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Pharmacological effect of combination therapy of Enalapril and Losartan on anti-inflammatory and antioxidant activity in albino rat

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***Abstract*---**Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activities through inhibition of cyclooxygenase (COX), the enzyme that makes prostaglandins (PGs). They share, to a greater or lesser degree, the same side effects, including gastric and renal toxicity. Recent research has shown that there are at least two COX isoenzymes. COX-1 is constitutive and makes PGs that protect the stomach and kidney from damage. COX-2 is induced by inflammatory stimuli, such as cytokines, and produces PGs that contribute to the pain and swelling of inflammation. Thus, selective COX-2 inhibitors should be anti-inflammatory without side effects on the kidney and stomach. Oxidative stress can affect vital molecules in human cells, including DNA and proteins, which are responsible for many processes in the body. In the human body, uncontrolled oxidation is commonly caused by highly reactive molecules known as free radicals. Free radicals, produced either by normal cellular metabolism or as an effect of pollution and exposure to other external factors, are responsible for premature aging of the body and play a pathogenic role in cardiovascular and degenerative diseases (e.g., cataracts, Alzheimer's disease, and cancer).

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Keywords---ACE inhibitors, Alzheimer's disease, Anti-inflammatory, Antioxidant, Cyclooxygenase, Cytotoxicity.

Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well-known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NASIDs and opiates [2]. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folk-loric used as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs [3]. A. vulgaris is commonly known as mugwort and it contains the constituent's volatile oil, flavonoids, a sesquiterpene lactone, coumarin derivatives, moxibustion and triterpenes [4]. Antioxidants are compounds that have capability of either delay or inhibit the oxidation processes. It occurs under the influence of oxygen or reactive oxygen species. Antioxidants are compound involved in the defence mechanism of the organism against the pathologies associated condition due to the attack of free radicals [5]. There are several benefits of antioxidants like antioxidants may boost our brain function, antioxidants decreases oxidative stress, antioxidants prevent cancer, promote liver health, treat urinary tract infection, can treat acne, delay aging, can help bodybuilders. Antioxidant prevent the free radical reaction and protect the muscles from being damaged [6]. Vitamin C have great role in tissue repair. The antioxidant activity of the drugs sample and the standard was measured on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity method. The stable DPPH radical method is a widely used, relatively quick, most accepted and precise method for the evaluation of the free radical scavenging activity of the drug sample [7]. The present work is undertaken to study the combined effect of ACE inhibitors on anti-inflammatory and antioxidant activity of animals for their establishment in AD. During literature survey I found that ACE inhibitor prevents memory impairment, oxidative stress. In present work the combination of ACE inhibitors will improve memory more than the individual.

Material and Methods

Animals for experimentation of in-vivo study

The in-vivo animal experimentation was performed on albino rats weight with 110-150 g and use individually for various experiments. Mice were utilize for learn all in-vivo capacity. The animals were domicile to animal quarters previous to testing at temperature of 25±2°C and 50±5% with relative humidity in polypropylene cages through a 12 hours light/dark cycle and allowable free of charge entrance to food and water. The experiments were achieve by subsequent rules and system of CPCSEA (Committee for the reason of Control and Supervision on Experimental Animals) approved by the IAEC, Bhopal.

In-vivo pharmacological screening for Memory enhancement activity

The drug powders i.e. enalapril and losartan potassium were evaluated for memory enhancement potentials using the following In-vivo models. These crude drug powders in various fractions were used for further study.

(a) Anti-inflammatory activity
(b) Antioxidant activity

Anti-inflammatory activity:

Animals

Adult male wistar rats (150-200 g) were employ to learning the anti-inflammatory movement. The animals having in five per cage were preserve in standard laboratory conditions having with access to food and water *ad libitum* with light period of 12 h/day and temperature 27 ± 2°C.

Procedure

The experimentation of anti-inflammatory activity was performed carrageenan-induced rat paw oedema method**.** The Male wistar rats (150-200 g) were arbitrarily dispersed into seven groups of five animals each. The first group acts as a control, second group act as the standard (containing as aceclofenac sodium 10 mg/kg, i.p). The third group of animals received enalapril 10 mg/kg body weight respectively. The fourth group of animals received enalapril 20 mg/kg, body weight. The fifth group of animals received losartan potassium 10 mg/kg body weight and the sixth group of animals received losartan potassium 20 mg/kg body weight. The seventh group of animals received enalapril (10 mg) and losartan potassium (10 mg) to get 20 mg/kg body weight. After 1 h, 0.1 ml of 1% w/v suspension of carrageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were calculated using plethysmometer (UGO Basile, 7140 Italy) each hour till 3 h after carrageenan injection [9-10]. The mean amount increase in paw volumes were noted, thus oedema volumes in control (Vc) and in groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated by using the formula

Percentage of inhibition = 100 (1-Vt / Vc)

Where, Vc = Edema volume in control and Vt = Edema volume in test/standard compound

Statistical analysis

The results are expressed as mean ± SEM. The statistical analysis was performed by analysis of variance (ANOVA) test.

Antioxidant activity (Superoxide scavenging)

Procedure: Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200 µl) was added to 2.8 ml of an aquous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (5-100 µg/ml) were added and absorbances were recorded at 560 nm against the control [11-12].

Result and Discussion

Anti-inflammatory activity

The result of enalapril and losartan potassium combination study against carrageenan-induced paw oedema. All the drug molecules in various doses gave significant (P < 0.01) reduction on rat paw oedema at predetermined time intervals. The combination of drugs was showed maximum inhibition of 54.58 % at the dose of 10 mg/kg after 2 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 56.72 % of inhibition. Carrageenan-induced paw oedema was applied as a prototype of exudative phase of acute inflammation during inflammation evaluation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin and the delayed phase is sustained by the leucotrienes and prostaglandins. Flavonoids and tannins are reported to inhibit PG synthesis. Most of the non-steroidal anti-inflammatory drugs (NSAIDs) have well balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity.

Table 1: in-vivo Anti-inflammatory activity animal study of drug material for Edema Volume

S. No.	Treatment	Dose	EV (ml) 1 h	EV (ml) 2 h	EV (ml) 3 h	EV (ml) 4 h
1	Control	-	1.79±0.61	1.63±0.33	1.59±0.24	1.51±0.11
2	Aceclofenac sodium	10 mg/kg	1.21±0.34	0.98±0.72	0.88±0.21	0.79±0.12
3	Enalapril	10 mg/kg	1.37±0.01	0.99±0.11	0.95±0.17	0.91±0.15
4	Enalapril	20 mg/kg	1.27±0.02	0.96±0.11	0.93±0.23	0.90±0.21
5	Losartan potassium	10 mg/kg	1.32±0.01	0.98±0.14	0.93±0.47	0.89±0.15
6	Losartan potassium	20 mg/kg	1.22±0.02	0.95±0.14	0.91±0.47	0.88±0.15
7	Enalapril: Losartan potassium (10mg:10mg)	20 mg/kg	1.11±0.21	0.92±0.11	0.86±0.47	0.84±0.15

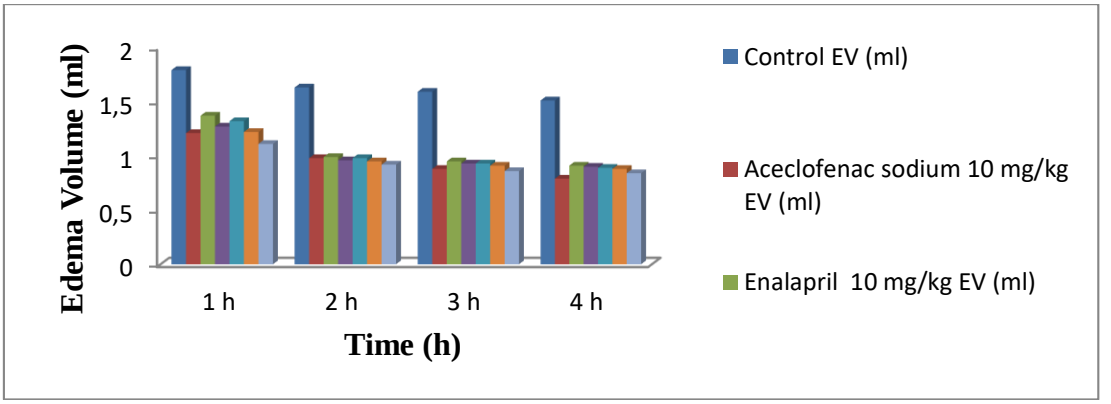

Figure 1: in-vivo Anti-inflammatory activity animal study of drug material for Edema Volume

Table 2: in-vivo Anti-inflammatory activity animal study of drug material for Edema Inhibition

S. No.	Treatment	Dose	EI (%) 1 h	EI (%) 2 h	EI (%) 3 h	EI (%) 4 h
1	Control	-	-	-	-	-
2	Aceclofenac sodium	10 mg/kg	36.18	53.72	60.70*	64.51
3	Enalapril	10 mg/kg	31.37	51.27	55.08*	59.75
4	Enalapril	20 mg/kg	37.17	56.11	57.01*	57.11
5	Losartan potassium	10 mg/kg	32.37	52.87	55.08*	61.01
6	Losartan potassium	20 mg/kg	38.17	56.27	58.01*	62.11
7	Enalapril : Losartan potassium (10mg:10mg)	20 mg/kg	42.37	58.99	61.58*	64.32

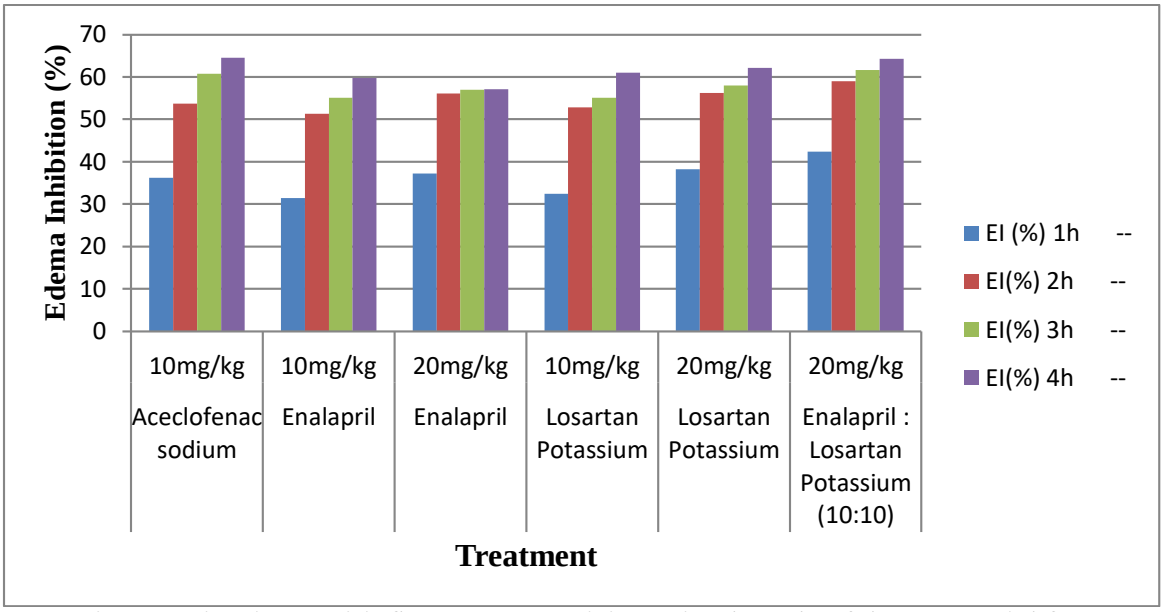

Figure 2: in-vivo Anti-inflammatory activity animal study of drug material for Edema Inhibition

Antioxidant activity (Superoxide scavenging)

Anti-oxidant activity by inhibition of DPPH radical

The potential decrease in the concentration of DPPH radial due to scavenging property of extract in combination of drugs and BHT showed significant free radical scavenging activity.

Table 3: in-vivo Antioxidant activity animal study of drug material (DPPH free radical inhibition)

S. No.	Drug material	Concentration (µg/ml)	DPPH free radical inhibition
1	Enalapril	5	12.11±0.13
2		10	32.06±0.11
3		25	47.21±0.04
4		50	60.12±0.19
5		100	71.10±0.14
6	Losartan potassium	5	13.21±0.11
7		10	33.26±0.14
8		25	49.23±0.14
9		50	62.52±0.09
10		100	74.14±0.04
11	Enalapril : Losartan potassium (1:1)	5	15.23±0.13
12		10	35.16±0.04
13		25	51.63±0.11
14		50	65.82±0.09
15		100	77.24±0.14

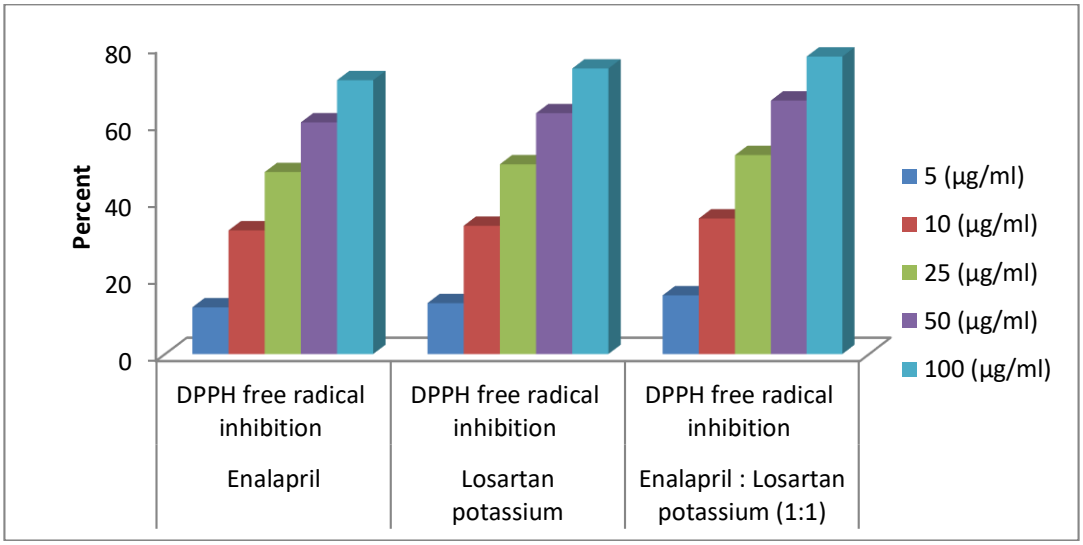

Figure 3: in-vivo Antioxidant activity animal study of drug material (DPPH free radical inhibition)

Table 4: in-vivo Antioxidant activity animal study of drug Nitric oxide inhibition

S. No.	Drug material	Concentration (µg/ml)	Nitric oxide
1	Enalapril	5	10.11±0.01
2		10	26.01±0.13
3		25	40.03±0.01
4		50	58.12±0.06
5		100	67.01±0.11
6	Losartan potassium	5	11.18±0.11
7		10	27.11±0.03
8		25	41.13±0.11
9		50	59.02±0.16
10		100	68.21±0.01
11	Enalapril: Losartan potassium (1:1)	5	12.18±0.11
12		10	29.31±0.13
13		25	44.03±0.12
14		50	61.52±0.06
15		100	71.11±0.21

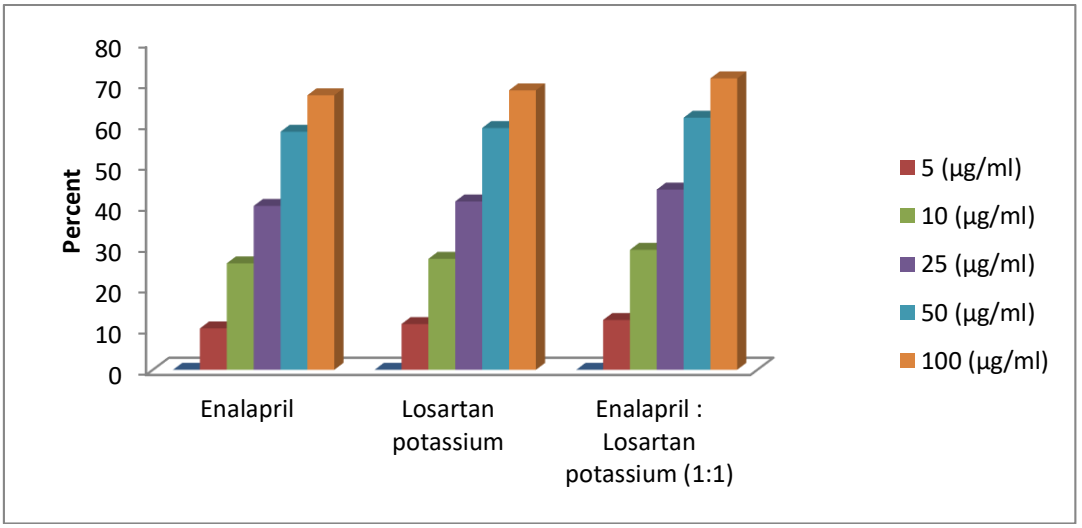

Figure 4: in-vivo Antioxidant activity animal study of drug material (Nitric oxide inhibition)

Table 5: in-vivo Antioxidant activity animal study of standard solution of Butylated hydroxytoluene (BHT)

S. No.	Standard solution	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
1	Butylated hydroxytoluene (BHT)	25	41.17±0.11	34.13±0.18
2		50	75.62±0.21	71.42±0.06
3		100	87.03±0.12	79.11±0.11

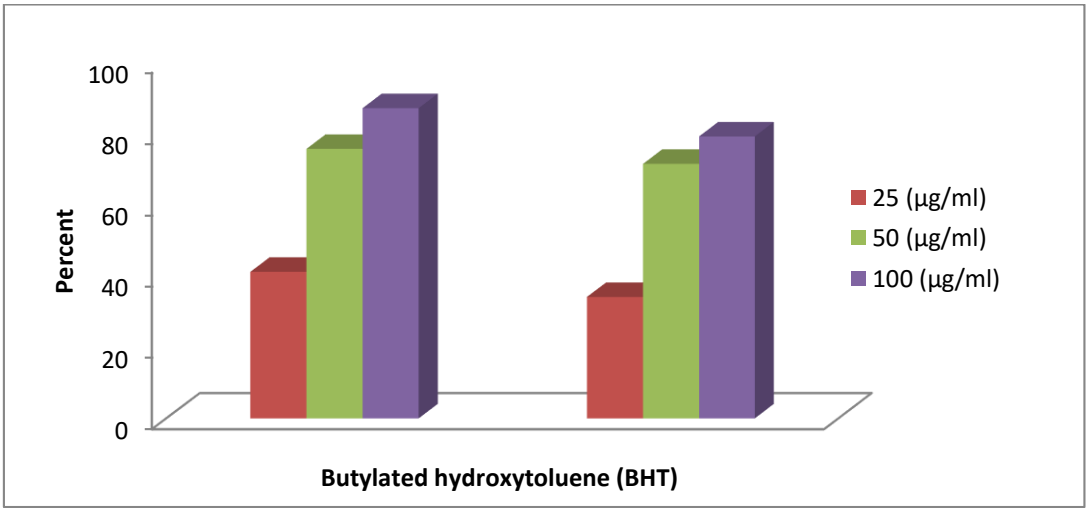

Figure 5: in-vivo Antioxidant activity animal study of standard solution of Butylated hydroxytoluene (BHT)

Summary and Conclusion

The results of both drug enalapril and losartan potassium combination study against carrageenan-induced paw oedema. All the drug molecules in various doses gave significant ($P < 0.01$) reduction on rat paw oedema at predetermined time intervals. The combination of drugs was showed maximum inhibition of 54.58 % at the dose of 10 mg/kg after 2 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 56.72 % of inhibition. Carrageenan-induced paw oedema was applied as a prototype of exudative phase of acute inflammation during inflammation evaluation. The potential decrease in the concentration of DPPH radial due to scavenging property of extract in combination of drugs and BHT showed significant free radical scavenging activity.

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