BIOCHEMICAL INVESTIGATION ON EUGENIA CARYOPHLLATA (CLOVE) WATER EXTRACT ON WISTER ALBINO RATS

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Declaration

This work has been conducted in the Department of Microbiology and Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum and Laboratory and Research Unit Khartoum Hospital.
Dedication

To my parents

To my Husband,

To my sisters

with love

To my colleagues and Friends

With best wishes.
ACKNOWLEDGEMENTS

First of all my thanks and praise to almighty Allah, the beneficent, the merciful for giving me health and strength to accomplish this work. I wish to express my indebtedness and sincere thankfulness to my supervisor Dr. Suliman Mohamed El Hassan for his keen guidance, valuable assistance, advice and encouragement. Indeed, his generous help and support is greatly appreciated.

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ABSTRACT

*Eugenia caryophyllata* is a plant which is believed by Sudanese herbalists to have antimicrobial effect, antiseptic and anesthetic effect.

This plant has been tested in the present study to investigate its toxic effect in Wister albino rats.

The plant was water extract. Distilled water was used to extract the polar compounds. Twenty Wister albino rats were divided into four groups (A, B, C, D) each consisted of five rats. Group A rats were kept as control and were given distilled water only. Rats in group B were given clove water extract, a dose of 10 drops, rats in group C were given 20 drops and rats in group D were given 30 drops.

Rats were killed after 3 weeks. There were no postmortem changes observed. The histopathological examination of the liver, kidney and spleen revealed no inflammatory or necrotic changes.

Bilirubin, ALT, AST and ALP enzymes were the parameters which were measured. The statistical analysis showed there were no significant differences between the three treatments (B, C, D) and the control (A).

In the present study *Eugenia caryophyllata* (clove) was found to be non-toxic. More investigations on *Eugenia caryophyllata* (clove) toxicity may support this study.
نحتاج إلى مساعدةٍ منك في قراءة السطر المحدد.
INTRODUCTION

Many cultures around the world have strong beliefs on the uses of many different plants as medicine which have rendered some culture, 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 caryophyllata) is an aromatic plant, which grows in the tropical regions of Africa, Asia and South America.
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 and ease arthritis inflammation.

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.

This lead us to investigate and study the toxic effects of this plant (Clove Eugenia coryophllata) in Wister albino rats.
CHAPTER ONE

1. LITERATURE REVIEW

1.1 Medicinal plants

Data on medicinal properties of Sudanese herbs are rare, although old traditional medicine is very well known in Sudanese history. So nowadays microbiology, chemistry, biochemistry, pharmacology and toxicology, all of this science are available to cover the ethanobotanical, chemical constituents, toxic and pharmacological aspects.

1.1.1 Uses of plants in medicine

The use of plants for the treatment of various diseases is universal and has been practiced by many people since ancient times. Hippocrates in the late fifth century B.C mentioned 300 to 400 medicinal plants (Schultes, 1978). In the first century A.D. Discorides wrote Demateria Medica, medicinal plant catalogs which become the prototype for modern pharmacology.

The use of medicinal plants varies from disease to disease. The Neem tree (*Azadirachta indica*) for example has been known in Asia for along time. The sun-dried seeds of the plant are used by Indians to control pests in house as well as stored cereal grains, and as a detergent
resembling shampoo, for the removal of lice from the head (Srivostova, 1984). The Neem tree has also been found to have an antimicrobial effect. In African countries, the ripe fruit of *Balanites aegytiaca*, Laloub is a popular medicinal plant and is used as a purgative and anthelmintic (Oliver, 1986). *Cassia senna* and *C. italica*, both of the family Legulminasceae was the first cassia species reported in literature for their therapeutic value as purgatives. The chemical constituents in the ripe pods of these plants are emodin, aloe and chrysophanol (Friedrich and Steftem, 1973).

*Cucurbita maxima* and *C. peppo* seeds kernel (Kousa) are used in many African countries including the Sudan as well as Europe and Asia as anti-helminthes for tape worms and as diuretic and the scraped fruit pulp is applied as a poultice to burns, swelling or as cooling application for headache (Oliver, 1986).

Garlic (*Allium sativum*) is antibacterial, antihistamine, anticoagulant, expectorants, antibacterial, anti-alternative diaphoretic, diuretic, stimulant and antispasmodic. Garlic has been used orally as an antioxidant to reduce cholesterol and triglycerides. Garlic has antibiotic, antiviral and antifungal properties; it is used to increase the effects of the
immune system, to reduce blood sugar levels, and to reduce menstrual pain. Topical application on the skin can help to treat corns, warts, calluses, ear infections, muscle pain, nerve pain, arthritis and sciatica. The antioxidant properties found in garlic may contribute to this effect by protecting against the cell damage by cancer-causing free radicals. Garlic may increase the number of natural killer cells, which destroy white cells cancerous or infected by viruses, it is also blocks the formation of powerful carcins called nitrosamines, which may be formed during the digestion of food. Garlic may have positive effects in preventing cardiovascular diseases, such as attack or stroke. It helps to prevent atherosclerosis through the actions of its ability to reduce the fatty substances, such as cholesterol in the blood stream. Garlic works as an immune system stimulant which helps the body to fight bacterial and fungal infections. *Pimpinella anisum* which belongs to apiceae family and pimpinella genus is included in medicinal plants which has antimicrobial activities against bacteria and fungi. Hexane and ethyl acetate extraction of pimpinella tuberous roots exhibit abroad spectrum antimicrobial activity and were analyzed for different photochemicals. Also it has an antioxidant activity which was determined by using the
free radical alpha-alpha-diphenyl-beta-picrylhy drazyl (DPPH) and preformed radical monocation 2.2-azino-bis (3-ethyl benzoline-b-sulphonicacid). Pimpinella has antioxidant capacities in arranged comparable to that of alpha tocophand, BHA, ascorbic acid and trolox, which were used as reference antioxidants.

In Peru Passiflora quadranglaris is used for external ulcer like sores and wounds (Ramirez et al., 1988). Oryza sativa is antidiarrhoeal used in Peru (Ramires et al., 1988). Mikania micrantha in Haiti is used for the joint and muscle pain (Weniger et al., 1986).

Ludwigia octovalis in Nicaragua is used to prevent miscarriage during child birth (Dennis, 1988).

In Sudan El Kamali and Khalid (1998) reported that, a decoction prepared from the balb of Allium cepa is used to treat constipation, the balb of Allium sativium is eaten to relieve fever, the powder of the herb Artemisa henba-alla (Sheeh) is administered as an anti-spasmodic, a decoction from the seed of Ocimum basilicam (Reihan) is used to treat jaundice. The decoction of the rhizome of Zingiber officinal (Zenjabeel) is mixed with milk to treat cough.
The same authors reported that, *Trigonella foenum-graecum* (Hilba), seeds swallowed with water to treat stomach disorders and dysentery. The powder of the seed is used externally as poultice to treat boils, abscesses and carbuncles, the decoction is used as anti-spasmodic in cases of malaria, hypertension and kidney disorders. *Acacia nilotica* (Garad) is used as an analgesic, antiseptic and antipyretic.

1.2 Clove (*Eugenia caryophyllata*)

1.2.1 Historical background:

During the Handy nasty (207 B.C. to 220 A.D.) those who addressed the Chinese emperor were required to hold cloves in their mouth to mask bad breath. Traditional Chinese physician have long used the herb to treat indigestion, diarrhoea, hernia and ring worm, as well as fungal infection. Indians traditional of ayurvedic healers have used clove since ancient time to treat respiratory and digestive tract infection.

Clove first arrived in Europe around the 4th century A.D. as a highly coveted luxury. The medical German herbalist used clove as part of anti-gout mixture. Once clove became easily available in Europe, it was prized as a treatment for indigestion, flatulence, nausea, vomiting and diarrhea. It was also used to treat cough, infertility, warts, wounds
and toothache. Early American physicians used clove to treat digestive complaints and added it to bitter herb-medicine preparation to make them more palatable. They were also the first to extract clove oil from the herbal buds. They used it on the gums to relieve toothache. Contemporary herbalist recommends clove for digestive complaints and its oil for toothache (Valero and Salmeron, 2003).

1.2.2 Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class : Magnoliopsida
Order : Myrtales
Family : Myrtaceae
Genus : Eugenia
Species : *Eugenia caryophyllata*
Synonyms: *Syzygium aromaticum*
- *Eugenia aromaticum*
- Clove
- Clou

Arabic name: ﻗﺭﻨﻔل

1.2.3 Plant description

Clove *Eugenia caryophyllata* Syn. (*Syzygium aromaticum*) or *Eugenia aromaticum* is the aromatic dried flower bud of a tree in the family Myrtaceae. It is native to Indonesia and used as a spice in medicine all over the world. The name derives from French clou, a nail, as the buds vaguely resemble small irregular nails in shape. Cloves are
harvested primarily in Indonesia and Madagascar, it is also grown in Zanzibar, India and Sri Lanka. The clove tree is an evergreen which grows to a height ranging from 10-20m, having large oval leaves and crimson flowers in numerous groups of terminal clusters. The flower buds are at first of a pale colour and gradually become green, after which they develop into a bright red, when they are ready for collecting. Cloves are harvested when 1.5-2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petal which form a small ball in centre (Turner and Jack, 2004).

At the start of the rainy seasons long greenish buds appear from the extremity of these. The corolla comes which is of a lovely rosy peach colour, as the corolla fades the calyx turns yellow, then red. The calyces with the embryo seed are at this stage beaten from the tree and when dried are the clove of commerce. The flowers have strong refreshing odor. If the seeds are allowed to mature, most of the pungency is lost. Each berry has only one seed. The trees fruit usually about eight to nine years after planting. The whole tree is highly aromatic. The spice was introduced to Europe from the fourth to sixth century (Turner and Jack, 2004).
The finest cloves come from Molucca and Pemba, where the trees grow better than anywhere else, but they are also imported from the east and west Indies, Mauritius and Brazil (Turner and Jack, 2004).

In commerce the varieties are known by the names of the localities in which they are grow. Formerly cloves were often adulterated, but as production increased the price lowered and fraud has decreased. Cloves contain a large amount of essential oil which is much used in medicine. When of good quality they are fat, oily and dark brown in colour, and give out their oil when squeezed with the finger-nail. When pale colour and dry, they are inferior quality and yield little oil. Clove stalks are sometimes imported and are said to be stronger and more pungent even than the cloves (Turner and Jack, 2004).

### 1.2.4 Phytochemistry:

Buds is the part of the clove used. Essential oil is also produced from the leaves. The leaves are certainly aromatic enough to make them potentially interesting. The ripe fruits (mother of clove) have only local use. Clove is strongly aromatic and very intensive fragrance fiery and burning taste. The content of essential oil in cloves of good quality may exceed 15%. The oil itself is dominated by eugenol (70 to 85%), eugenol
acetate (15%) and alpha and ß-caryophyllene (5 to 12%), which together make up 99% of the oil. Cloves contain about 2% of the triterpenoeleonolic acid. Volatile oil, gallotannic acid are two crystalline principles. Caryohyllin, which is odorless and appears to be a phylosterol, eugenin, gum, resin, fiber (Valero, 2003).

1.2.5 Clove active compound:

The compound responsible for the clove’s aroma is eugenol. It is the main component in the essential oil extracted from cloves, comprising 70-85%. Eugenol has pronounced antiseptic and anaesthetic properties (Turner and Jack, 2004).

1.2.6 Uses of clove in medicine:

Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria supporting its traditional use as a treatment for diarrhea, intestinal worms and other digestive ailments. Clove are said to have antiseptic and anaesthetic properties. Clove and clove oil combat fungal infection, relieve nausea and vomiting, improve digestion, fight intestinal parasites, stimulate uterine contraction, ease arthritis inflammation and stop migraine headache. Patients reported that clove oil may relieve gum and tooth pain and may be useful as a topical
antiseptic in mouth wash (Fetrow and Avila, 1999). The microbiological quality of drinking water is a major public health priority in developing countries. Various parts of the plants i.e. clove; seeds and fruits are used against *E. coli* in drinking water (Blech *et al.*, 1991). Cloves are generally considered safe, although a relatively small number of people may be allergic to eugenol (Zheng and Kenny, 1992).

Clove is the most stimulating and carminative of all aromatics, given in powder or infusion for nausea, emesis, flatulence, indigestion and used chiefly to assist the action of other medicines. The medicinal properties reside in the volatile oil. The oil must be kept in dark bottles in a cool place. If distilled with water, salt must be added to raise the temperature of boiling and the same cloves must be distilled over and over again to get their full essence. Clove oil as a local irritant, stimulates peristalsis. It is a powerful antiseptic, a feeble local anaesthetic applied to decayed teeth, and has been used with success as a stimulating expectorant in phthisis and bronchial troubles. Fresh infusion of clove contains a stringent matter as well as the volatile oil. The infusion and clove water are good vehicles for alkalis and aromatics (Grives, 1995).
1.3 Uses of essential oil in medicine:

The antimicrobial properties of essential oils have been known for many centuries. In recent years (1987-2001), along number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi. The classical methods commonly were used for the evaluation of essential oils antibacterial and antifungal activities. The agar diffusion methods and the dilution methods as well as turbidimetric and impedimetric monitoring of microorganisms growth in the presence of tested essential oils are described. Essential oils of use against microorganisms in the research are thyme, oregano, mint, cinnamon, salvia and clove (Kalemba and Kunicka, 2003).

Some essential oils such as clove, eucalyptus, lavender, mint, myrrh and mile folia are used for treatment against inflammatory disease including arthritis, rheumatism and skin allergy and ulcers (Dorshan and Doreswamy, 2004).

The antibacterial activity of 11 essential oils from aromatic plants against the food borne pathogen *Bacillus cereus* were examined. The
essential oils of clove, nutmeg, mint, oregano, cinnamon, sage and theme were used against Bacillus cereus (Valero, 2003).

1.4 Toxicity and safety

1.4.1 Toxicity of clove cigarette smoke in rats and hamsters

Eugenol, eugenol acetate, beta-caryophyllene and alpha-humulene are constituents of clove and clove cigarette smoke. The toxicity of these compounds was evaluated by intratracheal instillation in male F-344, rats and hamsters (Lavoie et al., 1986). Eugenol was the most toxic in this assay. The LD50 of eugenol was 11 mg/kg in male F.344 rats and 17 mg/kg in male Syrian golden hamsters (Lavoie et al., 1986). Congestion of the lung with interstitial hemorrhages, acute emphysema, and acute pulmonary edema were among the macroscopic and histologic findings observed in the rats and hamsters after intratracheal administration of eugenol (similar effects were not observed with male Syrian golden hamsters exposed to clove cigarette smoke). The estimated daily intake of eugenol for those hamsters exposed to clove cigarette smoke was below 2mg/kg (Lavoie et al., 1986).
1.4.2 Acute toxicity and anaesthetic effects of clove oil in *Penaeus semisulcatus*

Acute toxicity and anaesthetic effects of clove oil were studied in *P. semisulcatus* (1.8 – 2.1 g body weight). The EC$_{50}$th (the concentration mg/kg effective for 50% of test animals), LC$_{50}$th (the concentration lethal to 50% of test animals after 1 h) and LC$_{50}$ 24 h (the concentration lethal to 50% of test animals after 24 h) were calculated at concentrations of 25, 30 and 130 mg/l respectively, at 30°C, salinity 40 ppt, pH 8.6 and dissolved oxygen >6 mg/l. Generally, with increasing concentrations of clove oil, the times required for sedation and anaesthesia decreased, while the recovery times increased. At concentrations 50, 100, 150 and 200 mg/l under temperature of 30°C and salinity of 40 ppt, the time required for sedation were 6 ± 0.2, 2.5 ± 0.3, 2 ± 0.08 and 0.5 ± 0.08 minutes, while times required for complete recovery were calculated to be 4.5 ± 0.3, 5.5 ± 0.17, 6.5 ± 0.25 and 11 ± 0.38 minutes, respectively. Also, the times required for deep anaesthesia were 20 ± 1, 5 ± 0.5, 3 ± 0.4 and 2.2 ± 0. minutes in the above concentrations, while the times required for complete recovery were 10 ± 1, 11 ± 1.5, 14 ± 2.2 and 16 ± 3 minutes, respectively. Furthermore considering the times to sedation, deep anaesthesia and recovery at different temperatures of 20, 25, 30 and
35°C and salinities of 25, 30, 35, 40 and 48 ppt, the combinations of salinity plus temperature and clove oil concentration plus salinity had the greatest and the least effects (Soltani et al., 2004).

1.4.3 N. acetylcysteine for the treatment of clove oil induced fulminant hepatic failure

Three months old female developed fulminant hepatic failure after ingesting less than 8 ml of clove oil. Initial treatment involved gastrointestinal decontamination, supportive measures, and admission to hospital. She subsequently developed fulminant hepatic failure and was treated with intravenous N-acetylcysteine (N-AC) according to a protocol used for acetaminophen poisoning. Over the next 72 h her liver synthetic function and clinical status improved, and she made a complete recovery (Taylor and Francis, 2004).

1.5 Clove safety

Japanese researchers have discovered that like many spices clove contains antioxidants. Antioxidants help prevent the cell damage that scientists believe eventually causes cancer. On the other hand, in laboratory tests the chemical eugenol, has been found to be a weak tumor promoter, making clove one of many healing herbs with both pro-and anticancer effects. This point, scientists aren’t sure which way the
balance tilts. Until they are anyone with a history of cancer should not use medicinal amounts of clove for otherwise healthy non-pregnant, non-nursing adults, powdered clove is considered nontoxic. However, high doses of the oil may cause stomach upset when ingested. When used externally, it may develop a rash (Soltani et al., 2004).

Clove and clove oil in medicinal amounts should be consumed only under the supervision of qualified professional (Soltani et al., 2004).
CHAPTER TWO

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals

Twenty Wister Albino rats of either sex and age and different weights were used in this study. The rats were divided into four groups on the basis of body weight in such way that the average weight of each group was 475-490 g.

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 rats were left for 7 days adaptation period.

2.1.2 Plant

_Eugenia caryophyllata_ (clove) were purchased from local market. It was dried in the shade. The clove buds were the part used in experimentation.

2.1.3 Preparation of clove water extract

The concentration of clove water extract which 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 100ml of the water. Another hundred ml of distilled water were added to give extract 10% concentration.

**2.1.4 Experimental design**

The rats group were designated as A, B, C and D and five rats were included in each group. Hundred and fifty milliliter of water were supplied to each group. Different amount of clove water extract were added to the water.

- The rats of group A were kept as normal control.
- 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.

**2.1.5 Samples collection**

After the adaptation period rats were kept for twenty one days and blood was collected from the orbital vein at the end of the first week and second week from all groups to measure the level of billrubin and
Alanine amino transferase (ALT), Alkaline phosphatase (ALP), Aspartate amino transferase (AST). After blood was collected it was allowed to clot, it was centrifuged at 3000 rpm for 5 minutes to separate serum. Serum was kept at -20°C until used for above mentioned analysis.

At the day 21st after administration of clove, the experiment was terminated and rats were killed and the blood was collected from rats in group A, B, C, D, and the blood was allowed to clot and then centrifuged at 3000 rpm for 5 minutes to separate serum. The collected serum also kept at 20°C and used for above mentioned analysis.

At the end of the experiment when all rats were killed, liver, kidney, spleen were inspected and specimens were collected in 10% formal saline for histo-pathological examination.

2.2 Histopathological and biochemical techniques

2.2.1 Histopathological techniques

The method of Drury and Wallington (1980) was followed to prepared, tissue for histopathological examination.

Tissue samples from liver, spleen and kidney were collected and fixed in 10% formal saline and later processed and then embedded in
paraffin. Sections 4-6 mm thick were prepared fixed on glass slide and stained with haematoxylin and eosin (H and E) as follow:

2.2.1.1 Fixation

Formal saline 10% was used as fixatives. Samples were fixed for 48 hours or more.

2.2.1.2 Dehydration

First the tissues were cut (trimmed) into small pieces about one cubic cm, and labeled with a pencil, then washed in running tap water for 15 min to remove fixing agent. The dehydration was carried out by passing the samples through increasing concentration of alcohol 60, 70, 80, 90 and 100%.

2.2.1.3 Clearing

Clearing was carried out by chloroform, zylene, benzene and cedar wood oil.

2.2.1.4 Impregnation

Melted paraffin wax (two changes) was used to remove the clearing agent from the tissue and to penetrate the tissue to fill the intercellular spaces.
2.2.1.5 Blocking

Tissues were blocked in melted paraffin wax and quickly cooled.

2.2.1.6 Section cutting

Sections 5-6 microns thick were cut with rotary microtome.

2.2.1.7 Fixing section to slide

The sections were transferred and floated in warm water bath 50-60°C containing amount of gelatin powder. The sections were transferred and fixed to the glass slide and then incubated for 30 min at 60ºC to dry.

2.2.1.8 Staining

Routine stains, haematoxylin and eosin were used. Sections were stained by haematoxylin for 10 min, washed, differentiated in 1% acid alcohol, rinsed in running tap water for 10 min. Then counter stained with eosin for 2-3 min rinsed quickly in water and dehydrated in 70% - 90% absolute alcohol. Sections were then cleared in zylene.

2.2.1.9 Mounting

The section was covered with cover glass using mounting medium, Canada balsam. After overnight drying at room temperature, sections were examined microscopically.
2.2.2. Serum analysis

Sera constituents were determined using Roche Diagnostics Hitachi 902 Analyzer. It was fully automated, computerized and performs potentiometric and photometric assays.

2.2.2.1 Alkaline phosphatase (ALP)

The activity of ALP was measured by an optimized standard method according to Chemie (1972).

**Principle:**

Alkaline phosphatase in alkaline medium splits p-nitrophenyl phosphate in the presence of Mg\(^{2+}\) ions, into p-nitrophenol and phosphate.

\[
\text{P-nitrophenyl phosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}.
\]

**Procedure:**

Two ml of alkaline phosphatase and control were pipetted into two separated test tubes and were put into control and calibration positions into Hitachi apparatus which is full automatic analyzer model 902 which analyzed the sample. Calibration was according to the identification number (ID) for the alkaline phosphatase. Then the reading appeared immediately at screen at the top of the apparatus at wave length 902 nm.
Calculation:

\[ ALP = \text{A}405 \text{ nm/min } \times 2760 \text{ (I/U)} \]

\[ \text{A} \equiv \text{the mean sample absorbance reading} \]

\[ \text{I/U} \equiv \text{International unit} \]

2.2.2.2 Alanine amino transferase (ALT) Glutamic pyruvic transaminase (GPT), L-aspartate.

Principle

Glutamic pyruvic transaminase (GPT) was measured by enzymatic method according to Reitman and Frankel (1957). Alanine amino transferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl-hydrazine.

\[ \alpha \text{-oxoglutarate} + \text{L–alanine} \xrightarrow{\text{GPT}} \text{L glut} + \text{pyruvate}. \]

The GPT was measured in (I/U).

Procedure:

Two ml of alanine amino transferase and control were pipetted into two separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer model 902. Calibration was according to the identification number (ID) for the alanine amino transferase. Then the reading appeared immediately at screen at the top of the apparatus at wave length 902 nm.
2.2.2.3 Aspartate amino transferase (AST), Glutamate oxaloacetate Transaminase (GOT)

Principle:

Glutamic oxaloacetate (GOT) in sera was measured by enzymatic method according to Reitman and Frankel (1957). Aspartate amino transferase was measured by monitoring the concentration of oxaloacetate hydrozone formed with 2.4-dinitrophenyl hydrazine.

\[
\alpha\text{-oxoglutarate} + L\text{-aspartate} \xrightarrow{\text{GOT}} L\text{-glutamate} + \text{oxaloacetate.}
\]

Procedure:

Two ml of aspartate amino transferase and control were pipetted into two separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer 902. Calibration was according to the identification number (ID) for the aspartate amino transferase. Then the reading appeared immediately at screen at the top of the apparatus at wave length 902 nm.

AST was measured in I/U.

2.2.2.4 Total bilirubin

Principle:

Total bilirubin reacts with diazotized dichloroniline to form a colouredazo compound according to the method of Jendrassik and Grof
(1938). The intensity of the colour was proportional to the bilirubin concentration in the sample.

**Procedure:**

Two ml of bilirubin and control were pipetted into two separated test tubes and were put into control and calibration positions into Hitachi apparatus automatic analyzer. Calibration was according to the identification number (ID) for the bilirubin. Then the reading appeared immediately at screen at the top of the apparatus at wavelength 902 nm.

**Calculation:**

Total bilirubin (mg/dl) = total absorbance × 17.5.
CHAPTER THREE

3. RESULTS

3.1 Postmortem examination

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

3.2 Histopathological findings

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

3.2.1 Liver

Liver of group A control rats showed congestion, cytoplasmic vaculation.

Liver of group B rats showed congestion, sinusoidal dilatation, hepato-cyte cytoplasmic vaculation (hydropic degeneration). Some hepatocytes were swollen with only scanty cytoplasm around nucleus.

Liver of group C rats showed congestion, swollen hepatocytes and hydropic degeneration.
Liver of group D rats showed congestion, including sinusoidal dilatation. Hepatocytes showed cytoplasmic vaculations and in some places cytoplasmolysis.

3.2.2 Kidney

Kidney of group A control rats showed many shrunken glomerulie.

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

Kidney of group C rats showed congestion, dilated Bowman’s spaces, in many places, shrunken dark staining glomerular tufts was also seen.

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

3.2.3 Spleen

Spleens of group A control rats and group B and C rats were almost normal and no pathological changes were observed, however some haemosidrin deposit and congestion were seen in spleen sections of control rats.
3.2.4 General

Generally, the histo-pathological 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.

3.3 Serum biochemical analysis

3.3.1 First week

When *Eugenia caryophyllata* water extract was added to the drinking water of the rats, the bilirubin concentration, alanine amino transferase, aspartate amino transferase and alkaline phosphotase activities measured at end of the first week are shown in table 1. There was no significant difference in bilirubin level between the treated rats with clove water extract (group B, C and D) and control rats group A. However 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 (0.14 Vs 0.2).

There was no significant difference in ALT level between control rats drinking water only (group A) treated rats with 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) and control rats (group A).

There was no significant difference in AST level between treated rats (group B, C, and D) and control rats (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).

There was a significant difference in ALP level between control rats (group A) and that of treated rats (group B, C and D) (P< 0.05).

3.3.2 Second week

When Eugenia caryophyllata water extract was added to the drinking water of the rats, the bilirubin concentration, alanine amino transferase, aspartate amino transferase and alkaline phosphotase activities measured at the end of the second week are shown in table 2.
There was no significant difference in bilirubin level between control rats (group A) and treated rats (group B, C and D).

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

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

However, the ALP level of the treated rats in group C was numerically higher than the ALP level of the control rats in group A (269Vs 237.2).

3.3.3 Third week

When *Eugenia caryophyllata* water extract was added to the drinking water of the rats the bilirubin concentration, alanine amino transferase activities, aspartate amino transferase and alkaline phosphatase measured at the end of the third week are shown in table 3.
There was no significant difference in bilirubin level, ALT level and AST level between the treated rats drinking water with clove water extract (group B, C and D) and the control rats (group A).

However, the ALT levels of the rats in group B was numerically lower than the ALT level of the rats in group A (65.2 Vs 70.3), and the ALT level of the rats in group C was numerically higher than 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 (group B, C and D) and the control rats (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).
Table (1): The effect of administration of *Eugnial caryophllata* to Wister albino rats on bilirubin, alanine amino transferase, aspartate amino transferase and alkaline phosphotase levels measured at the end of the first week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>SE</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>BIL</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT</td>
<td>55.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST</td>
<td>505.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>257&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP</td>
<td>248.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>306.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean value have different superscript letters within each raw are significantly different (P< 0.05)

Table (2): The effect of administration of *Eugnial caryophllata* to Wister albino rats on bilirubin, alanine amino transferase, aspartate amino transferase and alkaline phosphotase levels measured at the end of the second week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>SE</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>BIL</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.142&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT</td>
<td>85.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST</td>
<td>232.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP</td>
<td>237.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>238.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>269&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean value have different superscript letters within each raw are significantly different at (P<0.05)
Table (3): The effect of administration of *Eugnial caryophllata* to Wister albino rats on bilirubin, alanine amino transferase, aspartate amino transferase and alkaline phosphotase levels measured at the end of the third week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>SE</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>BIL</td>
<td>0.19(^a)</td>
<td>0.124(^a)</td>
<td>0.172(^a)</td>
</tr>
<tr>
<td>ALT</td>
<td>70.3(^a)</td>
<td>65.2(^a)</td>
<td>79.2(^a)</td>
</tr>
<tr>
<td>AST</td>
<td>231(^a)</td>
<td>239.6(^a)</td>
<td>227(^a)</td>
</tr>
<tr>
<td>ALP</td>
<td>279.1(^a)</td>
<td>314.2(^a)</td>
<td>216(^a)</td>
</tr>
</tbody>
</table>

Mean Value have different superscript letter with each raw are significantly different (P<0.05)
Fig. (1): Single dried clove flower bud
Fig. (2): Clove (*Eugenia caryophyllata*) flowers and leaves
Fig. (3): Spleen section showing congestion H&E X 250.
Fig. (4): Liver section showing congestion and sinusoidal dilatation H&E X 250
Fig. (5): Kidney section showing slight glomerular congestion H&E X 250.
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 caryophyllata*, which is believed amongst herbal therapists as antimicrobial agent against bacteria and fungi, kills intestinal parasites and has antiseptic and anaesthetic effect (Fetrow, 1999; Blech, *et al.*, 1991), was examined to reveal whether it has a toxic effect or not. Water, was used in study for the extraction of the soluble and polar compounds. Fresh infusion of clove contain astringent matter as well as volatile oil. The infusion and clove water extract are good vehicles for alkalis and aromatics. When *Eugenia caryophyllata* water extract was administered to rats and rats were killed when the experiment was terminated after three weeks no postmortem changes were observed in any organ of treated or control rats examined. The water extract of *Eugenia caryophyllata* in this study did not cause
inflammatory or necrotic changes in the liver, kidney and spleen. Similar changes were observed in the sections of all groups when compared with the control group with some variations, mainly hydropic degeneration and congestion in liver and spleen sections and shrunken glomerulie in kidney section.

In this study, control rats revealed no necrotic or inflammatory changes or congestion in liver and no shrunken glomemrlie 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 *Eugenia caryophyllata* had no toxic effects on treated Wister albino rats when compared with the control rats. This indicates that water soluble compounds of the clove (*Eugenia caryophyllata*) 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 belief that clove is generally considered safe and has no toxic effects, however, there were previous studies (Lavoie et al., 1986; Soltani et al., 2004; Taylor and Francis, 2004) which reported that clove extracts have a different toxic effect when administered by different routes whether by injection or ingestion (drinking, feeding).

Although this study did not examined 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 constituents of clove and clove cigarettes smoke in rats and hamsters, (Soltani et 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 alinine 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 (P< 0.05) in control and treated rats. Serum bilirubin concentration increases, if removal of bilirubin by hepatocellular transport is decreased (Coles, 1986). The suggests *E. carryophlalata* has no deleterious effect on the liver.

Previous study (Zheng and Kenny, 1992) together with the present 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).

In the present investigation the histopathological examination of the liver, kidney and spleen, together with serum analysis revealed *Eugenia caryophllata* has no deleterious effect on liver, kidney and spleen. Thus it can be concluded that *Eugenia caryophllata* is a safe plant and can be use in different aspects safely.
CONCLUSION AND RECOMMENDATION

Conclusion:

From the results of this study it can be concluded that:

1. *Eugenia caryophyllata* water extract had no toxic effects on liver, kidney and spleen of the wister albino rats.

2. *Eugenia caryophyllata* water extract had no effect on the serum level of billirubin, ALT, AST and ALP.

3. *Eugenia caryophyllata* plant was not toxic to Wister albino rats in the present study and this indicate that *Eugenia caryophyllata* is a safe medicinal plant.
Recommendation:

From the results and discussion of this study it is recommended that:

1. Further investigation on extract of *Eugenia caryophyllata* other than water should be carried out.

2. Fractionization of *Eugenia caryophyllata* extracts should be carried and therapeutic and toxicity of each fraction should be examined separately.
REFERENCES


