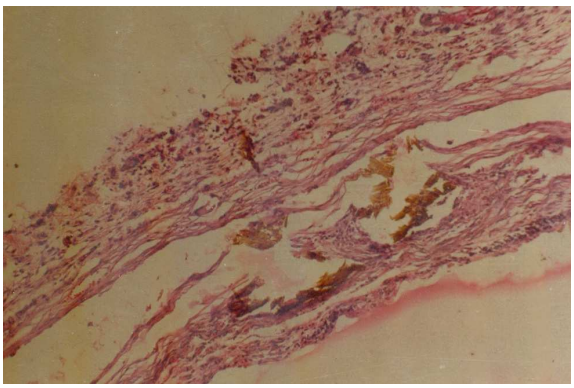
a) Control (vehicle)



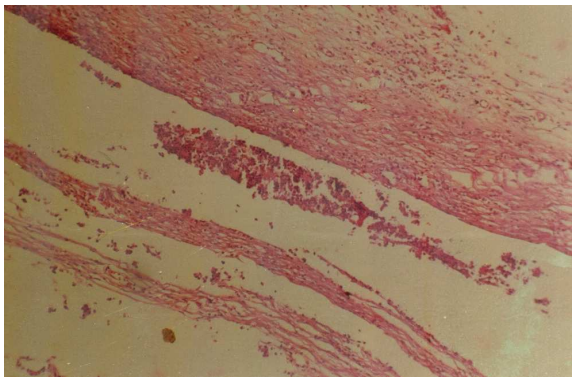
b) Aspirin (200 mg/kg)



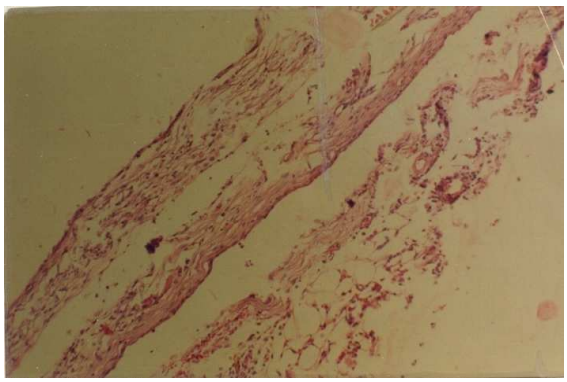
c) Vitamin E (36 mg/kg)



d) Vitamin E (72 mg/kg)



e) VitaminE (9 mg/kg) with aspirin (54mg/kg)



Note: All the treatment groups (b to e) revealed a decrease in the thickness of granulation tissue, proliferation of capillaries, infiltration of leucocytes and in the fibroblast number in all the treated groups when compared with that of control group (a).

DISCUSSION

Findings of the present study clearly indicate that vitamin E in the dose of 18,36 and 72 mg/kg suppressed inflammation significantly both in carrageenan as well as foreign body induced inflammation. These findings are in agreement with the earlier reports where vitamin E suppressed dextran induced inflammation[13] and cotton pellet granuloma formation[24].Another article indicates the anti-inflammatory activity of vitamin E in baboons[25].Inability of vitamin E in the dose of 9 mg/kg (lowest) to suppress inflammation in the present study is also in agreement with the earlier report that 10 mg of alpha tocopheryl failed to produce significant anti-inflammatory activity[13].

Combination of sub anti-inflammatory (SAI) dose of vitamin E (9 mg/kg) with SAI dose of aspirin (54 mg/kg) exerted significant anti-inflammatory activity which could be compared almost to that of aspirin (200 mg/kg).Such interactions involving Vitamin E on acute and subacute inflammation is not well documented. However, its synergistic interaction with large doses of aspirin[18] in chronic inflammation has been reported. The observed anti-inflammatory activity of vitamin E and its combination with aspirin in the present study were confirmed by histological studies on granulation tissues.

In the present study, except 9 mg/kg of vitamin E all other doses of vitamin E used (18,36 and 72 mg/kg) produced significant analgesic activity which could be compared to that of aspirin. Similarly vitamin E in combination with aspirin also produced significant analgesic activity.

Present findings clearly indicate that vitamin E not only potentiates analgesic activity of aspirin but also possesses analgesic activity by itself. Analgesic activity of vitamin E is poorly documented in the literature except a clinical study wherein vitamin E in the dose of 600 mg three times a day has been reported to reduce myalgia significantly[26].

Gastric mucosal studies in the animals treated with aspirin (200 mg/kg), vitamin E (36 and 72 mg/kg) and the combination of SAI dose of vitamin E with that of aspirin revealed, maximum ulcer index in aspirin treated group where as ulcer index in other treated groups did not differ significantly from that of control. The ulcerogenic property of aspirin is well documented, while the interaction of vitamin E with low dose of aspirin on gastric mucosa is poorly documented. While vitamin E has been shown to provide significant protection against NSAIDs induced gastric ulceration[27].

The present study was not intended to reveal the exact mechanisms involved in the present findings. Based on earlier reports about vitamin E, several mechanisms can be proposed to explain its anti-inflammatory activity such as free radicals scavenging[10,28],PG synthesis inhibition [12,29], inhibition of PAF synthesis [30], antagonism of PAF activity [31] and suppressions of phagocytic activity granulocytes[32].

The potentiation of anti-inflammatory activity of aspirin by dl-alpha-tocopheryl acetate appears to involve more of pharmacodynamic rather than pharmacokinetic interactions. Since the plasma levels of vitamin E and aspirin have not been monitored in the present study, pharmacokinetic interactions cannot be completely ruled out.

The analgesic activity of vitamin E could be due to its suppression of PG synthesis directly [12] or indirectly through scavenging the free radicals [33].

The gastroprotective effect of Vitamin E may be due to increased mucus production and interference with oxidative stress development by decreasing plasma and gastric mucosal malondialdehyde (MDA) levels [34].

CONCLUSIONS

The findings of the present study indicate the anti-inflammatory and analgesic activity of dl-alpha-tocopheryl acetate. In interaction studies, dl-alpha-tocopheryl acetate acts synergistically with aspirin towards reducing inflammation and pain. When co-administered, dl-alpha-tocopheryl acetate reduced the gastric ulcerogenic potential of the aspirin. If these findings are extrapolated to human beings, vitamin E can be used individually or else as an adjuvant to NSAIDs in the treatment inflammatory conditions. Clinical studies in this regard are really worthwhile.

REFERENCES

1) Holsapple MP, Malave A, Lowy MT,Yim GK.Central and peripheral anti-inflammatory actions by clonidine and a structurally related imidazoline analog. *J Pharmacol Exp Ther*.1984;229: 399-403.
2) Spector WG, Willoughby DA. Vasoactive amines in acute inflammation. *Ann N Y Acad Sci*. 1964;116:839–846.
3) Kasi Viswanadham C, Patil PA. Enhancement of anti-inflammatory activity of aspirin by verapamil. *Ind J Med Res*.1993;98(B):34-36.
4) McConkey B, Davies P, Crockson RA, Crockson AP, Butler M, Constable TJ, Amos RS. Effects of gold, dapsone, and prednisone on serum C-reactive protein and haptoglobin and the erythrocyte sedimentation rate in rheumatoid arthritis. *Ann Rheum Dis*.1979;38(2): 141–144.
5) Hiremath SV, Gouripur VV, Patil PA. The anti-inflammatory activity of some sulphonamides in albino rats. *Indian J Med Res.* 1996;103:120-125.
6) McCord JM, Wong K. Phagocyte-produced free radicals: roles in cytotoxicity and inflammation.*Ciba Found Symp.*1978;(65):343-360.
7) Winrow VR, Winyard PG, Morris CJ, Blake DR Free radicals in inflammation: second messengers and mediators of tissue destruction. *Br Med Bull* . 1993; 49: 506-522.
8) Mc Cord JM.Free radicals and inflammation: protection of synovial fluid by superoxide dismutase.*Science*. 1974;185(150):529-531.
9) Wannamethee SG, Lowe GDO, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. *Am J Clin Nutr*. 2006; 83:567–574.
10) Kaiser S**.** DiMascio P. Murphy ME. Sies H. Physical and chemical scavenging of singlet molecular oxygen by tocopherols. *Arch Biochem Biophys*.1990;277:101-108.
11) Cheesman KH**,** Slatter TF. An introduction to free radical biochemistry. *Brit Med Bull.* 1993**;** 49: 481-493.
12) Likoff RO, Guptill DR, Lawrence LM, McKay CC, Mathias MM, Nockels CF, Tengerdy RP Vitamin E and aspirin depress prostaglandins in protection of chickens against Escherichia coli infection. *Am J Clin Nutr.*1981;34(2):245-51.
13) Kamimura M. Antiinflammatory activity of vitamin E.*J Vitaminol.*1972;18(4):204-209.
14) Cid L, Pararajasegaram G, Sevanian A, Gauderman W, Romero JL, Marak GE, Jr, Rao NA. Anti-inflammatory effects of vitamin E on experimental lens-induced uveitis. *Int Ophthalmol.* 1992 ; 16(1):27–32.
15) Yücel I, Paksoy N, Yücel G, Aksu G, Aksu TA Effect of vitamin E in the treatment of bovine-albumin-induced uveitis in rabbits.*Ophthalmic Res.* 1992;24(3):129-133.
16) Berkenkopf JW, Lutsky BN. Vitamin E-induced chronic inflammation in rats. *Agents Actions.* 1979;9(4):350-357.
17) Steiner M. Effect of alpha-tocopherol administration on platelet function in man. *Thromb Haemost*. 1983;49(2):73–77.
18) Brandt K, Elmadfa I, Sobirey M, Schlotzer E. Effect of various combinations of DL-alpha-tocopherol and acetylsalicylic acid on adjuvant arthritis in the rat. *Z Rheumatol.* 1988;47(6):381-387.
19) Paget GE and Barnes JM., In evaluation of drug activities: Pharmacometrics, Vol.1, Eds.,Lawrence D.R. and Bacharach A.L., New York: Academic Press,1965,155.
20) Ravikumar, Patil PA, Majagi SI. Antiinflammatory and analgesic activity of dopamine antagonist and

their interaction with aspirin in wistar rats.*Pharmacologyonline.*2009;2:1358-1366.

21) Turner RA., Ed.,"Screening methods in pharmacology" New York and London: Academic Press Inc.,1965, pp.323.
22) Turner RA., Ed.,"Screening methods in pharmacology" New York and London: Academic Press Inc.,1965, pp.112-113.
23) Bhowmick S, Bose R, Pal M, Pal SP. Antiulcer activity on N-phthalolyl GABA-A new GABA mimetic agent.Indian.J.Exp.Biol.1990;28:190-192.
24) Yoshida K, Mizukawa H, Honmura A, Uchiyama Y, Nakajima S, Haruki E.The effect of sho-saiko-to on concentration of vitamin E in serum and on granuloma formation in carrageenan cotton pellet-induced granuloma rats. *Am J Chin Med.* 1994;22(2):183-189.
25) Wang XL, Rainwater DL, Mahaney MC, Stocker R. Cosupplementation with vitamin E and coenzyme Q10 reduces circulating markers of inflammation in baboons.*Am J Clin Nutr.*2004; 80(3):649-655
26) McLoughlin C, Elliott P, McCarthy G, Mirakhur RK. Muscle pains and biochemical changes following suxamethonium administration after six pretreatment regimens.*Anaesthesia.*1992; 47(3):202-206.
27) Javor T**,** Tarnok F, Past T, Nagy S. Cytoprotective effect of free radical scavengers against mucosal damage produced by different anti-rheumatic drugs. *Int J Tissue React*. 1986**;**8**:**35-40**.**
28) Hladovec J. Protective effect of oxygen-derived free radical scavengers on the endothelium in vivo. *Physiol Bohemoslov.*1986;35(2):97-103.
29) Hope WC, Dalton C, Machlin LJ, Filipski RJ, Vane FM. Influence of dietary vitamin E on prostaglandin biosynthesis in rat blood. *Prostaglandins.* 1975;10(4):557-567.
30) Kakishita E, Suehiro A, Oura Y, Nagai K. Inhibitory effect of vitamin E (alpha-tocopherol) on spontaneous platelet aggregation in whole blood. *Thromb Res.* 1990;60(6):489-499.
31) Violi F, Pratico D, Ghiselli A, Alessandri C, Iuliano L, Cordova C, Balsano F.Inhibition of cyclooxygenase-independent platelet aggregation by low vitamin E concentration. *Atherosclerosis.*1990;82(3):247-252.
32) Szczepańska I, Kwiatkowska J, Przybyszewski WM, Sitarska E, Malec J.Amelioration of hydroxyurea-induced suppression of phagocytosis in human granulocytes by free radical scavengers. *Scand J Haematol.* 1985;34(1):35-38.
33) Lands WEM. Actions of anti-inflammatory drugs. *TIPS.*1981; 2: 78-80.
34) Valcheva-Kuzmanova S, Krasnaliev I, Galunska B, Belcheva A.Influence of DL-alpha-tocopherol acetate on indomethacin-induced gastric mucosal injury in rats. *Auton Autacoid Pharmacol.* 2007;27(3):131-136.