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Effect of hexane extract of *Syzygium aromaticum* on haematological profile of rats



Authors Info

S. Arora¹, M. Pal², M. Bordoloi³
and S. P. Nandi^{1*}¹Amity Institute of Biotechnology,
Amity University, Noida-201 313,
India²Division of Forensic Science,
School of Basic & Applied
Sciences, Galgotias University,
Greater Noida-201 306, India³Natural Product Chemistry
Division, CSIR-North East
Institute of Science & Technology,
Jorhat-785 006, India*Corresponding Author Email :
spaul@amity.edu

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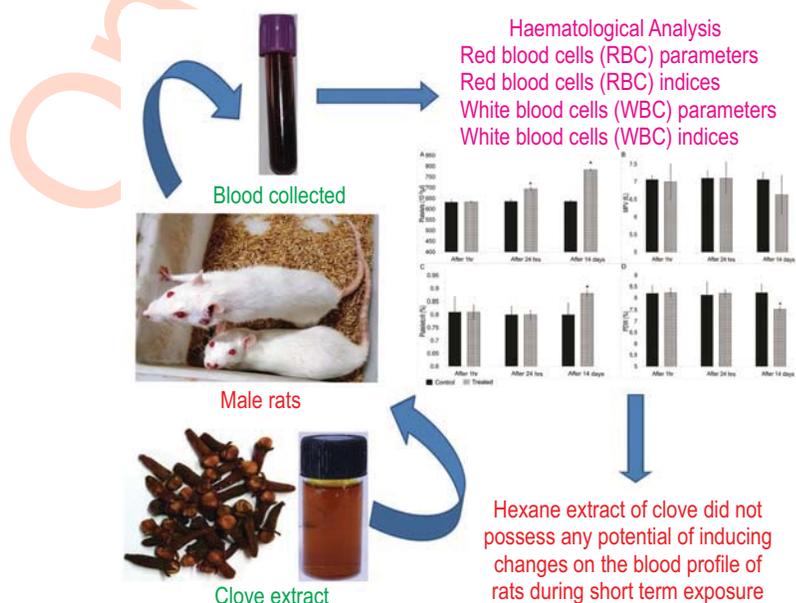
Abstract

Aim : To analyse the effect of hexane extract of *Syzygium aromaticum* on haematological profile of rats.

Methodology : Previously, hexane extract of *S. aromaticum* buds at a high dose of 5000 mg kg⁻¹ was given to one rat and the corresponding control received saline water as vehicle. It was repeated and no mortality was observed. Based on the result, 2000 mg kg⁻¹ was selected for the main study. The rats were prorated into two groups (control and treated). Each group consisted of six rats. After administration of hexane extract, blood was collected from three rats per group at an interval of 1 hr (retero-orbital plexus); 24 hrs and 14 day (cardiac puncture) in ethylenediaminetetraacetic acid coated vials and signs of toxicity and mortality were observed in animals. Haematological parameters were estimated from collected blood samples using automatic haematological analyser (Model Melet Schloesing MS4).

Results : The hexane extract of *S. aromaticum* at a dose of 2000 mg kg⁻¹ b. wt. to male rats did not produce any change in haematological profile for 1 hr exposure. The changes were observed in monocytes and white blood cells after 24 hrs. Significant (P<0.05) changes were observed in red blood cells, haemoglobin, hematocrit, platelets, plateletcrit and platelet distribution widths after 14 days of treatment. The treated rats neither exhibited any signs of toxicity nor death during 14 days of study.

Interpretation : The results revealed that a single dose of 2000 mg kg⁻¹ of *Syzygium aromaticum* did not induce any impact on haematological profile of rats till 14 day after administration of hexane extract.



Introduction

Natural products play an important role in traditional healing from centuries till date (Petrovska, 2012). The majority of population depends on herbal medicines rather than opting for other treatments due to high cost and side-effects associated with it. Eighty percent of the world population prefers to use medicinal plants to treat their ailments (Ekor, 2014). Herbal products are being consumed for centuries without knowing the risk of its usage and also documentation is still scanty on the toxicity and dosage of these products.

Syzygium aromaticum (clove) is an evergreen tree (8-12 m tall) belonging to family Myrtaceae native to Maluku Islands in Indonesia and harvested in different Asian Countries like India, Sri Lanka, Bangladesh, Pakistan and Tanzania (Kamatou et al., 2012). *S. aromaticum* buds have antibacterial (Sofia et al., 2007; Dorman et al., 2000) antioxidant (Shan et al., 2005; Gulcin et al., 2012; Dudonne et al., 2009; Gulcin et al., 2004), antifungal (Rana et al., 2011) and anticarcinogenic properties (Aggarwal et al., 2006; Ghosh et al., 2005). It is commonly used for toothache and throat inflammation (Cai and Wu, 1996; Jadhav et al., 2004). Other uses of clove oil include treatment of intestinal diseases, cure against impotency, infertility (Nadkarni, 2000) and relaxant property of smooth muscles (Damiani et al., 2003). Clove oil consist of gallic acid and acetyleugenol, methyl-n-amyl ketone, sesquiterpenes, vanillin, furfural and oleanolic acid, flavonoids, lipids, resin rhamnetin, carbohydrates, vitamins, tannins and gums (Chaieb et al., 2007). Bioactive constituents present in *S. aromaticum* are essential oil 12-16 % (v/w) and phenolic compounds. Halder et al. (2011) reported that clove oil is capable of reverting back memory and learning deficits by reducing oxidative stress. Presence of large number of phytochemicals and secondary metabolites have encouraged the researchers to explore many new applications of clove oil (Vijayasteltar et al., 2016).

Oral administration of water extract of *S. aromaticum* buds at different doses has been reported to be toxic but not fatal, emphasising its effect on growth, serological, pathological and haematological features in Wistar rats (Adam et al., 2013). Determination of effect of foreign component (plant extract) in blood can be estimated using haematological parameters (Olson et al., 2000). Investigation on blood parameters provides immediate information on the health impact of a product. Studies on haematological parameters are therefore important to establish a relationship between test products with physiology of body (Parma et al., 2007). The objective of the present research was to estimate various haematological parameters in male rats after oral administration of hexane extract of *S. aromaticum*.

Materials and Methods

Plant extract preparation : Dry flower buds of *S. aromaticum* were washed thoroughly with water and air dried at room temperature. The extraction procedure as described by Handa et al. (2008) was followed. Ten gram of clove was powdered using mortar and pestle and immersed in 40 ml hexane at room

temperature for 16 hrs. The extract was then filtered. The procedure was repeated three times. The combined filtrate was evaporated with the help of vacuum at 50°C using rotary evaporator to achieve a concentrate that could be stored at 4°C for future use.

Test animals : Healthy Sprague-Dawley rats (n=12) weighing 140-160 g were selected for the study. The rats were housed in cages at controlled room temperature of 22-24°C, relative humidity between 40-60 % and a constant schedule of light : dark : 12 hr : 12 hr cycles. The animals were fed with pelleted food supplied by Golden Feeds Delhi, India and water *ad libitum*. The experimental protocol was approved through Institutional Animal Ethics Committee (IAEC) of Institute of Nuclear Medicine and Allied Sciences (INMAS), Defence Research and Development Organization (DRDO), Delhi.

Experimental procedure : Experimental animals were divided into two groups (control and treated) containing six rats in each group. Before the actual experiment, two rats were taken, one of them was treated with 5000 mg kg⁻¹ of hexane extract of clove and the other one received saline water as vehicle and both were checked for mortality. The experiment was repeated twice. In both sets of experiment the animals survived. Based on the observation, 2000 mg kg⁻¹ of dose was selected for the main study. Hexane extract was orally administered to the experimental groups, whereas control group was treated with saline. Blood was withdrawn into EDTA coated vials after 1 hr of treatment from retro-orbital plexus of three rats, one each from experimental and control group. After 24 hrs, blood was redrawn from the same rats by cardiac puncture and the rats were sacrificed. Similar steps were repeated after 14 day. Rats were observed daily for signs of toxicity, if any, during 14 days of experiment.

Haematological investigations : The collected blood was used for analysis of Red Blood Cell (RBC) counts, Haemoglobin (HGB) concentration, White Blood Cell (WBC) counts, Lymphocytes (LYM), Monocytes (MCT), Granulocytes (GRA), Platelets Count (PTL), Haematocrit (HCT) level, Red Cell Distribution Width (RDW), Mean Platelet Volume (MPV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Plateletcrit (PCT) and Platelet Distribution Widths (PDWs) using automatic haematolyzer (Model Melet Schloesing MS4 in Department of Biochemical Sciences, Defence Institute of Physiology and Allied Sciences, Delhi, India).

Statistical analysis : The values obtained for different haematological parameters were expressed as mean \pm SD. Student's t-test was used to determine statistical significance between the difference of mean of treated and control groups.

Results and Discussion

Analysis of haematological parameters is used to determine the physiological response in blood. It also provides

immediate information of undesirable effect of chemical compounds or herbal products and these systematic researches have been reported to be extremely accurate, sensitive and reliable (Olson *et al.*, 2000). In view of this, the present study was conducted to assess the effect of hexane extract of *S. aromaticum* on haematological profiles.

The dose (2000 mg of hexane extract of *S. aromaticum* kg⁻¹ body wt.) was selected based on previous study where no mortality was observed when treated with 5000 mg kg⁻¹ in two sets of experiment. Moreover, Agbaje *et al.* (2009) also reported 2500 mg kg⁻¹ b. w.t. as oral LD 50 value in rats for *S. aromaticum*.

Administration of *S. aromaticum* hexane extract did not produce any significant ($P > 0.05$) change in lymphocytes or granulocytes whereas significant increase of 15% in monocytes was observed after 24 hrs in treated group and 1% decrease in white blood cells was observed in treated group after 14 day of treatment when compared with control ($P < 0.05$) (Table 1). The response could be due to the demand for tissue macrophages which helps in regulating homeostatic condition created due to chemical compounds present in the extract (Gordon *et al.*, 2017). The presence of certain phytochemicals with the ability to stimulate the production of white blood cells could be another reason for improved level of monocytes (Oyedemi *et al.*, 2011). Short term exposure and low dose of hexane extract of *S. aromaticum* in our study showed no significant changes in white blood cells, lymphocytes or granulocytes thereby indicating no immune boosting effect. Earlier reports indicated that some commonly chosen medicinal plants like mistletoe, round-leaved

vine contain factors that trigger the production of white blood cells (Imoru *et al.*, 2005; Al-Mamary, 2002; Ladokun *et al.*, 2015).

RBC and HGB play a key role in transferring respiratory gases (De Gruchy, 1976). The hexane extract of *S. aromaticum* has significant ($P < 0.05$) increase of 37% in RBC, 21% in HGB and 25% in HCT after 14 days of treatment when compared with the control, whereas no significant ($P > 0.05$) change in RDW was observed in treated group when compared with the control group (Table 2). Agbaje *et al.* (2009) has reported change in RBC, HCT and HGB by the *S. aromaticum* extract due to free radical scavenging effect of flavonoids. A possible condition in such case may be that there is competition between the active constituent flavonoids and haemoglobin in RBC for oxygen and hypoxial condition accelerated the production of RBC. Another important activity possessed by the product of *S. aromaticum* is the direct stimulation of kidney which causes formation and secretion of erythropoietin (Sanchez-Elsner *et al.*, 2004). It is interesting to note that Adam *et al.* (2013) also reported increase in RBC and haemoglobin using aqueous extract of *S. aromaticum*.

No significant difference of the hexane extract of *S. aromaticum* was found there on red blood cell indices (RBC indices) – MCV, MCH and MCHC (Fig. 1) when compared with the control group ($P > 0.05$). These RBC indices were not affected because may be no change on haemoglobin weight per red blood cell and also in average weight of red blood cell (Agbaje *et al.*, 2009). In most of the animals MCV, MCH and MCHC - red blood cell indices have a particular role in diagnosis of anaemia (Coles,

Table 1 : Effect of *S. aromaticum* hexane extract on various parameters of white blood cells in rats

Parameters	After 1hr		After 24 hrs		After 14 days	
	Control	Treated	Control	Treated	Control	Treated
WBC ($10^3/\mu\text{l}$)	7.42±0.04	7.46±0.12	7.42±0.08	7.67±0.09	7.46±0.02	7.39±0.01*
Lymphocytes (%)	82.96±1.17	84.03±1.44	82.36±2.44	81.56±2.10	81.7±1.63	82.96±1.13
Monocytes (%)	0.81±0.01	0.86±0.02	0.79±0.01	0.91±0.03*	0.81±0.01	0.87±0.02
Granulocytes (%)	2.16±0.18	1.96±0.11	2.26±0.18	2.20±0.28	2.26±0.19	2.20±0.24

Values are mean ± SD of three animals. *indicates significant difference ($P < 0.05$) between control and treated as obtained by Student's t-test

Table 2 : Effect of *S. aromaticum* hexane extract on various parameters of red blood cells in rats

Parameters	After 1hr		After 24 hrs		After 14 days	
	Control	Treated	Control	Treated	Control	Treated
RBC ($10^6/\mu\text{l}$)	5.47±0.05	5.58±0.01	5.38±0.06	6.5±0.25	5.58±0.05	7.63±0.11*
HCT (%)	35.9±1.4	35.8±0.49	36.16±2.1	38.7±0.42	36.86±2.05	46.1±0.46*
RDW (%)	8.23±0.15	8.33±0.01	8.43±0.08	8.56±0.18	8.33±0.04	8.3±0.14
HGB (g/dl)	12.43±0.27	12.6±0.05	12.46±0.23	13.66±0.80	12.56±0.18	15.23±0.13*

Values are mean ± SD of three animals. *indicates significant difference ($P < 0.05$) between control and treated as obtained by Student's t-test

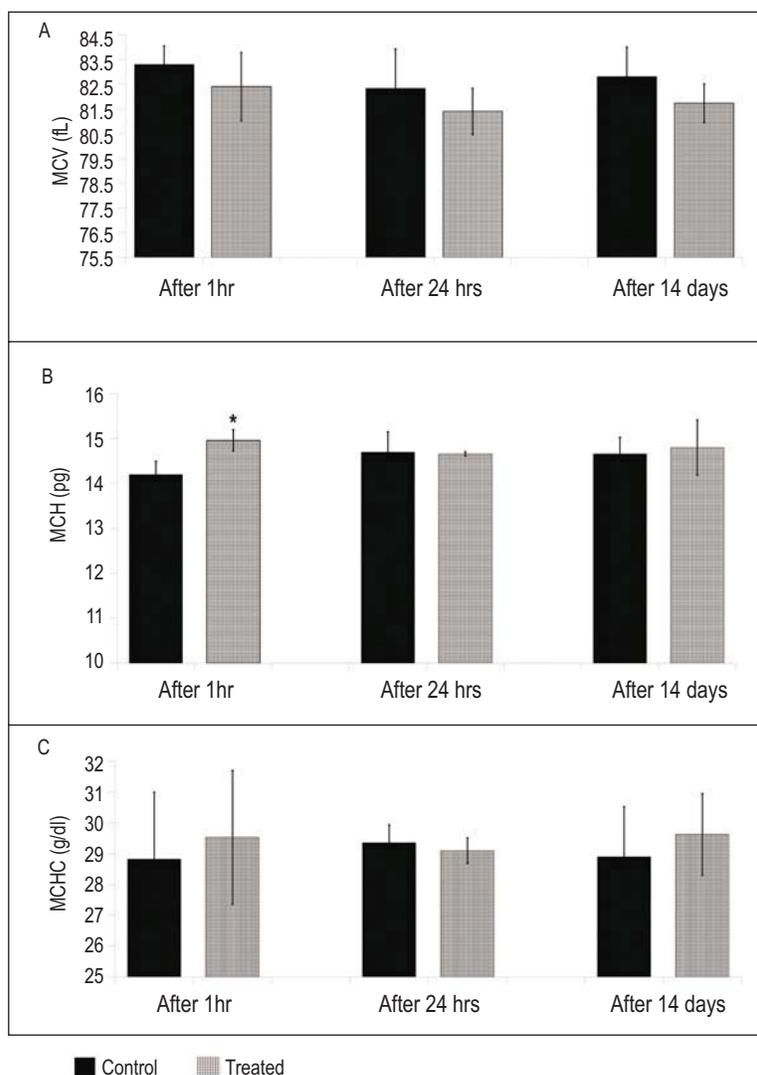


Fig. 1 : Effect of *S. aromaticum* hexane extract on red blood cell indices in rats. A : Mean Corpuscular Volume (MCV), B : Mean Corpuscular Haemoglobin (MCH) and C : Mean Corpuscular Haemoglobin Concentration (MCHC). Values are mean \pm SD of three animals. *indicates significant difference ($P < 0.05$) between control and treated as obtained by Student's t-test

1986). The insignificant effect on these indices relating to red blood cell proposes that there was no change on haemoglobin weight per red blood cell and also in average weight of red blood cell. This suggests that the hexane extract did not induce anaemia throughout the period of treatment.

There was significant increase of 9% and 23% in platelets count after 24 hrs and 14 days of treatment respectively and 10% increase in plateletcrit after 14 days of treatment; whereas 8.5% decrease was seen in PDW percentage after 14 days of treatment with hexane extract of *S. aromaticum* when compared with control ($P < 0.05$). There is no significant difference in MPV ($P > 0.05$) (Fig. 2). Such alterations were also observed in the study reported by Appidi *et al.* (2009) and Devaki *et al.* (2012) where *Hermania incana* leaves produced mild effect on

haematological parameters. The haematological parameters like HGB, RBC and PCV are linked with the total population of red cells; on the other hand MCV, MCH and MCHC are associated with RBC alone. The in non-toxic effect of extract on these haematological parameters proposes that it does not regulate the inclusion of haemoglobin inside red cells and also do not alter the structural features and osmotic brittleness of red blood cells (Adebayo *et al.*, 2005).

The aroma and flavour of *S. aromaticum* buds is due to the presence of volatile oil which is approximately 15 to 20%. The main component phenylpropanoids consists of eugenol, carvacrol, cinnamaldehyde and thymol constitute 60 – 90% of distilled clove bud oil (Adam *et al.*, 2013). Treatment with a foreign herbal compounds may bring about significant

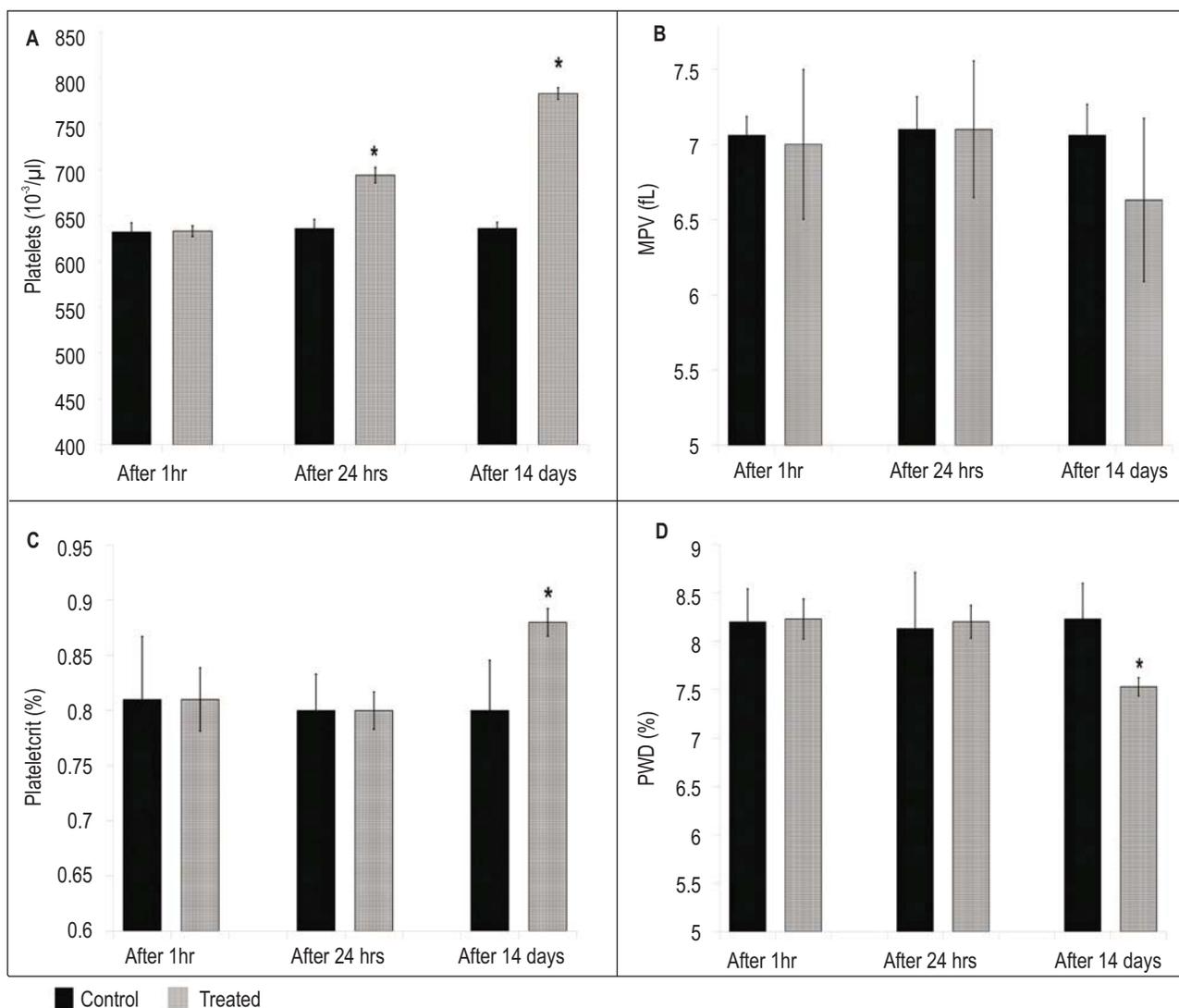


Fig. 2 : Effect of *S. aromaticum* hexane extract on platelet indices in rats. A : Platelets (PTL), B : Mean Platelet Volume (MPV), C : Plateletcrit (PCT) and D : Platelet Distribution Widths (PDWs). Values are mean \pm SD of three animals. *indicates significant difference ($P < 0.05$) between control and treated as obtained by Student's t-test

alterations in the metabolic conversion and concentration of enzymes, biomolecules and also in certain pathways (Oyewole *et al.*, 2007). United States Food and Drug Administration declared clove oil as "Generally Regarded as Safe" substance when administered at a level of 1500 ppm in all food categories. Additionally the acceptable daily intake (ADI) of this oil was set as 2.5 mg kg^{-1} body weight for humans by WHO Expert Committee on food additives (Gulchin *et al.*, 2012). It has been concluded that the rats exposed to hexane extract of *S. aromaticum* neither showed any signs of toxicity nor mortality during the course of study. Further the hexane extract of *S. aromaticum* did not induce any change on the blood profile of rats during short term exposure of 14 days.

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