

## Effects of the Aqueous Extract of Clove (*Syzygium aromaticum*) on Wistar Rats

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**Abstract:** The present study was planned in order to investigate the effects of various oral doses of water extract of *Syzygium aromaticum* buds, emphases of its effect were placed on changes of the growth, serological, hematological and pathological characteristics of Wistar rats. Water extract of *Syzygium aromaticum* was fed to Wistar rats at 50, 200, 400 and 800 mg/kg/day for two weeks. Impairment of growth and hepatonephrotoxicity were observed in the rats of all groups. These changes were correlated with alterations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and total protein, cholesterol and urea concentration and hematology.

**Keywords:** Hepatonephrotoxicity, *Syzygium aromaticum*, wistar rats

### INTRODUCTION

Cloves are the dried flower buds of *Syzygium aromaticum* (L.) Merr & Perry, a tree of the myrtle family (Myrtaceae), which is an evergreen tropical plant that flowers twice every year and harvested when the outer green leaves (calyx) have changed from green to a yellow pink (Fig. 1). It has been grown in Indonesia, India, Malaysia and Sri Lanka, but production in the Asian countries is quite small as compared to that in countries in other regions, namely Madagascar, Tanzania (especially in Zanzibar) and the West Indies (Chomchalow, 1996).

Clove buds yield approximately 15 to 20% of a volatile oil that is responsible for the characteristic aroma and flavor. The principal constituents of distilled clove bud oil (60 to 90%) are the phenylpropanoids, including primarily eugenol (4-allyl-2-methoxyphenol) and carvacrol, thymol and cinnamaldehyde.

The oil also contains approximately 10% acetyleugenol and small quantities of gallic acid, sesquiterpenes, furfural, vanillin and methyl-n-amyl ketone. Other constituents include flavonoids, carbohydrates, lipids, oleanolic acid, rhamnetin and vitamins. In addition, the bud contains a tannin complex, gum and resin and a number of glucosides of sterols (Chaieb *et al.*, 2007).

The buds are used for symptomatic relief of toothache and inflammation-pain in the mouth and throat as they are sources of anti-microbial agents against oral bacteria commonly associated with dental caries and periodontal disease (Cai and Wu, 1996). A main aroma constituent of clove buds, eugenol, was reported to have antifungal activity (Martini *et al.*, 1996).



Fig. 1: *Syzygium aromaticum* buds

Further uses of cloves as carminative and tonic application and also cream (combined with other aromatic drugs), against inflammation-pain in toes and legs have been described. It is also used in many therapeutic fields such the treatment of kidney and intestinal diseases, against impotence, genital-pain and infertility and is reported as stomachic (Nadkarni, 2000) and has smooth muscle relaxant property (Damiani *et al.*, 2003).

### MATERIALS AND METHODS

**Animals:** Thirty two-month-old, male Wistar rats, with average body weight ranged from 100-110g. The rats were clinically healthy and housed within the premises of Al-Neelain University animal house under standard husbandry conditions, light/dark cycle with feed and drinking water provided *ad libitum*.

**Plant material:** *Syzygium aromaticum* buds were purchased from a local market in Khartoum, Sudan on July, 2012. The plant tissues were cleaned, shade-dried, ground by a mechanical grinder and the aqueous extract was used in the present study.

**Experimental design:** The rats were allotted at random to five groups, each of 6 rats. Group 1 continued to be fed the normal diet and served as control. Groups 2, 3, 4 and 5 were given water extract of the clove at 50, 200, 400 and 800 mg/kg/day orally; respectively. All rats were dosed their designated experimental oral doses for 2 weeks. Average, body weight and body weight gain for each group were recorded weekly. After 2 weeks all groups were anaesthetized with diethyl ether and humanely killed. Blood samples were collected at slaughter. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidneys, heart, spleen and intestines were immediately fixed in 10% neutral buffered formalin and processed for histopathology.

**Haematological parameters:** Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood cells (RBCs), White Blood cells (WBCs), differential WBCs counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), were determined.

**Serobiochemical parameters:** Serum samples were Analyzed for the activities of Aspartate Transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and for concentration of total protein, albumin, globulin, bilirubin, cholesterol and urea.

#### Methods:

**Preparation of plant extracts:** The pieces of grounded plant tissue were extracted separately. A sample (500 g) was accurately weighted in a covered glass bottle and separately macerated with water (at room temperature 25-30°C for 72 h), filtered and freeze dried.

**Haematological methods:** Whole blood for hematological parameter collected in EDTA anticoagulant blood container and examined for Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), white blood cells (WBCs) and differential WBCs count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and mean Corpuscular Haemoglobin Concentration (MCHC) and the measuring techniques were performed according to an Automated Hematology Analyzer (Human GambH, Max-Planck-Ring 21,D-65205 Wiesbaden, Germany).

**Serobiochemical methods:** Blood samples were collected and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 min and stored at -20°C until analyzed. The following methods for enzyme activity of control and test rats were performed according to the instructions in the manual of the Roche Diagnostic Hitachi 902 Analyzer (Germany, 1996).

Here we measured aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentrations of total protein, albumin, globulin, bilirubin, cholesterol and urea.

**Pathological methods:** Necropsy was conducted to identify gross lesion, after anesthetizing, the rats were dissected. Specimens of the liver, kidneys, heart, spleen and intestines were collected and immediately fixed in 10% neutral buffered formalin. The organs were embedded in paraffin wax, sectioned at 5 µm diameter and stained routinely with Hematoxylin and Eosin (H and E) (Andrew *et al.*, 2008).

**Statistical analysis:** Statistical Package for Social Science (SPSS, version 16) was used for the analysis of the data. The significance of differences between means (Mean±Standarderror (Mean±S.E.) were compared at each for all groups, using Duncan's simple T-test (Snedecor and Cochran, 1989).

## RESULTS

**Growth changes:** This data is presented in Table 1. After one week of dosing, the body weight gain of Groups 2, 3 and 4 were lower ( $p<0.05$ ) and that of Group 5 showed no significant changes when compared to control (Group 1). At the end of the experimental period (2 weeks), the body weight gain of rats in Group 3 was significantly lower ( $p<0.05$ ) than the controls, but no change was observed concerning other test groups.

**Hematological changes:** Two weeks after treatment, Group 2 receiving the lowest dose of clove bud water extract had the lower values ( $p<0.05$ ) of MCH, MCHC and WBCs count than the controls. Significantly higher ( $p<0.05$ ) values of Hb was recorded in Groups 3 and 4, RBCs in groups 2, 3 and 4 and WBCs count was higher ( $p<0.05$ ) in Group 4 than the controls. The values of PCV were high ( $p<0.05$ ) in Groups 2, 3 and 4 than those of group 1 (controls). The Lymphocytes counts were higher ( $p<0.05$ ) and of the neutrophils were lower ( $p<0.05$ ) in the test groups than the control Group 1. The values of MCV remained unchanged (Table 2).

**Serobiochemical changes:** Table 3 showed the serobiochemical changes in rats given daily oral doses of *S. aromaticum* buds water extract at 50, 200, 400, 800 mg/kg for 2 weeks. There was a significant decrease ( $p<0.05$ ) in AST activity in Groups 2 and 4 and increased activity ( $p<0.05$ ) in Group 3. Increased activity ( $p<0.05$ ) of ALT and cholesterol concentration and decreased activity of ALP and urea and cholesterol concentrations were observed in the test groups when compared to the controls. Total protein was higher ( $p<0.05$ ) in Groups 4 and 5 and that of bilirubin was higher in Group 4 than the control.

Table 1: Body weight and body weight gain in rats orally given *S. aromaticum* extracts for 2 weeks

Groups/Parameter	Body weight (g) 0 week	Body weight gain (g) one week	Body weight gain (g) two week
1. Control (normal diet)	105.0±2.2	12.6±2.5	5.2±3.2
2.50 mg/kg/day water extract	104.3±2.8	3.0±3.5*	6.0±1.9 <sup>NS</sup>
3.200 mg/kg/day water extract	105.0±3.2	5.0±2.7*	2.1±1.0*
4.400 mg/kg/day water extract	105.0±3.2	5.0±2.1*	7.0±2.9 <sup>NS</sup>
5.800 mg/kg /day water extract	105.0±1.7	11.0±3.4 <sup>NS</sup>	7.2±3.9 <sup>NS</sup>

Value are expressed as mean±S.E; not Significant; \* = Significant = (p<0.05)

Table 2: Heamatological change of rats given *S. aromaticum* aqueous extracts orally for 2 weeks

Parameters	1. Control (Normal diet)	2. <i>S. aromaticum</i> (50 mg/kg/day)	3. <i>S. aromaticum</i> (200 mg/kg/day)	4. <i>S. aromaticum</i> (400 mg/kg/day)	5. <i>S. aromaticum</i> (800 mg/kg/day)
Hb (g/d)	9.5±1.2	8.9±1.9 <sup>NS</sup>	11.8±0.9*	11.7±0.2*	10.9±0.6 <sup>NS</sup>
RBC (X10 <sup>6</sup> mm <sup>3</sup> )	4.5±0.6	6.0±0.1*	6.8±0.3*	6.5±0.1*	5.7±0.4 <sup>NS</sup>
PCV (%)	30.7±3.5	33.2±0.7*	38.6±1.6*	36.4±1.5*	31.9±2.1 <sup>NS</sup>
MCV (mm <sup>3</sup> )	55.7±1.1	55.0±0.7 <sup>NS</sup>	56.8±0.7 <sup>NS</sup>	55.9±0.9 <sup>NS</sup>	55.5±0.5 <sup>NS</sup>
MCH (pg)	17.4±0.8	14.1±2.9*	17.3±0.9 <sup>NS</sup>	18.0±0.3 <sup>NS</sup>	16.1±0.9 <sup>NS</sup>
MCHC (%)	31.2±1.2	25.5±1.4*	30.4±1.5 <sup>NS</sup>	32.2±0.5 <sup>NS</sup>	34.3±2.3*
WBC (X10 <sup>3</sup> mm <sup>3</sup> )	5.9±0.8	3.7±0.8*	6.5±1.5 <sup>NS</sup>	7.2±0.4*	6.7±1.3 <sup>NS</sup>
Lymphocytes (%)	41.0±3.6	65.0±2.4*	52.2±3.9*	51.5±3.2*	52.6±2.7*
Neutrophil (%)	59.0± 2.6	34.6±7.4*	47.1±2.6*	48.3±1.2*	47.4±2.7*

Values are expressed as mean±S.E; NS = not significant; \* = Significant = (p<0.05)

Table 3: Serobiochemical analysis of rats given *S. aromaticum* aqueous extracts orally for 2 weeks

Parameters	1. Control (Normal diet)	2. <i>S. aromaticum</i> (50 mg/kg/day)	3. <i>S. aromaticum</i> (200 mg/kg/day)	4. <i>S. aromaticum</i> (400 mg/kg/day)	5. <i>S. aromaticum</i> (800 mg/kg/day)
AST (iu)	74.5±1.7	45.9±4.3*	87.6±1.4*	69.8±0.9*	73.3±7.1 <sup>NS</sup>
ALT (iu)	33.4±2.5	48.3±2.4*	51.2±3.2*	38.9±1.2*	42.1±3.6*
ALP(iu)	141.7±1.8	135.2±6.7*	133.2±7.0*	134.5±8.7*	130.20±9.2*
Total protein (g/dL)	6.9±0.1	7.3±0.1 <sup>NS</sup>	7.5±0.3 <sup>NS</sup>	7.9±0.2*	7.9±0.2*
Albumin (g/dL)	2.8±0.3	3.2±0.4 <sup>NS</sup>	3.0±0.1 <sup>NS</sup>	3.5±0.2 <sup>NS</sup>	3.5±0.1 <sup>NS</sup>
Globulin (g/dL)	4.1±0.2	4.1±0.5 <sup>NS</sup>	4.5± 0.3 <sup>NS</sup>	4.4±0.2 <sup>NS</sup>	4.4±1.0 <sup>NS</sup>
Bilirubin (mg/dL)	0.1± 0.0	0.2±0.0 <sup>NS</sup>	0.1±0.0 <sup>NS</sup>	0.4±0.2*	0.1±0.1 <sup>NS</sup>
Urea (mg/dL)	41.0±1.9	34.8±3.9*	37.8±1.8*	39.0±0.1*	39.8±0.5*
Cholesterol(mg/dL)	52.7±2.0	62.4± 1.7*	62.8±2.8*	57.8±0.4*	57.6±1.0*

Values are expressed as mean±S.E; NS = not significant; \* = Significant = (p<0.05)

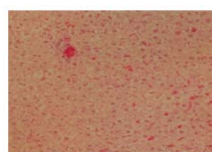


Fig. 2: Cytoplasmic fatty vacuolation and petechial hemorrhage of the centrilobular hepatocytes in a rat given daily oral dose of *S. aromaticum* bud aqueous extract at 200 mg/kg for 2 weeks H&E x100

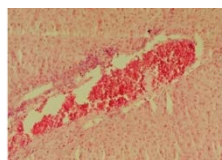


Fig. 3: Hepatocellular fatty vacuolation, necrosis and hemorrhage in a rat receiving daily oral doses of *S. aromaticum* buds aqueous extract at 400 mg/kg for 2 weeks, H&E x100

other vital organs of the control rats (Group1). There is fatty cytoplasmic vacuolation of the centrilobular

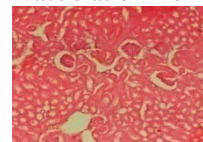


Fig. 4: Glomerular packing and necrosis and tubular necrosis in a rat given daily oral dose of *S. aromaticum* buds aqueous extract at 200 mg/kg for 2 weeks, H&E x100

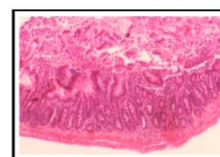


Fig. 5: Mild desquamation of the intestinal epithelium and lymphocytic infiltration in a rat daily orally dosed with *S. aromaticum* buds aqueous extract at 800 mg/kg for 2 weeks, H&E x100

**Pathological changes:** After the end of the treatment period, no lesions were seen in the spleen, heart and

hepatocytes in the liver of 200 mg/kg aqueous extract (Fig. 2) and fatty cytoplasmic vacuolation of the centrilobular hepatocytes and hemorrhage in the liver of

400 mg/kg aqueous extract (Fig. 3), packing of the glomerular tubules, dilatation and necrosis of the renal tubules in the kidney of 400 mg/kg aqueous extract (Fig. 4a, b), infiltration of lymphocytes and disquamation of the intestinal epithelium in the intestine of 800 mg/kg aqueous extract (Fig. 5).

## DISCUSSION

Although the lethal dose (LD<sub>50</sub>) of *Syzygium aromaticum* was recorded to be 2500 mg/kg which showed its relative safety as classified by the Organization for Economic Cooperation and Development (OECD, Paris-France) (Walum, 1998), the oral doses administered at 50, 200, 400 and 800 mg/kg/day were considered hepatonephrotoxic. LD<sub>50</sub> is not a biological constant because many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature, caging conditions and time of the day can all affect its value; hence there are considerable uncertainties in extrapolating its value obtained for a species to other species. On the other hand the toxicity of the plant material seems dependent of the types of active principles, the concentration added to the diet and the rate of their metabolic conversion in the liver to metabolites and their consequent excretion.

In the present study and after 2 weeks of treatment, only Group 3 had the lowest growth rate and no significant change was observed in other test groups. Inability of the *S. aromaticum* treated groups to gain as much weight as the control might be due either to the effect of the extract on the internal organs e.g. injured liver and kidneys, or to the general discomfort which lead to a low feeding rate in the treated rats. Administration of a foreign chemical compound may bring about significant changes in the metabolic transformation and concentration of biomolecules, enzymes and even metabolic pathways (Oyewole *et al.*, 2007).

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood. The extract significantly changed PCV, RBC and Hb of some treatment groups, *S. aromaticum* has been found to contain flavonoids (Agbaje *et al.*, 2009), which are free radical scavengers. It is therefore possible that these components compete with Hb in RBC for oxygen, resulting in hypoxia, which then stimulates synthesis and RBC production. It is also possible that the end product of *S. aromaticum* metabolism in the body stimulates the kidney directly to cause formation and secretion of erythropoietin, which is the humoral regulator of RBC production (Sánchez-Elsner *et al.*, 2004). It is also likely that *S. aromaticum* contains erythropoietin-like principle(s), which is responsible for the high RBC and PCV values recorded in this study.

The calculated blood indices-MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (Coles, 1986). Normocytic hypochromic anemia, normocytic normochromic and normocytic hyperchromic anemia was observed in group 2, groups 3 and 4 and in group 5 respectively after two weeks of treatment, a conclusion indicated by normal unchanged MCV in all the test groups.

On the other hand, *S. aromaticum* extract produced significant reduction of WBCs at the lower dose level Group 2 and neutrophil count in the test groups. This reduction could be attributed to the fact that the extract might contain some bioactive agents that could cause destruction or impair production of WBCs or contain some components that reduced the production of these regulatory factors or interfere with the sensitivity of the committed stem cells responsible for the production of white blood cells (Adebayo *et al.*, 2010). Leukocytosis observed in Group 4 is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The leucocytosis indicates a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections, or it could be attributed to an increase in leukocyte mobilization (Celik and Suzek, 2008).

Changes in AST, ALT and ALP activities and cholesterol concentration indicate hepatotoxicity in rats. It seems that nephrotoxicity is a feature of *S. aromaticum* in rats as evidenced by the presence of renal lesions and of the decrease in urea concentration in serum. The damage of centrilobular hepatocytes without significant biliumaemia was observed in rats which had been fed on *Artemisia abyssinica* (Adam *et al.*, 2000) or *Francoeuria crispata* (Adam, 1998).

## CONCLUSION

Oral administration of *Syzygium aromaticum* buds water extract at 50, 200, 300 and 400 mg/kg/day is toxic but not fatal and caused damage of vital organ exemplified by cytoplasmic vacuolation and necroses of the centrilobular hepatocytes, packing of the nephrons, dilatation of renal tubules and intestinal Lymphocytic infiltration and desquamation.

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