

Estimation of Eugenol Derived Chemical Compound Activity on Intestine Tissue and Some Enzymes of Infected Mice

Noor .A .M. Ajeel¹, Abdul Latif M. Jawad²

^{1,2}Collage of Science, Baghdad University, Iraq,Baghdad

Abstract: This research aimed to determine the effect of synthesized chemical derivative of plant extracted and purified eugenol on the intestine of the albino mice infected by *Escherichia coli* in addition to its effect on liver enzyme ALP and creatinine. Phenolic compound eugenol was isolated chemically in laboratory, its purity was confirmed by HPLC technology in percent of 90%- 100%. The purified eugenol was chemically derived producing three new synthesized derivatives: derived eugenol compound 1(D.E.C.1) (methoxy -1-bromoprop-phenol acetate). Experimental animal were used to detect the inhibitory effect of eugenol derivative on pathogenic bacteria *E-coli* was by injection in vivo. Experiment design included use of 10 mice divided in 5 groups the target organ was (intestine) which was isolated from dissected mice , blood test and biochemical test (Creatinine, Liver enzyme ALP) were performed. Results showed that mice treated twice with D.E.C.1 10% for ALP and creatinine in mice treated twice with D.E.C.1 at 10% was nearly normal value as in the control group. Histological results showed changes in the intestineranging from hemorrhage, degeneration and lymphocyte accumulation in infected non- treated mice while the treated mice with D.E.C.1 derivative's showed gradual regeneration after one week.

Keywords: Eugenol, histopathology, biochemical test

1. Introduction

Plants have been used for medicinal purposes for as long as history has been recorded. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the amalgamation of useful drugs. There are between 35,000 and 70,000 plant species that have been used for medicinal purposes in the world [1]

The circulatory of eugenol (upto 0.5 ml) to hound mutts caused a drop in arterial blood pressure. Increased blood flow observed after intra-arterial that blood vessel are the focal site of action within the cardiovascular system. Eugenol affects the central nervous system. It is an aesthetic in mice and dogs. Greater doses elongate the sleeping time [2]

2. Materials and Methods

Plant identificationand extraction:

Clove bud *Eugenia caryophyllata* was used which identified in the national herbarium, The oils extracted from clove was prepared by using the Soxhlet apparatus.

Oils concentrations: Different oils concentrations were used in this study (0.01%, 0.5%, 0.1%, 1%, 10%) and prepared by using the formula

$$C1V1=C2V2$$

Ethanol was the solvent which was used as oils diluent

Formation of D.E.C.1 derived eugenol compound (methoxy-1- bromopro phenolacetate) desiccated CaCl_2 38.8 g (0.206 mol) of eugenol and 0.17 g of benzoyl peroxide in 130 mL of carbon tetrachloride, 24.1 g (0.12 mol) of bromo-succinimide were placed in funnel flask for 5 h at -5 to 2°C the liquid was dissolved in methanol (24 mL). The

mixture was refrigerated in ice bath. To the mixture were added 37% acetic acid solution (3.0 mL, 36.5 mmol) and 40% methylamine solution (1.84 mL, 24.3 mmol). The mixture was concentrated by evaporation of the solvent. The product was purified using unscrambling funnel to give The solvent was removed under water suction distilled under reduced pressure to present a the compound [3]

Vivo examination

The natural world were house under sterilized conditions and feed with complete watch your waistline to grow in the wanted manner .Ten mature albino mice, males prejudiced (23 – 25.7) gram were used , animals were isolated in a relatively prohibited environment by using 10 mice divided in to 5 groups, 2 mice in each one as in, each group were injected intra- peritoneal most effective derivative with different concentrations each group was infected pathogenic *Escherichia coli* except control group



Figure 1: Injected mice intra- peritoneal

Blood Collecting and sampling:

After 7days of the effective derivative treatment, 1ml of blood was directly strained from the tail vein of the mice using 1 ml syringe. Blood was centrifuged at 3000 rpm for 10 min. Serum was transferred to 1 ml eppendorof tube using micropipette, and then kept according to n deep freezer -30 until biochemical and immunological analyses were superior[4]

Biochemicaltests:

Renal functions (creatinine) liver function enzymes (Alkaline phosphatase ALP

Histopathology of mice:

unchanging samples were flooded in ethanol all night and then dehydrated by graded alcohol 2 h. for each concentration followed by placing organs samples, sectioning occurs, further applying of alcohol to remove aggregate wax, then slides were standing by to examine[5]



Figure 2: Dissected mice

Statistical analysis

Complete Randomized Design (C.R.D.) was the preferred as an untried design, to study the effect of different factors on the diameter of inhibition zones. Least noteworthy difference (LSD) was used to balance the significant difference between means at $P \leq 0.05$.

3. Results and Discussions

Results

Biochemical test of animals

Many differences were noticed among results each test showed high significant for group 1,2,3,4,5 ,and the interactions shown between each one of biochemical test accomplished on blood of tested animals and group number according to table1

Table 1: Biochemical test

Test	Groups	ALP u/L	Creatinine u/L
Group 1		201.840 c	128.920 c
		±	±
		2.087	1.526
Group 2		247.800 b	143.140 b
		±	±
		14.772	3.148
Group 3		234.560 bc	145.320 b
		±	±
		4.706	3.015
Group 4		264.580 ab	142.940 b
		±	±

	17.786	1.716
Group 5 Control	295.180 a	154.940 a
	±	±
	8.595	3.273
General Normal value	64-306 u\l	<145 u\l

ALP and Creatinine U\L:

According to the used procedure which was used the group 1 was with 2mice with D.E.C.1 at 0.5% , group2 treated with 1% group 3 treated with 5% group4 treated twice with 10% of D.E.C.1 and group 5 were with 2 mice that is control not infected not treated The results were the highest concentration shown in group4 which were treated twice with D.E.C.1

Histopathology



Figure 3: Group 1 showed mass degeneration

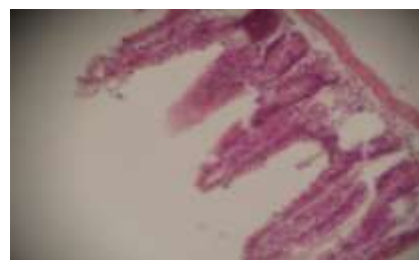


Figure 4: Group2 showed necrosis tissue texture



Figure 6: Group 3 showed small degeneration

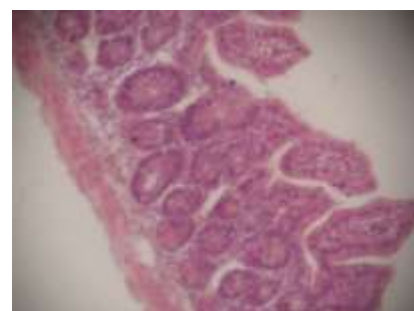


Figure 6: Group4 showed normal tissue texture

4. Discussion

The clove oil is mostly extract from part of clove plants (*Eugenia caryophyllata*) such as leaves, flower, and stem. The quality of cloves oil is resolute by the content of phenol compound, especially eugenol which is still considered as the main difficulty of clove oil in Indonesia. Currently, the clove oil still has high content of eugenol [6]

Eugenol derivatives shows highly consequence on bacterial growth that because the presence of active groups in the chemical. That amuse yourself in the inhibitory effect due to the content of the carbonyl group of atoms oxygen that the corporation of the two of electronic that react or link with enzymes of active groups in the body, which cause to the inhibitions of some imperative enzymes and its aromatic ring [7]

5. Histological tests affected by Eugenol Derivative Treatment

ALP in the serum of mice were tested elevated in treated one and the activity of ALP was significantly decreased show injury to the liver in non-treated one this result with [8]. Previous studies have showed that bacterial infection causes a disturbance in liver function well by an increase in serum ALT and a decrease in ALP activity [9]. The detailed mechanism by which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known. Experimental studies have shown that subtle membrane changes are enough to allow passage of intracellular enzymes to the extracellular cavity [10] Reduction form in the level of liver enzyme is mark of the stabilization of plasma membranes as well as fix of hepatic tissue damage. This in effect conforms to the commonly agreed on view reported earlier by that serum levels of transaminase back to normal with healing of hepatic parenchyma and the enzyme [11,12].

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