Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Vol.5, No.4, 2023, pp - 01 - 13

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Research Paper

Effect of Eugenol – An Active Component of Clove Oil - On Platelet Aggregation and Lipid Peroxidation in Healthy Individuals

Mehboob R. Chippa[1], Y.K.Bolya[1], S.K.Verma[2]*

[1]Department of Medicine
R.N.T. Medical College, Udaipur, Rajasthan, Bharat

[2]Department of Medicine
Pacific Medical College and Hospital, Udaipur, Rajasthan, Bharat

***Corresponding Author Email:** drskverma77@gmail.com

ABSTRACT

The present study was designed to observe the effect of different doses of eugenol on platelet aggregation and lipid peroxidation in healthy individuals.

The study was conducted on 30 healthy individuals divided in two groups. In group I (N = 15), the effect of single dose of eugenol was observed. The group was further divided into three sub-groups of five each and given plain eugenol in 10 mg/kg, 15 mg/kg and 20 mg/kg of body weight respectively. The effect of plain eugenol observed was compared with the effect of placebo given one day prior to eugenol on platelet aggregation and lipoprotein oxidation susceptibility. Group II (N=15) was similarly subdivided and eugenol was administered in 10, 15 and 20 mg/kg of body weight in single dose. But in this group eugenol was administered with 25 gm of butter and the effect was compared with butter and placebo given one day prior to the fat and eugenol. In both groups blood samples were collected initially and ninety minutes after ingestion of eugenol or placebo with or without fat and analysed for platelet aggregation and lipid peroxidation.

It has been observed that administration of eugenol in different doses to healthy volunteers variably affect platelet aggregation induced by ADP and epinephrine. The magnitude of responsiveness increases as the dose is increased from 10 mg/kg to 20 mg/kg body weight and at higher dosage (20 mg/kg) it is statistically significant ($P<0.05$). However, lipid peroxidation remains unaltered even in higher doses.

Similarly, in group II, when fat was administered with eugenol, the response of platelet towards aggregating agents remained same as in plain eugenol group. Here also the response is dose related and statistically significant ($P < 0.05$) at 20 mg/kg body weight dose. Lipoprotein oxidation susceptibility however remains significantly unaltered in all three doses schedules.

In both the study groups, therefore one observation is common, that eugenol has some potential of lowering platelet aggregation, but the effect is greatly modified by the doses administered. Secondly, as the effect is more or less same in both the groups the absorption of eugenol is practically same and not affected by fat.

KEYWORDS: Acetyl eugenol, Clove oil, Spices, Antioxidant

INTRODUCTION

Herbs have been extensively used in Ayurvedic, Unani and Chinese system of medicine for centuries. The Great Indian Physician Charak had described the medicinal value of more than 1500 plants. Voluminous literature on herbal medicine is available in books like Charak Samhita, Sushruta Samhita, Dhanvantri and Indian materia-medica. Incidentally many of these plants in some form or other are in common use. Few of them are used as condiments while others for the purpose of treating various diseases. Clove has been used as a spice and medicine from early times by Chinese and Indians for various ailments[1].

Spices have been consumed in mostly all the cultures over centuries. It is common assumption that their consumption is related to taste and aroma. However, there is more to spices consumption than mere taste or smell.

In recent times many spices have made cross cultural penetrations which in turn has generated curiosity about their biological effects, mode of action and isolation of active components. Garlic, onion, ginger, clove and curcumin are the few examples. They were used by ancient civilizations millenniaago and were known to their various medicinal effects in these cultures. Yet scientific evidence confirming their medicinal properties have been provided only recently[2-8]. Spices are now known to possess antithrombotic, anti-sclerotic, hypoglycemic, hypolipedemic, anti-inflammatory, anti-arthritic and platelets aggregation inhibiting properties[2-9].

The spices form a part of daily diet in the Indian subcontinent and the Far East. As they were found to influence the oxygenation of arachidonic acid, their consumption might have preventive effect in diseases where elevated levels of ecosanoids have been reported. The spices contribute in preventing the occurrence of diseases mediated by peroxidation of lipids as low incidence of cardiovascular diseases. Inflammatory disorders etc. is incidentally observed in regions where spices are consumed regularly. Dietary intake of natural antioxidants some of which might originate from spices could be an important aspect of body's defence mechanisms against the above mentioned diseases in such populations. Many anti-oxidants have been lately identified as anticarcinogens. Plant xenobiotics may be important in inhibiting carcinogen induced tumorigenesis in humans. Eugenol may be one such compound[10].

Clove (*Syzygium aromaticum*) is rich in volatile compounds and antioxidants such as eugenol, B-caryophyllin and alpha-Lumulene. Eugenol is the major compound accounting for at least 50% while remaining 10-40% consists of eugenol acetate, B-caryophyllin and alpha-Lumulene. Besides its application in the perfume, cosmetic, flavouring, as a food spice, it does have biological activity relevant to human health such as antimicrobial, antioxidant, insecticidal, anti-inflammatory, analgesic, anticancer etc.11 (Haro-Gonjalez.Molecules 2021; 26:6387-6411)

The antiplatelet components eugenol and acetyl eugenol have been isolated and identified. They were potent in inhibiting the platelet aggregation. Their inhibitory effect was reversible. They inhibited arachidonate, adrenaline and collagen induced platelet aggregation[12].

Eugenol (4 Allyl-2 methoxy phenol), is a naturally occurring phenolic compound. It is a major component of clove oil and is also present in oils of cinnamon, basil and nutmeg. It is used mainly as flavouring agent up to a level of 0.01 per cent in foods such as baked products, beverages, sweets and frozen dairy products. It is extensively used along with zinc oxide as a component of several dental materials. [Joint F.A.O./W.H.O. Expert Committee on food additives has established a conditional acceptable daily intake (ADI) of eugenol up to 5 mg/kg for humans[13]. It is considered non-mutagenic, non-carcinogenic and generally recognized. as a safe (GRAS) and is approved by FDA[14]]

Eugenol and acetyleugenol are the known substances present in the clove. Though clove has traditionally been used as aromatic and stomachic, its pharmacological study is meagre. It has been shown that clove and its active principle possess cholagogue effect[15]. The inhibition by eugenol of glucuronic acid conjugation in the stomach of rats and guinea pigs and of dogs may have some bearing on reported mucinogenic activity of eugenol and its beneficial effect in gastric ulcer formation[16-17]. Eugenol also inhibited tumour promotion caused by Anthracene derivatives in mice[18].

Lately, eugenol has been subjected to some more scientific scrutiny. It was found to be anti- aggregatory in ex-vivo experiments. It abolishes arachidonate induced aggregations and second phase of epinephrine induced aggregation. Inhibition of aggregation seemed to be mediated by a reduced formation of thromboxane and increased formation of 12-hydro- peroxy-eicoso-tetraenoic acid (HPETE). It was inferred that possibly some enzymes of arachidonic acid cascade was inhibited by eugenol[10].

In animal experimental study eugenol and acetyl eugenol (100 mg/kg) given orally to rabbits, decreased significantly formation of thromboxane B2 during blood clotting. It has more pronounced inhibiting action 92±6 per cent and biological half life 72±30 minutes. Acetyl eugenol is less active. Eugenol remains most active compound and has reversible action on plateletfunction[19].

Antioxidant activity of eugenol has also been evaluated in vivo, at a concentration similar to recommended level of dietary intake (2.5 mg/kg body weight)[20]. Eugenol inhibited superoxide formation and lipid peroxidation and the radical scavenging activity may be responsible for its chemopreventive action[18]. Lipid peroxidation is known to be initiated by reactive oxygen species[21].

On the basis of above in vitro and ex-vivo studies, it is evident that eugenol is an important naturally occurring phenolic compound to possess strong antioxidant and platelet aggregation inhibiting property. However there is no human study available so far as the literature is concerned regarding the effect of eugenol on platelet aggregation and lipoprotein oxidation susceptibility.

In view of these observations it is considered pertinent to observe the effect of eugenol on platelet aggregation and lipid peroxidation in healthy individuals.

MATERIALAND METHODS

The present study was conducted on 30 male volunteers between age 25-40 years who were non-smoker, nontobacco chewer and not taking any non-steroidal anti-inflammatory drugs for last 15 days. Hypertension, diabetes mellitus and coronary artery disease were excluded by detailed clinical history and relevant laboratory investigations. The study was approved by institutional ethical committee and after informed consent the selected individuals were divided into two groups of 15 each.

Group I (N=15) - Eugenol group

Group II (N=15) - Fat + Eugenol group.

Group I (Eugenol group):

After an overnight fast first blood sample was collected. They were then randomly divided into three subgroups of 5 each and received eugenol on one occasion and placebo on other occasion in different doses.

Group IA (N = 5) - Eugenol 10 mg/kg body Wt. orally

Group IB (N = 5) - Eugenol 15 mg/Kg body wt. orally

Group IC (N=5) - Eugenol 20 mg/kg body wt. orally.

Second blood sample was collected 90 minutes after administration of eugenol or placebo.

Group II (Fat + Eugenol group):

This group was also randomly divided into 3 sub-groups of five each as in group 1 and also received eugenol in same three different doses schedules. But this group in addition received 25 gms. of butter with four pieces of breads with eugenol.

Group IIA (N = 5) - fat + 10 mg/kg body wt. eugenol orally.

Group IIB (N = 5) - fat + 15 mg/kg body wt. eugenol orally.

Group IC (N = 5) - fat + 20 mg/kg body wt. eugenol orally.

On the first day, initially fasting blood sample was collected and then 25 gms. butter with four pieces of bread was fed. Second sample was collected after 90 minutes. On the second day same procedure was repeated but in addition of different doses of eugenol with bread and butter.

All the blood samples were subjected for platelet aggregation using ADP and Epinephrine as aggregating agent and lipid peroxidation.

Preparation of eugenol capsules:

In present study eugenol supplied by Sigma (USA) was used. The calculated amount of eugenol was mixed with lactose powder filled in gelatine capsule. The placebo contained only lactose powder and was given in the same doses.

Platelet aggregation

Platelet-rich plasma (PRP) and Platelet-poor plasma (PPP) was obtained by differential centrifugation. Platelet aggregation was measured turbidimetrically on ELVI-840 aggregometer and Omniscribe chart recorder. The measurement of aggregation was performed exactly after 30 minutes of sample collection to avoid differences of the aggregation due to altered status of the platelet resulting from ex-vivo conditions. After 10 minutes of equilibrium at 37°C and constant stirring at 1,000 rpm, the aggregation of PRP was induced by ADP or epinephrine (Sigma), the response was recorded for 5 minutes, and the results were expressed as percentage aggregation[22].

Lipoprotein Oxidation Susceptibility Test (LOS):

The LOS was measured based on the principle that the hydrolysis of plasma lipoperoxide forms MDA (Malondialdehyde) which reacts with thiobarbituric acid to form a red 2:1 Thiobarbituric acid Malondialdehyde adduct, this coloured complex absorbs maximally at 532 nm and optical density will be measured by spectrophotometry [23].

Reagents used:

1. Dextran sulphate
2. Copper chloride
3. Magnesium chloride
4. Bovine albumin
5. Barbituric acid
6. Trichloracetic acid
7. Hydrochloric acid
8. Sodium chloride
9. n-Butanol.

Reagents:

1. **Dextran sulphate:** 1.0 gm of dextran sulphate was dissolved in 100 ml of distilled water and 0.2 mM of solution of dextrans sulphate was prepared.
2. **Copper chloride:** 8.52 mg of copper chloride dissolved in 100 ml of distilled water. 0.5 nm of copper chloride was prepared.
3. **Magnesium chloride:** 10.2 mg of magnesium chloride dissolved in 100 ml of distilled water and 0.5 M magnesium chloride solution was prepared.
4. **Bovine albumin:** 3 gm of bovine albumin dissolved in 50 ml of distilled water thus 6.0 per cent of bovine albumin solution was prepared.
5. **Hydrochloric acid:** 2.2 ml of HCL dissolved in distilled water and 0.25 N of HCI solution was prepared.

6. **TBARS reagent:** This reagent contained 26 nm of Thiobarbituric acid (TBA) and 0.92 M trichloroacetic acid in 0.25N HCI. The TBA was added first, heated and stirred. After it was dissolved, the TCA was added to the solution and was brought to volume with HCI. This reagent was stored in a dark bottle at room temperature.
7. **Sodium chloride:** 4 gm of sodium chloride dissolved in 100 ml of distilled water to give 4.0 per cent of sodium chloride solution.

Procedure:

Step I: LOS test serum (not plasma) was used in this test. A 500 µ lit serum sample was treated with 50µ lit solution containing 0.2 nm dextran sulphate (MW = 50,000 Genzyme, Cambridge MA) and 0.5 M $MgCl_2.H_2O$ to precipitate the apo. B containing lipoproteins (LDL and VLDL).

Step II: After centrifugation at 3,000 rpm at 20°C for 10 minutes. The supernatant was removed.

Step III: 1 ml of 6% bovine serum albumin (BSA) and another 50µ lit. of dextran sulphate magnesium solution was added.

Step IV: Solution was vortexed and recentrifuged as above to wash away any residual serum proteins and HDL.

Step V: The supernatant was again removed and the remaining precipitate (containing LDL and VLDL) was dissolved in 4% NACI.

Step VI: A volume of redissolved precipitate containing 100 mg of non-HDL cholesterol was combined with sufficient 4 % NaCl to give a total volume 500µ lit.

Step VII: 50µ lit of 0.5 mM CuCl2.2H2O solution was added (Final Copper Concentration was 46 µM).

Step VIII: Samples were incubated at 37°C in a shaking water bath for 3 hours.

Step IX: Next 2 ml of TBARS reagent was added to each tube.

Step X: The mixture was heated at 100°C in a water bath for 15 minutes.

Step XI: After removing and cooling the tubes 2.5 ml n-Butanol was added. The tubes were vortexed and then centrifuged for 15 minutes at 3,000 rpm at room temperature.

Step XII: The pink upper layer was removed and the optical density was determined in a spectrophotometer at 532 nm.

The coefficient of variation of method was 4% intra assay and 9% inter assay.

Normal value: 66±22 nmol. MDA/mg. of non HDL-C.

Statistical Analysis

All data are expressed as mean ± SE. The results were analyzed with Student's t test for paired data. p value of <0.05 was considered statistically significant[24].

RESULTS

TABLE-1
EFFECT OF PLACEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP I-A

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after placebo	Initial	90 minutes after placebo	Initial	90 minutes after placebo
Mean	62.00	64.25	60.25	60.50	51.00	53.00
% Change	-	3.62	-	0.4149	-	3.92
SD ±	10.9807	8.7766	8.5421	9.6257	8.9442	4.4721
SE ±	4.9108	3.9251	3.8202	4.3048	4.0000	2.0000
p		NS		NS		NS

P = As compared with initial

NS = Not significant

Administration of placebo in 5 healthy individuals led to alter ADP induced platelet aggregation from mean level of 62± 10.98 to 64 ± 8.77 per cent and was statistically not significant. Similarly the platelet aggregation induced by epinephrine was also remained unaffected (P=NS). Administration of placebo in 5 healthy individuals led to change in lipid peroxidation from a mean of 51±8.94 to 53±4.7 nmol MDA/mg non-HDL-C which was statistically not significant. (Table 1)

TABLE-2

EFFECT OF SINGLE DOSE (10 mg/kg.) OF EUGENOL ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS

GROUP I-A

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after Eugenol (10mg/kg)	Initial	90 minutes after Eugenol (10mg/kg)	Initial	90 minutes after Eugenol (10mg/kg)
Mean	66.75	67.75	60.75	61.25	47.00	47.40
% Change	-	1.4981	-	0.8230	-	0.8510
SD ±	5.9027	4.1963	4.2019	5.5198	8.3666	6.4265
SE ±	2.6398	1.8766	1.8792	2.4686	3.7417	2.8741
p		NS		NS		NS

P = As compared with initial

NS = Not significant

The effect of single dose (10 mg/Kg BW) of eugenol on platelet aggregation and lipid peroxidation on healthy individuals showed that the platelet aggregation induced by ADP and epinephrine did not change significantly. Similarly, lipid peroxidation did not alter to any significant extent. (Table 2)

TABLE-3

EFFECT OF FAT (25 gm) PLUS PLACEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS

GROUP II-A

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat + placebo	Initial	90 minutes after fat + placebo	Initial	90 minutes after fat + placebo
Mean	63.50	66.00	54.25	55.25	54.00	54.00
% Change	-	3.9370	-	1.8433	-	0.00
SD ±	12.099	7.0378	18.0286	18.7374	11.2249	8.9492
SE ±	5.4110	3.1474	8.0628	8.3798	5.020	4.000
p		NS		NS		NS

P = As compared with initial

NS = Not significant

The effect of 25 gm fat plus placebo administration in healthy individuals altered ADP and epinephrine induced platelet aggregation from a mean of 63.50 ± 12.09 to 66.07 ± 7.03 per cent and 54.25 ± 18.02 to 55.25 ± 18.73 per cent respectively, which was statistically not significant. Likewise no significant change was observed in lipoprotein susceptibility (LOS) after fat administration. (Table 3)

TABLE-4
EFFECT OF SINGLE DOSE OF FAT PLUS EUGENOL (10 mg/kg) ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS GROUP II-A

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat + eugenol	Initial	90 minutes after fat + eugenol	Initial	90 minutes after fat + eugenol
Mean	53.25	53.75	47.50	48.25	53.00	52.00
% Change	-	0.9389		1.5789	-	1.8867
SD ±	13.1576	11.4222	6.1870	7.4791	9.7467	10.9544
SE ±	5.8844	5.1083	2.7669	3.3448	4.3589	4.8991
p		NS		NS		NS

P = As compared with initial

NS = Not significant

After 90 minutes of administration of fat ± eugenol (10 mg/kg), ADP and epinephrine induced platelet aggregation showed a change of mean from 53.25 ± 13.15 to 53.75 ± 11.42 per cent and 47.50 ± 6.18 to 48.25 ± 7.47 per cent respectively which was not significant statistically. Similarly, lipid peroxidation decreased from a mean of 53 9.74 to 52 ± 10.95 nmol MDA/mg of non HDL-C which is around 1 per cent and was not significant statistically. (Table 4)

TABLE-5
EFFECT OF PLACEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS GROUP I-B

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after placebo	Initial	90 minutes after placebo	Initial	90 minutes after placebo
Mean	58.70	61.45	58.84	62.59	52.00	48.00
% Change	-	4.6848	-	6.3732	-	7.6923
SD ±	10.8489	6.7137	10.9200	13.6243	7.5828	5.7008
SE ±	4.8519	3.0025	4.8837	6.0931	3.3912	2.5495
p		NS		NS		NS

P = As compared with initial

NS = Not significant

Single dose of placebo, increased ADP and epinephrine induced platelet aggregation from a mean of 58.70 ± 10.84 to 61.45 ± 6.71 and 58.84 ± 10.92 to 62.59±13.62 per cent respectively, which was not significant statistically. Similarly placebo reduced lipid peroxidation from a mean of 52.00 ± 7.58 to 48.00 ± 5.70 nmol MDA/mg non-HDL-C which was also not significant statistically (Table 5).

TABLE-6

EFFECT OF SINGLE DOSE OF EUGENOL (15 mg/kg.) ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP I-B

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after eugenol	Initial	90 minutes after eugenol	Initial	90 minutes after eugenol
Mean	68.7	66.50	65.95	66.50	54.00	54.60
% change	-	3.2727	-	0.8339	-	1.1111
SD ±	5.1536	7.9744	6.4591	6.5192	6.3835	10.5261
SE ±	2.3048	3.5663	2.8886	2.9155	2.8570	4.7075
p		NS		NS		NS

P = As compared with initial

NS = Not significant

Administration of single dose (15 mg/kg BW) of eugenol in healthy individuals decreased ADP induced platelet aggregation was from a mean of 68.75 ± 5.15 to 66.5 ± 7.97 per cent which is around 3.27 per cent which was statistically not significant. However, epinephrine induced platelet aggregation remained unaffected. Lipid peroxidation was altered by 1.1 per cent from a mean of 54 ± 6.38 to 54.6 ± 10.52 nmol MDA/mg non-HDL-C and was not significant. (Table 6)

TABLE-7

EFFECT OF FAT (25 gm) PLUS PLACEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP II-B

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat+placebo	Initial	90 minutes after fat+placebo	Initial	90 minutes after fat+placebo
Mean	56.65	58.30	48.65	58.68	53.00	54.00
% Change	-	2.9126	-	20.6166	-	1.8867
SD ±	13.9379	7.6472	13.6959	19.4408	12.0415	19.4935
SE ±	6.2334	3.4200	6.1251	8.6944	5.3852	8.7180
p		NS		NS		NS

P = As compared with initial

NS = Not significant

ADP and epinephrine induced platelet aggregation altered from the mean of 56.65±13.93 to 58.30 ± 7.64 and 48.65 ± 13.6 to 58.68± 19.44 per cent respectively, which was statistically not significant (P = NS). Similarly lipid peroxidation altered from 53 ± 12.04 to 54.00 ± 19.49 which is about 1.88 per cent and was also not significant statistically (Table 7)

TABLE-8
EFFECT OF FAT PLUS EUGENOL (15 mg/kg BW) ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP II-B

Case no.	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat+eugenol	Initial	90 minutes after fat+eugenol	Initial	90 minutes after fat+eugenol
Mean	68.75	63.90	54.75	52.50	54.00	60.00
% Change	-	7.0545	-	4.1095	-	11.1111
SD ±	8.4731	6.2962	7.9252	6.4346	11.4017	15.8113
SE ±	3.7894	2.8158	3.5443	2.8777	5.0991	7.0712
p		NS		NS		NS

P = As compared with initial

NS = Not significant

Administration of single dose (15 mg/kg) of eugenol fed with 25 gm of fat in 5 healthy individuals. Both concomitantly reduced ADP and epinephrine induced platelet aggregation from a mean of 68.75 ± 8.47 to 63.9 ± 6.29 and 54.75 ±7.92 to 52.5 ± 6.43 per cent respectively. It is evident that ADP induced platelet aggregation was reduced to marked extent about 7.0545 per cent. Same dose of fat plus eugenol altered lipid peroxidation from a mean of 54.00 ± 11.40 to 60.00 ± 15.80 nmol MDA/mg non HDL-C which is around 11.1 per cent and statistically not significant.(Table 8)

TABLE-9
EFFECT OF PLACEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP I-C

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after placebo	Initial	90 minutes after placebo	Initial	90 minutes after placebo
Mean	58.95	59.75	55.65	56.75	48.40	47.60
% Change	-	1.3570	-	1.9766	-	1.6528
SD ±	8.5920	11.7614	11.6250	7.5684	8.6775	5.5946
SE ±	3.8425	5.2600	5.1990	3.3847	3.8808	2.5020
p		NS		NS		NS

P = As compared with initial

NS = Not significant

ADP and epinephrine induced platelet aggregation showed 1.35 per cent and 1.97 per cent change which was not significant statistically. On the other hand lipid peroxidation was reduced from 48.40 ± 8.67 to 47.60 ± 5.59 nmol MDA/mg nonHDL-C. However it was not significant. (Table 9)

TABLE-10
EFFECT OF SINGLE DOSE (20 mg/kg) OF EUGENOL ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP I-C

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after eugenol	Initial	90 minutes after eugenol	Initial	90 minutes after eugenol
Mean	68.50	59.50	64.50	65.25	55.00	56.00
% Change	-	13.13	-	1.1627	-	1.8181
SD ±	13.8654	13.5092	9.7867	9.3706	10.00	13.8744
SE ±	6.2009	6.0417	4.3768	4.1907	4.4722	6.2050
p		< 0.05		NS		NS

P = As compared with initial

NS = Not significant

Effect of single dose (20 mg/kg BW) of eugenol has been shown in Table 10 on platelet aggregation and lipid peroxidation in healthy individuals. ADP induced platelet aggregation decreased from a mean 68.50 ± 13.86 to 59.50 ± 13.50 per cent which is statistically significant ($p < 0.05$). However, epinephrine induced platelet aggregation remain unaffected. Alteration in lipid peroxidation was statistically not significant.

TABLE-11
EFFECT OF FAT (25 gm) PLUS PLECEBO ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP II-C

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat+ placebo	Initial	90 minutes after fat+ placebo	Initial	90 minutes after fat+ placebo
Mean	57.55	57.10	58.60	59.25	55.00	54.00
% Change	-	0.7819	-	1.1092	-	1.8181
SD ±	10.2077	3.9314	9.7397	7.6013	14.1421	11.4017
SE ±	4.565	1.7582	4.3558	3.3995	6.3247	5.0991
p		NS		NS		NS

P = As compared with initial

NS = Not significant

ADP and epinephrine induced platelet aggregation varied from 57.55 ± 10.20 to 57.10 ± 3.93 per cent and from 58.6 ± 9.73 to 59.28 ± 7.60 per cent respectively which were not significant. Lipid peroxidation also decreased from a mean of 55±14.14 to 54±11.40 nmol MDA/mg non HDL-C but was also not significant. (Table 11)

TABLE-12
EFFECT OF SINGLE DOSE OF FAT PLUS EUGENOL (20 mg/kg.) ON PLATELET AGGREGATION AND LIPID PEROXIDATION IN HEALTHY INDIVIDUALS
GROUP II-C

	PLATELET AGGREGATION (PERCENT)				Lipid peroxidation susceptibility (nmol MDA/mg of non-HDL-C)	
	ADP		Epinephrine			
	Initial	90 minutes after fat+ eugenol	Initial	90 minutes after fat+ eugenol	Initial	90 minutes after fat+ eugenol
Mean	59.50	53.50	60.25	60.00	58.00	56.00
%change	-	10.0840	-	0.4149	-	3.4482
SD ±	13.5091	12.1642	7.0377	7.0710	8.3666	11.4017
SE ±	6.0416	5.4401	3.1474	3.1623	3.7417	5.0991
p		<0.05		NS		NS

P = As compared with initial

NS = Not significant

After single dose administration of fat (25 gm) plus eugenol (20 mg/kg BW) in 5 healthy individuals lead to reduce ADP induced platelet aggregation up to 10.08 per cent from a mean of 59.5 ± 13.5 to 53.5 ± 12.16 per cent and was statistically significant ($p < 0.05$). However epinephrine induced platelet aggregation remained almost unaffected from a mean of 60.25 ± 7.03 to 60.0 ± 7.07 per cent. Lipid peroxidation also decreased from a mean of 58 ± 8.36 to 56.0 ± 11.4 which is around 3.44 per cent. However the decrease was not statistically significant. (Table 12)

DISCUSSION

The present study was designed to observe the effect of different doses of eugenol on platelet aggregation and lipid peroxidation in healthy individuals.

The study was conducted on 30 healthy individuals divided in two groups. In group I (N = 15), the effect of single dose of eugenol was observed. The group was further divided into three sub-groups of five each and given plain eugenol in 10 mg/kg, 15 mg/kg and 20 mg/kg of body weight respectively. The effect of plain eugenol observed was compared with the effect of placebo given one day prior to eugenol on platelet aggregation and lipoprotein oxidation susceptibility.

Group II (N=15) was similarly subdivided and eugenol was administered in 10, 15 and 20 mg/kg of body weight in single dose. But in this group eugenol was administered with 25 gm of butter and the effect was compared with butter and placebo given one day prior to the fat and eugenol.

In both groups blood samples were collected initially and ninety minutes after ingestion of eugenol or placebo with or without fat and analysed for platelet aggregation and lipid peroxidation.

It has been observed that administration of eugenol in different doses to healthy volunteers variably affect platelet aggregation induced by ADP and epinephrine. The magnitude of responsiveness increases as the dose is increased from 10 mg/kg to 20 mg/kg body weight and at higher dosage (20 mg/kg) it is statistically significant ($P<0.05$). However, lipid peroxidation remains unaltered even in higher doses.

Similarly in group II when fat was administered with eugenol, the response of platelet towards aggregating agents remained same as in plain eugenol group. Here also the response is dose related and statistically significant ($P < 0.05$) at 20 mg/kg body weight dose. Lipoprotein oxidation susceptibility however remains significantly unaltered in all three doses schedules.

In both the study groups, therefore one observation is common, that is eugenol has some potential of lowering platelet aggregation, but the effect is greatly modified by the doses administered. Secondly, as the effect is more or less same in both the groups the absorption of eugenol is practically same and not affected by fat. This observation is similar to those observed in experimental study where eugenol and its metabolites were excreted more than 80 per cent in the urine, after oral ingestion[10]. Except few gastrointestinal side effects such as nausea, epigastric burning and bloating at higher doses, the drug was tolerated well by most of the study subjects.

Eugenol is an important naturally occurring phenolic compound. It has been reported to possess strong antioxidant and platelet aggregation inhibiting property in animal experiments[9].

Eugenol or sodium eugenol acetate inhibited arachidonic acid, collagen, epinephrine and ADP induced platelet aggregation on concentration dependent manner of washed rabbit platelets. The platelet aggregation inhibition effect was resulted by

inhibition of arachidonic acid induced thromboxane B2 and prostaglandin E2 formation[25]. Saed *et al.* Studied eugenol, the active principle of clove oil on human platelet aggregation, arachidonic acid (AA) and platelet- activating factor (PAF) metabolism and in vivo effect on AA and PAF induced shock in rabbits. It was observed that eugenol strongly inhibited PAF induced platelet aggregation with lesser effect against AA and collagen. It inhibited AA metabolism via cyclooxygenase and lipo-oxygenase pathways. Both thronboxane A2 and 12-hydroxy-eicosatetraenoic acid were inhibited by eugenol [26].

Oxidative stress, induced by hydrogen peroxide in islets of Langerhans and its inhibition by eugenol was studied in male mouse. The study revealed that H_2O_2 induced oxidation stress and lipid peroxidation in the isolated islets of Langerhans recovered by administration of eugenol and the recovery was achieved by raising the level of total antioxidant capacity (TAC) and Catalase and reducing malondialdehyde (MDA). It was interesting that low dose administration of eugenol was potent to achieve a therapeutic effect[27]. Antioxidant activity of eugenol was also assessed by *in vitro* and in vivo methods. The study results showed that eugenol inhibited iron and OH radical initiated lipid peroxidation. The inhibitory activity of eugenol was 5 times more than alpha-tocopherol and 10 times less than BHT, the synthetic antioxidant. The mechanism of antioxidant effect was interesting in the sense that eugenol incorporates into mitochondrial membrane and inhibited lipid peroxidation activity as a chain breaking agent[28]. The eugenol abolishes arachidonate, adrenaline and epinephrine induced aggregation *in vitro.* It is mediated by a reduced formation of thromboxane and increased formation of 12-HPETE. This is based on the following experimental evidences: (1) Eugenol inhibits thromboxane B2 formation from added arachidonate to washed platelets, and (2) it inhibits the formation of thromboxane B2 from arachidonic acid labelled platelets after stimulation with calcium ionophore A 23187. The formation of lipoxygenase derived products increased in both the experimental conditions. Some enzymes, the cyclooxygenase of the Arecadonic acid cascade was inhibited by the eugenol[10].

In animal experimental study it was observed that administration of eugenol and acetyl eugenol (100 mg/kg) given orally to rabbits decreases significantly the formation of thromboxane B2 during blood clotting. The effect of eugenol was more pronounced than acetyl eugenol with a biological half life of 72 ± 30 minutes[19]. This probably explains the observation of thepresent work that why the platelets were unresponsive in 10 and 15 mg/kg body weight doses. The eugenol inhibited thromboxane B2 in the dose of 100 mg/kg in animal study while in the present human study the maximum dose administered was only 20 mg/kg and at this dose there is significant inhibition of platelet aggregation induced by ADP but not by epinephrine. Therefore, it might be possible that higher doses are required to block the thromboxane B2 in order to achieve inhibition of platelet aggregation.

Recently, a pharmacodynamic study was performed to determine the level of eugenol in body fluids in human volunteers by HPLC assay. Six volunteers were administered oral dose of 150 mg eugenol once and 24 hours urine, serum and bile samples were collected.

Free eugenol never appeared in measurable concentration in the serum. It was present only as eugenol conjugates with a peak level at 40 minutes. While in the urine less than 0.1 per cent of the drug was excreted as free eugenol. Therefore the study brings a very important conclusion that eugenol undergoes a pronounced first pass effect in the blood. Therefore unconjugated eugenol was not detected after an oral dose of 150 mg and only eugenol conjugate was present. More than 80 per cent of the drug was excreted within 6 hours after oral administration and that too as eugenol conjugates [29].

The work of Fischer and Dengler therefore explains that why the effect of eugenol in the present study is observed at relatively higher doses. Higher dose is probably required to produce inhibition of platelet aggregation as the eugenol in blood is mostly present as conjugates and that conjugates may not have pronounced effect on thromboxane B_2 or aggregation of platelets[28].Same mechanism may explain the failure of lipoprotein oxidation susceptibility to be effected even in higher doses administered in this study.

Antioxidants activity of eugenol has been studied using in-vitro methods where the effect of eugenol on microsomal mixed function oxidase mediated peroxidation using - F^{+3}-ADP-NADPH, carbontetrachloride (CC14) - NADPH and Cumenehydroperoxide (CuMOOH) system was examined. Eugenol clearly inhibited iron or iron chelates induced non-enzymatic peroxidation by scavenging both initiating and propagating radicals. The study shows that eugenol did not inhibit cytochrome P-450 reductase activity but it inhibited P-450-linked mono-oxygenase activities so on the basis of above study eugenol has the potential to be used as therapeutic antioxidant[20].The present study however has failed to demonstrate any observable effect on lipoprotein oxidation susceptibility. This may require a long term administration of eugenol in higher dosage.

CONCLUSION

The present study therefore demonstrates that eugenol, an active ingredient of clove and other essential oils, has some tendency to decrease platelet aggregation in human beings when administered at higher doses (20 mg/kg body weight). Its effect on lipoprotein oxidation susceptibility is not statistically significant. Its use in vivo at present will probably be limited by the quick turnover and relatively short biological half life. However, further studies are needed in high doses to assess its action on platelet aggregation and lipoprotein oxidation susceptibility. Furthermore, pharmacodynamic research is required to keep this molecule active and free (unconjugated) in the blood for a longer time in order to achieve its effective inhibition on platelet aggregation.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1. Chopra, R.N.: Indigenous drugs of India. 2nd Ed. U.N. Dhar and Sons, Calcutta, 1958, p. 313.
2. Verma, S.K., Singh, J., Khamesera R. and Bordia, A. Effect of ginger on platelet aggregation in man. Indian J. Med. Res. 98: 240, 1993.
3. Bordia, A., Verma, S.K. Effect of garlic feeding on regression of experimental atherosclerosis in Rabbits. Artery 7: 428, 1980.
4. Verma, S.K., Joshi, J.K. and Bordia, A. Antispasmodic property of clove oil An experimental and clinical study. SDMH J. 14: 291, 1991.
5. Srivastava, K.C., Bordia. A and Verma, S.K.: Curcumin a major component of food spices turmeric (curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human platelets. Prostagl. Leukotr Essential Fatty Acids. 52: 223, 1995.
6. Srivastava, K.C., Bordia, A. and Verma, S.K. Garlic (Allium sativum) for disease prevention. South African J. Science 91: 68, 1995.
7. Bordia, A., Srivastava, K.C. and Verma, S.K.: Effect of garlic on platelet aggregation in humans. A study in healthy subjects in patients with coronary artery disease. Prostogl. Leukotr. and Essential Fatty Acids. 55: 201, 1996.
8. Srivastava, K.C. and Mustafa, T. Ginger (Zingiberofficinale, and rheumatic disorders. Medical hypothesis. 29: 25, 1989.
9. Verma, S.K. and Bordia, A. Commonly used condiments in north India their medicinal properties and relevant information. Project report submitted to Spices Board (Ministry of Commerce, Govt. of India), Cochin, 1992.
10. Srivastava, K.C. and Malhotra, N. Characterization and effects of a component isolated from a common spice clove (Caryophylliflos) on platelet aggregation and eicosanoid production. Thrombo haemorrhagic disorder. 1/2: 59, 1990.
11. Haro-Gonzalez, JN, Castillo-Harrera,GA, Martinez-Velazquez, M Epinosa-Andrews,H.Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, Chemical composition, Food application, and Essential bioactivity for human health. Molecules 2021; 26:6387-6411.
12. Srivastava, K.C. Antiplatelet principles from a food spice clove (Syzygiumaromaticum). Prostogl. Leukotr. and Essential Fatty Acids. 48: 363, 1993.
13. Joint FAO/WHO Expert Committee on food additives. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agent FAO nutritional Mtg. Rep. Jer. No. 44. Geneva p. 41: WHO/Food Add/68. 33.
14. Opdyka, D.L.: Monographs on fragrance raw materials. Food and cosmetics. Toxicology. 13: 545, 1975.
15. Yamahara, J., Kobayashi, M., Saiki, Y.Swada, T. and Fugimura, H. Biological active principles of crude drugs. Pharmacological evaluation of cholagogue substances in clove and its properties. J. Pharm. Dyn. 6:281, 1983.
16. Hartiala, K.J.W., Pulkkinen, M. and Ball, P.: Inhibition of glucosiduronic acid conjugation by eugenol. Nature 210 739, 1960.
17. Raussi, M. and Hartiala, K. The effect of eugenol on the formation of salicylamide glucuronide in dogs. Acta physiol. Scand 59 (Suppl. 213): 125, 1963.
18. Sukumaran, K., Unnikrishnan, MC, Kuttan R: Inhibition of tumour promotion in mice by eugenol. Indian J. Physiol. Pharmacol. 38: 306, 1994.
19. Lackman, G.M., Herman, A.G. and Vlietinick A.J.: Eugenol and Anologus as antiplatelet compounds. Pharmacodyn. 278 171, 1985.
20. Nagababu, E. The antioxidants effect of eugenol. Thesis submitted for award of the degree of Doctor of Philosophy in Bio-chemistry to Osmania University. 1994.
21. Reddy, A.C., Lokesh, B.R.: Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous ion. Mol. Cell Biochem 137: 1, 1994.
22. Harms, C.S. and Triplett D.A. Platelet aggregation in laboratory management (ASCP Press Chicago), 1977, 34:.
23. Knight JA, Pieper RK. AND Mc Cellan. Specificity of TBA reaction use in studies of lipid peroxidation. Clin. Chem. 1988; 34:2433.
24. Mahajan, B.K. Methods in biostatistics for medical students 1st Ed. Published by Kum. Aruna B. Mahajan. Jamnagar, 1967.
25. Chen SJ, Wang MH, Chen IJ. Antiplatelet and calcium inhibitory properties of eugenol and sodium eugenol acetate.Gen Pharmacol 1966; 27(4):629-633.
26. Saeed SA, Simjee RU, Shamim G, Gilani AH. Eugenol: A dual inhibitor of platelet-activating factor and arachidonic acid metabolism. Phytomedicine 1995; 2(1):23-28
27. Oroojan AK, Chenani N, and Anaam M. Antioxidant effects of eugenol on oxidative stress induced by hydrogen peroxide in islets of Langerhans isolated from male mouse.Int.J. Hepatol.2020.https://doi.org/10.1155/2020/5890378.

28. Nagababu E, Rifkind JM, Sesikeran B, Lakshmaiah N. Assessment of antioxidant activities of eugenol by *in vitro* and *in vivo* methods. Methods Mol Biol.2010; 610:165-180.

29. 46. Fischer. I.U. and Dengler, H.J. Sensitive high Performance liquid chromatographic assay for the determination of eugenol in body fluids. J. Chromatography 525: 369, 1990.