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Study on Toxicity of Clove *Eugenia caryophllata* Water Extract on Wister Albino Rats

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Abstract

Eugenia caryophllata is a plant which is believed by Sudanese herbalists to have antimicrobial, antiseptic and anesthetic effect. This plant has been tested in the present study to investigate its toxic effect in twenty Wister albino rats. The plant was extracted with water as a solvent. Distilled water was used to extract the polar compounds. In this study twenty Wister albino rats were divided into four groups (A, B, C, D) each consisted of five rats 100 ml of distilled water was added to 20 g of clove. Group A: was kept as a control in which rats were given distilled water only (the solvent). Group B: was given a dose of 10 drops of the clove water extract. Group C: was given a dose of 20 drops of the clove water extract. Group D: was given a dose of 30 drops of clove water extract. Bilirubin, ALT, AST and ALP enzymes were the parameters which were measured among the three groups B, C, D compared with the control in group (A). According to the statistic analysis there were no significant differences between the three treatments (B, C, D) and the control (A). In the present study *Eugenia caryophllata* (clove) was found to be non-toxic. More investigations on *Eygenia caryphllata* (clove) toxicity may support this study. It was concluded that *Eugenia caryophllata* water extract was administrated to rats, and rats were killed when the experiment was terminated after three weeks, no postmortem change were observed in any organ of treated or control rats.

Keywords

Clove Toxicity, *Eygenia caryphllata*, Water Extract

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1. Introduction

Many cultures around the world have strong believes on the uses of many different plants as medicine which have rendered some cultures, up to date, almost completely depend on the use of plants in medicine rather than common commercial medicinal products (stockwell, 1988). Historically, plants have played an important role in medicine. Medicinal plants in general, have been an issue of great controversy through the history of mankind.

For early peoples, they came easily to hand and were intricately connected to diet and healing. Through observation and experimentation they learned which plants promoted health and well-being. Actually, the use of plants and herbs as medicine has almost become a differentiating aspect between first world and third world countries and cultures. This seems ironic as many of the modern medicinal products are actually derived or extracted from plants (Cowan, 1999).

The clove (*Eugenia caryophllata*) is an aromatic plant, which grows in the tropical regions of Africa, Asia and South America. There are reports that clove oil may relieve gum and tooth pain and may be useful as a topical antiseptic in mouth wash.

Generally it is believed that clove is antiseptic and has anesthetic properties. Clove and clove oil combat some microbial infection, relieve nausea, vomiting, improve digestion, ease arthritis inflammation.

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Commission E (German regulatory agency for herbs) approved clove oil for use as an antiseptic. It has long been believed in the Sudanese herbal and medicinal plants culture that have anti-microbial effects. This plant is actually used by the Sudanese herbalists for treatment of many bacterial infections. There are local uses of plants in the Sudan to treat abdominal pain, diarrhea, wounds and mouth pain. Clove oil had long been recognized as safe and used in foods, beverages and tooth pastes, but toxicities which could be life-threatening were reported much later (Lane et al., 1991; Brow et al., 1992; PTCL 2005; and Prashar et al., 2006). The systemic use of the essential oil has been restricted to three drops per day for an adult as excessive use can cause severe kidney damage (Bensky et al., 2004).

This study aim to investigate the toxic effects of this plant clove *Eugenia oryphllata* in Wister albino rats.

2. Materials and Methods

Twenty Wister Albino rats of either sex and age and different weights were used in this study. Eugenia caryophllata (clove) were purchased from local market. It was dried in the shade. The concentration of clove water extract was used in the experiment, was 10% which was prepared by adding twenty grams of clove buds to hundred ml of distilled water and left for twenty four hours during which clove buds absorbed the 100 ml of water, then another hundred ml of distilled water were added to give extract 10% concentration.

The rats were designated as A, B, C and D and five rats were included in each group.

- The rats of group A were kept as normal controls.
- The rats of group B were given clove water extract at a dose of 10 drops\day.
- The rats of group C were given a dose of 20 drops\day of clove water extract.
- The rats of group D were given a dose of 30 drops\day of clove water extract. Each group was kept in a cage and was supplied with feed composed of meat and flour plus salt. The feed was available at libidum in each cage. The drinking program was as followed:

Group A: received water only.

Group B: received water plus 10 drops \day of clove water extract of 10% concentration, added to daily drinking water.

Group C: received water plus 20 drops\day of clove water extract of 10% concentration, added to daily drinking water.

Group D: received water plus 30 drops\day of clove water extract of 10% concentration, added to daily drinking water.

Rats were kept for one month then blood samples was collected, to measure the level of the hepatic billrubin and Alanine amino transfers. Serum was used to estimate billrubin ALT, AST, ALP levels. Then, clove extract was administered in drinking water of the rats in group, B, C and D as described before. The rats were weight at the beginning and the end of the experiment.

The method of drury and Wallington (1980) was followed to prepare tissue for histopathological examination.

3. Results

When *Eugenia caryophllata* water extract was administrated to rats, and rats were killed when the experiment was terminated after three weeks, no postmortem change were observed in any organ of treated or control rats.

3.1. Histopathological Finding

The histopathological examination of H&E stained tissue sections showed the following:

Liver in group A showed congestion, cytoplasmic vaculation.

Liver in group B showed congestion, sinusoidal, hepatocyte cytoplasmic vaculation (hydropic degeneration). Some hepato cytes were swollen with only scanty cytoplasm around nucleus.

Liver in group C showed congestion, swollen hepatocytes and hydropic degeneration.

Liver in group D showed congestion, including sinusoidal dilatation. Hepatocytes showed cytoplasmic vaculations and in some places cytoplasmolysis.

Kidney in group A showed many shrunken glomerulie.

Kidney in group B was almost normal, at some places glomerular tufts were shrunken or absent with dilated bowman's space.

Kidney in group C showed congestion, dilated bowman's spaces, in many places, shrunken dark staining glomerular tufts was also seen.

Kidney in group D showed general hyperemia including glomerular capillaries, dark staining shrunken tufts and dilated urinary spaces were seen in some areas, arteries showed mural thickening.

Generally, the histopathological examination revealed no inflammatory or necrotic changes. Similar changes were observed in liver section in all groups with some variations, mainly hydropic degeneration and congestion.

Similarly, no necrotic or inflammatory changes were seen in kidney sections. Shrunken glomerulie, dilatated urinary

spaces and congestion were observed in all groups. Spleen sections showed no clear changes.

Serum biochemical analysis

First week

When Eugenia caryophllata water extract was added to the drinking water of the rats, the bilirubin, alanine amino transferase, aspartate amino transferase and alkaline phosphotase measured at the end of the first week are shown in table 1. At the end of the first week there was no significant difference in bilirubin level between the treated rats drinking water plus clove water extract (group B, C and D) and control rats group A. although the bilirubin level of treated rats drinking clove water extract (group B) was numerically lower than that of (group A) control rats drinking water only (group A), (0.14 Vs 0.2).

At the end of the first week there was no significant difference in ALT level between control rats drinking water only (group A) and treated rats drinking water, plus clove water extract (group B, C, and D).

However, there was numerical variation in ALT level between the treated rats (group B, C and D) drinking water plus clove water extract and control rats drinking water only (group A).

At the end of the first week there was no significant difference in AST level between treated rats drinking water plus clove water extract(group B, C, and D) and control rats drinking water only (group A).

However, there was numerical variation in AST level when treated rats (group B, C, and D) were compared with control rats (group A), (257, 155.6 204.4 Vs 505.7).

At the end of the first week there was a significant difference in ALP level between control rats drinking water only (group A) and that of treated rats drinking water plus clove water extract (group B, C and D) ($P<0.05$).

Second week

When Eugenia caryophllata water extract was added to the drinking water of the rats, the bilirubin, alanine amino transferase, aspartate amino transferase and alkaline phosphotase measured at the end of the second week are shown in table 2.

At the end of the second week there was no significant difference in bilirubin level between control rats drinking water only (group A) and treated rats drinking water plus clove water extract (group B, C and D).

There was no significant difference in ALT level between control rats drinking water only (group A) and treated rats drinking water plus clove water extract (group B, C, and D) although the ALT level of normal rats drinking water plus clove extract group (B and D) was numerically lower than the level of ALT of the control rats drinking water only (group A) (67.6 and 80.8 Vs 85.7). The ALT level of treated rats drinking water plus clove water extract (group C) was numerically higher than the normal control rats drinking water only (group A). (108.6 Vs 85.7).

Also there was no significant difference in AST and ALP level between the treated rats drinking water plus clove water extract (group B, c and D) and the control rats drinking water only (group A).

Although the ALP level of the rats in group C was numerically higher than ALP level of the rats in group A (269 Vs 237.2).

Third week

When Eugenia caryophllata water extract was added to the drinking water of the rats the bilirubin alanine amino transferase, aspartate amino transferase and alkaline phosphotase measured at the end of the third week are shown in table 3.

At the end of the third week there was no significant difference in bilirubin level. ALT level and AST level between the treated rats drinking water plus clove water extract (B, C and D) and the control rats drinking water only (group A).

However, the ALT levels of the rats in group B was numerically lower than the ALT levels of the rats in group A (65.2 Vs 70.3), and the ALT level of the rats in group A (79.2 Vs 70.30).

Also there was numerical variation in AST level between treated rats (group B, C and D) and control rats (group A).

There was no significant difference in ALP level between treated rats drinking water only (group A). However, there was numerical variation in ALP level when treated rats (group B, C and D) were compared with the normal control rats (group A).

3.2. Statistical Analysis According to ANOVA Method

Table 1. The effect of administration of Eugenia caryophllata Wister albino rats on bilirubin , amino transferase aspartate eamino transferees and alkaline phosphates levels measured at the end of the first week.

Parameter	Treatment				SE	Prob
	A	B	C	D		
BIL	0.2^a	0.14^a	0.2^a	0.29^a	0.0787	0.647
ALT	55.1^a	0.49^a	46.6^a	51.6^a	15.72	0.759
AST	505.7^a	257^a	155.6^a	204.4^a	42.31	0.276
ALP	248.1^a	306.2^b	163.8^c	$274..6^d$	33.85	0.028

Mean value have different superscript letters within eac raw are significantly different ($p<0.05$).

Table 2. The effect of administration of Eugenia caryphllata to wister albino rats on bilirubin alanine amino transferase aspartate Amino transferase and alkaline phosphotase levels measured at the end of the second week.

parameter	Treatment				SE	Prob.
	A	B	C	D		
BIL	0.15^a	0.142^a	0.14^a	0.156^a	0.0306	0.922
ALT	85.7^a	67.6^a	108.6^a	80.8^d	31.52	0.654
AST	232.9^a	263.2^a	207.6^a	227.8^a	31.24	0.467
ALP	237.2^a	238.6^a	269^a	204.0^a	48.83	0.653

Mean value have different superscript letters within each raw are significantly different at ($p<0.05$).

Table 3. The effect of administration of Eugenia caryophllata to wister albino rats bilirubin alanine amino transferase, apartate amino transferase and alkaline phosphotase levels measured at the end of third week.

parameter	Treatment				SE	Prob.
	A	B	C	D		
BIL	0.19^a	0.124^a	0.172^a	0.28^a	0.0651	0.226
ALT	70.3^a	65.2^a	79.2^a	66.4^a	16.88	0.821
AST	231^a	239.6^a	227^a	226.4^a	23.36	0.904
ALP	279.1^a	314.2^a	216^a	307.2^a	45.34	0.271

Mean value have different superscript letter with each raw are significantly different ($p<0.05$)

4. Discussion

Some plants have been used for centuries as a treatment of infections and other illness in human and animals. Some of them are believed having antimicrobial activities (Stockwell, 1988). In the present study the plant Eugenia caryophllata, which is believed amongst herbal therapists as antimicrobial agent bacteria and fungi, kills intestinal parasites and has Antiseptic and anaethtic effect (fetrow and avila .1991,blech , et al. 1991), was examined to reveal whether it has toxic effect or not. Water, was used for the extraction of the soluable and polar compounds.

The current study control rats revealed no necrotic or inflammatory changes or congestion in liver and no shrunken glometrulie was seen in kidney sections, however, some haemosidrin deposit and congestion were seen in spleen sections of control rats.

The histopathological examinations in this study revealed that the water extract of eugina caryophllata had no toxic effects on treated wister albino rats when compared with the control rats which drinking water only throughout the experiment. Thios indicates that water soluble compounds of the clove (Eugina caryophllata) has no toxic effects on wister albino rats.

Similar to the previous study of zheng and kenny (1992), this study showed that clove in general could be considered safe, although a relatively small number of people may be allergic to clove extracts specially eugenol component.

Although this study and previous one (zheng and kenny, 1992) support the believe that clove is generally consider safe and has no toxic effects, however, there were precious studies (lavoie et al., 1986; soltani et al., 2004; taylor and Francis, 2004) which reported that clove extracts have a different toxic effects when administered by different routes whether by injection or ingestion (drinking, feeding).

Although this study did not examine the toxic effects of oil extracts on wister albino rats, but other studies reported the toxicity of the oil extracts (lavoie et al., 1986). Other study reported the toxicity of eugenol, eugenol acetate, beta caryophylene and alpha- humelene constitents of clove and clove cigarettes smoke in rats and hamsters, (soltaniet al., 2004) they also reported that clove oil has a toxic effect on penaeusse misulcatus.

In this investigation, statistical analysis of the serum levels of alanine amino transferase, aspartate amino transferase and alkaline phosphatase of the treated wister albino rats revealed that there was no significant difference in ALT, AST and ALP levels. Since serum levels of alanine amino transferase and aspartate amino transferase and alkaline phosphatase are associated with pathological change in the liver and kidney when cellular degeneration or destruction in the liver or kidney occur (coles, 1986). This means that the clove water extract has no toxic effects on wister albino rats. The serum bilirubin level was not significantly different in control and treated rats. Serum bilirubin concentration increse, if removal of bilirubin by hepatocellular transport is decresed (coles, 1986). This suggests E. carryophllata has no deleterious effect on the liver. Previous study (zheng and kenny, 1992) together with this one support the belief of that clove is safe and has no toxicity. Generally, clove considered safe, although a relatively small number of people may be allergic to eugenol (zheng and kanny, 1992).

5. Conclusion

Thus it can be concluded that *Eugenia caryophllata* is a safe plant and can be used in different aspects safely according to our system. Although this study did not examine the toxic effects of oil extracts on wister albino rats, but other studies reported the toxicity of the oil extracts. In this *Eugenia caryophllata* water extract was administrated to rats, and rats were killed when the experiment was terminated after three weeks, no postmortem change were observed in any organ of treated or control rats.

References

[1] Bensky DS, Clavey S, Stoger E. Chinese Herbal Medicine: Materia Medica. Seattle, W.A: Eastland Press; 2004. ISBN 0939616424.

[2] Brow SA, Biggerstaff J, Savidge GF. Disseminated intravascular coagulation and hepatocellular necrosis due to clove oil. Blood Coagul Fibrinolysis. 1992; 3:665–668.

[3] Cowan, M. M. (1999). Plants products as antimicrobial agents. Clinical microbiology review, 2: 564 – 582.

[4] Drury, R.A.B and wallington, E.A. (1980). Careton's histological technique. Filth edition, Oxford, Newyork, Toronto.

[5] Fetrow C.W. and Arial. J.R. (1999) professionals hand book of complementary and alternative medicines. Publish springhouse, pa: springhouse corp.

[6] Lane BW, Ellenhorn MJ, Hulbert TV, McCarron M. Clove oil ingestion in an infant. Hum Exp Toxicol. 1991;10(4):291–294.

[7] Lavoie, E.J, Adams, J.D, renhardt, J., rivenson, A. and haffmann, D. (1986). Arch. Toxicol, 59 (2): 78 – 81.

[8] Physical and Theoretical Chemistry Laboratory at Oxford (PTLC), author Safety data for clove oil. Oxford: Physical and Theoretical Chemistry Laboratory; 2005. Retrieved April 6, 2008.

[9] Prashar A, Locke IC, Evans CS. Cytotoxicity of clove (Syzigium aromaticum) oil and its major components to human skin cells. Cell Prolif. 2006; 39:241–248.

[10] Soltni, M. I., Marmari, G.H.; Mehvabi, M.R. (2004). Aquaculture international, volume 12, Numbers 4 -5, pp. 457 - 466, publisher/ springer.

[11] Stockwell, C. (1988). Nature's pharmacy. Century hutchinson Ltd., London United Kingdom.

[12] Zheng, G.Q. and kenney, P.N. (1992). Sesquiterpenes from clove (Eugenia caryophllata) as potential ant carcinogenic agents, J. nat. prod., 55: 999-1003.