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The Effect of Combined Aqueous Extract of Turmeric and Curry Leaves on Haematological parameters and Histology of the Lungs in High Salt fed Wistar Rat.

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Abstract
This study investigated the effect of combined aqueous extract of Turmeric and curry leaves extract on the Haematological parameters and histology of the lungs in high salt fed Wistar rats. A total of 36 male Wistar rats were selected and they were randomly divided into 6 groups of 6 animals each. After acclimatization for 14 days, group one served as the control, and received no treatment other than food and water. Group two; received salt (8%). Group three received salt and vitamin C, group four received a low dose of the extract with salt, group five received medium dose of the extract and salt, group six received high dose of the extract and salt. The administration was done once daily for 28 days. Twelve animals were sacrificed on the 14th and 28th day respectively. Blood samples and lungs were collected from each animal to analyze the haematological parameters and histology of the lungs respectively. The results of this study showed that antioxidant levels are present in turmeric and curry leaves, and vitamin C is able to reduce the damaging effect of high salt intake on the lungs of Wistar rats. The results of this study show that high salt intake causes an increase on haematological parameters, combined extract of turmeric and curry leaves help reverse its increase.

Keywords: Curry leaves, Hematology, High salt intake, Histology, Turmeric

Introduction

Salt (NaCl) is important in human foods as it adds flavor and also acts as an ingredient for preservation. Salt (sodium chloride) is a vital component of our diets, and it is important for the proper functioning of different parts of the body. It has been estimated that 75% of the salt intake in the United State is derived from salt added during food processing or manufacturing, rather than from salt added at the table or during cooking. The lowest salt intakes are associated with diets that emphasize unprocessed foods, especially fruits, vegetables and legumes (FNBIM/SC, 2005; Westphal *et al.,* 2012; WHO, 2013). High sodium intake decreases renal calcium reabsorption which in turn leads to greater urinary calcium excretions, osteoporosis and kidney stones (Heller, 1999; Audran and Legrand, 2000). Gastric cancer is another condition linked with high salt intake (Tsugane, 2005; Liu and Russell, 2008). Sodium intake is also associated with high blood pressure (hypertension) (Denton *et al.,* 1995). Death may also result from attempted use of salt solutions as emetics, forced salt intake and accidental mix-up of salt with sugar in child food. High salt loading in humans and experimental animals increases the stiffness of conduit arteries and the activity of resistance arteries (Simon and Illyes, 2001). Stroke is another effect of high salt intake (Xie *et al.,* 1992). High salt intake leads to platelet aggregation (Gow *et al.,* 1992).

The role of salt intake as a risk factor for asthma, bronchial hyper responsiveness, and other bronchial symptoms has been addressed in a number of studies as reviewed by Mickleborough and Fogarty (2006) and as collectively, these studies indicate an increased risk of bronchial symptoms with high consumption of salt. To our knowledge, no

prospective epidemiological studies have specifically focused on dietary salt intake as a risk factor for chronic bronchitis (CB).

The use of herbs for prevention and/ or treatment of diseases are as old as humankind and so many synthetic drugs are of plant origin. The medicinal values of these plants are usually due to the presence of phytochemical content (Essien *et al.,* 2012) and the most important of these phytochemicals include alkaloids, tannins, flavonoids and phenolic compounds. When these compounds are extracted and administered to animals, they exert some biochemical and pharmacological actions in them.

Natural plant products have been used throughout human history for various purposes. Having co-evolved with animal life, many of the plants from which these natural products are derived are billions of years old. Tens of thousands of these products are produced as secondary metabolites by higher plants as a natural defense mechanism against disease and infection. Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design. Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman and Cragg, 2007).

The Indian system of holistic medicine known as Ayurveda uses mainly plant-based drugs or formulations to treat various ailments, including cancer. Of the at least 877 small-molecule drugs introduced worldwide between 1981 and 2002, the origins of most (61%) can be traced to natural products (Newman and Cragg, 2007). Although many synthetic drugs are produced through combinatorial chemistry, plant-based drugs are more suitable, at least in biochemical terms, for human use.

Turmeric, referred to as the queen of spices, is a rhizomatous herbaceous perennial plant (curcuma longa) of the ginger family Zingiberaceae. It is considered as a principal ingredient in many types of dishes originated from Bangladesh and India due to its attractive color, flavor and taste (Gupta *et al.,* 2013). Its rhizomes contain approximately 2%, volatile oils 5%, curcuminoids as well as approximately 69.43% carbohydrates 6.30% protein, 5.10% oil, and another important element in dry turmeric (Khan *et al.,* 2015).

Turmeric has been put to use as a foodstuff, cosmetic, and medicine. It is widely used as a spice in South Asian and Middle Eastern cooking. It lends curry its distinctive yellow color and flavor. It is used as a coloring agent in cheese, butter, and other foods (Ammon and Wahl, 1991).

Today, turmeric is promoted as a dietary supplement for a variety of conditions, including arthritis, digestive disorders, respiratory infections, allergies, liver disease, depression, and many others. Turmeric is a common spice and a major ingredient in curry powder. Traditionally, turmeric has been used as a medicinal plant with its various biological activities such as strengthening energy, antioxidant, antibacterial, anti-inflammatory, anticancer, and wound healing.

Curry leaf (*Murraya koenigii*) belongs to the family rutaceace consisting of 150 genera and 1600 species. It is found to be native to South Asia particularly India, Sri Lanka and Bangladesh. The use curry leaf dates back to 1st and 4th century AD. Tamil and Kannada literature describes curry leaf as Kari used as a flavoring agent. It is considered as one of the important ingredients in South Asian cuisine for its fragrance and aroma (Budha-Magar *et al.,* 2020). It maintains its flavor and other qualities even after drying, making it to be used as a popular spice and condiment in tropical countries (Verma, 2020). The leading component for flavor and aroma of curry leaf includes pinene, sabinene, caryophyllene, cardinol, and cardinene. The whole plant is regarded as tonic and stomachic and has traditional uses.

Murraya koenigii has been found to have bioactive phytochemicals like alkaloids, essential oils, phenolics, minerals and proteins, terpenoids, tocopherol, β-carotene and lutein. It can be used fresh, dried, powdered or in cooked form. It has many name forms, that is, Curry Leaf in English, Mitha Neem in Hindi, Karuveppilai in Tamil and Surabhinimba in Sanskrit (Henry,

2015). Curry Leaf plants can also be used as hedge and ornamental shrub due to their compound leaves. *Murraya koenigii* is distributed in the moist forest of Asian regions particularly Nepal, Bhutan, Loas, Pakistan, Thailand and cultivated all over India. It is rarely observed outside the Indian sphere of influence (Kumar *et al.,* 2013). Herbal drugs are highly effective with minimum side effects and are to be used extensively in treating various disease and they are relatively low cost.

It is obvious that high salt loading will impact negatively on the blood homeostasis which may lead to deleterious effect on the body, with the existing scarcity of scientific information on the combined effects of turmeric and curry leaves extract on high salt loading. It is, therefore, the main aim of this study which is to evaluate the effects of combined aqueous extract of curry and turmeric leaves on the hematological and histological parameters of the lungs of Wistar rat fed with high quantity of salt.

Materials and Methods

Procurement of Animals

Thirty-six (36) adults male Wistar rats weighing 180 200g were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The rats were served as the animal model for the study. The rats were housed in clean, disinfected wooden cages with sawdust as bedding. The animal house was provided in a controlled environment with a 12-hour light/dark cycle, 50 60% humidity, and a temperature of roughly 30°C. These conditions were maintained throughout the acclimatization and experimental periods. The rats were allowed to acclimatize for two weeks in the animal house. During this period, they had free access to clean water and standard animal feed. This acclimatization period ensures that the rats adapt to their new environment and stabilize before the commencement of the experiment.

Collection and Identification of Plant Materials

Fresh turmeric rhizomes and curry leaves were purchased from the local market within the University of Port Harcourt environment. The turmeric rhizomes and curry leaves were being taken to the Department of Plant Science and Biotechnology for identification and authentication before use for this study.

Collection of Plant Materials

Fresh turmeric rhizomes and curry leaves were purchased from Rumuokoro market within the University of Port Harcourt environment.

Preparation of Aqueous Extracts

The turmeric rhizomes were carefully cleaned to remove any dirt or debris, using clean water and a soft brush. The curry leaves were plucked from the stems and thoroughly washed to eliminate any impurities. The cleaned turmeric rhizomes and curry leaves were spread out in a single layer on clean trays. They were placed in a well-ventilated area with controlled temperature and airflow to facilitate drying. The turmeric rhizomes and curry leaves were left to dry naturally for 14 days, until they become completely dry and brittle. Once dried, the turmeric rhizomes and curry leaves were sent to the pharmacy department for extraction. The plants were separately ground into fine powders using an electric grinder. The powders will be carefully sieved through a fine mesh to obtain a consistent and uniform texture, ensuring the removal of any coarse particles. 60g of the powdered turmeric and curry leaves were accurately weighed using a digital scale and was put into a maceration jar, distilled water was added in a ratio of 1:1. The mixture was kept for 72 hours, with the maceration jar tightly closed. The mixture was agitated on a daily basis. After 72 hours the mixture was filtered, and the chaff was discarded.

Preparation of high salt diet

20g of sodium chloride was mixed with 80ml of water, making salt solution 1ml of 20%. following the method described by Obiefuna and Obiefuna (2001). The rats included in the study were classified based on their systolic blood pressure (SBP) measurements. Rats with SBP levels equal to or higher than 150 mmHg were categorized as hypertension (HTN) rats, rats with SBP values ranging from 130 to 150 mmHg will be identified as hypertension-prone (HP) rats, and rats with SBP levels below 130 mmHg will be classified as hypertension-resistant (HR) rats, as per the classification method described

by Li *et al*. (2021). This classification system based on SBP was allowed for the selection of rats with different hypertensive phenotypes, enabling the investigation of the effects of the combined aqueous extract of turmeric and curry leaves in the context of high-salt diet-induced hypertension. The high-salt group were given 1 ml of 20% NaCl solution by intragastric administration (gavage) (Dong *et al.,* 2020).

Experimental Design

Groups	Description	No. of Rats	Treatment Protocol
Group 1	Normal control	6	Will be adm inistered 1ml of normal saline and feed for 28 days
Group 2	Negative control	6	Will be administered high-salt diet containing 1ml of 20% sodium chloride without treatment for 28 days
Group 3	Vitamin C	6	Will be administered high-salt diet containing 1ml of 20% sodium chloride and 30mg/kg of vita min C for 28 days
Group 4	Low Dose	6	Will be administered high-salt diet containing 1ml of 20% sodium chloride and 250mg/kg of combined extract for 28 days
Group 5	Medium Dose	6	Will be administered high-salt diet containing 1ml of 20% sodium chloride and 500mg/kg of combined extract for 28 days
Group 6	High Dose	6	Will be administered high-salt diet containing 1ml of 20% sodium chloride and 1000mg/kg of combined extract for 28 days

Dose Administration

The determined dose of the combined aqueous extract was administered orally to the rats in the experimental group.

Sample collection

Sample collected involved lungs and blood samples from the rats on days 14 and 28 of the experiment. These samples were collected using standard techniques and handled with appropriate precautions to ensure accurate and reliable results.

Data Analysis

The data obtained from the hematological parameters was subjected to appropriate statistical analyses using the Statistical Package for Social Sciences (SPSS). Statistical tests, such as analysis of variance (ANOVA) or Student's t-test, was employed to determine significant differences between the experimental groups. The level of significance was set at $p < 0.05$.

Haematological Analysis

This analysis was performed using automated method as described by Randox Laboratories Limited, United Kingdom. Blood sample was collected and placed in an EDTA bottle and was mixed properly by inverting the EDTA bottle intermittently but gently (to avoid in vitro haemolysis), for several minutes. The machine was switched on and allows completing the booting process with the display of the various parameters on the screen. After the booting/warming for 5 minutes, the machine aspirator button (for reagent) was pressed to aspirate the three-basic reagent (the E-Z cleaner, cell lyse and diluents) from their respective containers linked to the machine. The aspiration button for sample was pressed to permit the probe cover to fall out the EDTA bottle containing the blood sample was

upright below the probe in a manner that probe became linked with the blood sample, to allow for aspiration sample of blood into the machine. The aspirated sample of blood was left to properly mix with this different reagent within the counting chambers. The counting if various blood parameters was done automatically within a few seconds with results displayed on the screen and printed by pressing the print button or the lead of the machine. The procedure for shutting down the machine will be initiated by pressing the menu button which then displayed a command to aspire the E-Z cleanser through the probe and this subsequently, cleaned up the whole chambers of the machine. Thereafter the machine will display a final command to depress the switch off button to finish the shutting down process

Histological Examination

The staining procedure for H&E follows a basic protocol:

Histopathology studies: The rats were dissected following diethyl ether anesthetic access the lungs. The lungs were harvested into 10% formaldehyde with subsequent trimming of the lungs to a seize range of 2-4mm. The tissue then undergoes the following: fixation, dehydration, clearing, impregnation, embedding, sectioning and staining.

Fixation: It was performed with 10% phosphate buffered formaldehyde solution at PH 7 for six to twelve hours to preserve the various cellular components in their normal micro-anatomical positions and to prevent it from any form of dehydration.

Dehydration: Dehydration of the fixed tissues was done to remove water from the tissue in an ascending order of 50% alcohol for 3hours, 70% alcohol for 1-3hours, 90-95% alcohol for 12 hours and 100% alcohol for 1-2hours.

Clearing: After dehydration, tissues were infiltrated with xylene for two hours to allow miscibility or solubility in paraffin at a temperature of 60 Celsius for two hours each in two changes.

Impregnation: This removes traces of agents from tissues. The tissues were transferred from xylene into a molten paraffin at a temperature of 60 Celsius for two hours each in two changes.

Embedding: the tissue block was being oriented so that the sections were cut in a desired plane of tissues. Tissues were immersed in the molten paraffin wax at 60 degrees Celsius and allowed to cool and solidify.

Sectioning, Mounting and Staining: The tissue block will be taken to a microtome for sectioning. The sectioned ribbons will be warned at 37^0C to straighten wrinkles. Haematoxylin and Eosin were used to stain the tissues, and the slides sent to histopathology laboratory for examination and evaluation of Histological changes. The slide was put in xylene for 5 to 10 minutes, then transferred to absolute alcohol, and to 95% alcohol and finally to 70% alcohol in seconds. The slide was rinsed in water and stained with haematoxylin for 12 to 20 minutes. It was then rinsed in water to remove excess stain, then differentiated and put in 1% acid alcohol for 5 seconds. This removes excess stain and helps the nucleus to absorb the stain. It was then rinsed in two changes of water for 2 to 5 seconds. This is called bluing, and it gives the stain tissues its characteristics background. The section was stained with Eosin for 3 to 5 minutes, rinsed in water to remove excess stain and dehydrated in absolute alcohol. It was then mounted with DPX and cover slip. Each of the slides was viewed under the microscope and was photographed under the magnification of x400 H&E and later printed into photo micrograph pictures.

Statistical Analysis: The result of the measurements was shown as means and the standard error of the mean. The mean difference was obtained by using ANOVA and post hoc with the least significant difference.

Results

The Effect of vitamin C and combined Extract of tumeric and curry leaves (TCL) on haemoglobin (HB)(g/dl) leaves in high salt fed Wistar rats is shown in Table 1. It was observed that haemoglobin was lowest for group 6 (11.90±0.50) followed by group 4 (12.32±0.64) and was highest in group 2 (18.72±2.48) followed by group 1(13.87±0.29) while on day 28, the haemoglobin was lowest in group 5 (12.40±0.50) followed by group 6(12.53±0.28) and was highest in group 2 (21.28±2.05) followed by group 1(13.87±0.29). Haemoglobin levels were significantly raised in NT group compared with control group at ($p<0.05$). There's no significant increase in TCL group compared to NT control group.

Table 1. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves (TCL) on Haemoglobin (Hb) (g/dL) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	13.87±0.29[b]	13.87±0.29[b]
1ml of 20% NaCl	18.72±2.48[a]	21.28±2.05[a]
Vitamin C	12.83±0.30[b]	13.14±0.19[b]
250mg/kg Extract of TCL	12.32±0.64[b]	12.58±0.35[b]
500mg/kg Extract of TCL	13.43±0.28[b]	12.40±0.50[b]
1000mg/kg Extract of TCL	11.90±0.50[b]	12.52±0.28[b]

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

The Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Packed Cell Volume (PCV) (%) levels in High salt-fed Wistar rats is shown in Table 2. It was observed that the value of PCV was lowest in group 1(38.50±2.92) followed by group 3 (39.20±3.93) on day 14, while on day 28, the PCV was lowest in group 1(38.40±2.92). Followed by group 4 (38.54±4.28) and was highest in group 2(45.20±10.28) followed by group 4 (42.62±4.90). There is significant increase in NT group compared to Control group ($p<0.05$). There is no significant increase in TCL group compared to NT group ($p>0.05$).

Table 2. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Packed Cell Volume (PCV) (%) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	38.50±2.92[b]	38.50±2.92[b]
1ml of 20% NaCl	45.20±10.29[a]	49.25±5.01[a]
Vitamin C	39.20±3.93[b]	43.40±3.63[a,b]
250mg/kg Extract of TCL	42.62±4.90	38.54±4.28[b]
500mg/kg Extract of TCL	40.82±6.42[b]	42.60±5.10[b]
1000mg/kg Extract of TCL	39.48±3.48[b]	42.45±4.60[b]

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

The Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Red Blood Cell (RBC) (million cells/mcL) levels in High salt-fed Wistar rats is shown below in Table 3. It was observed that the value of RBC was lowest for group 2 (5.98±0.39) followed by group 6 (6.02±0.29) and was highest in group 1 (6.90±0.21) followed by group 5 (6.80±0.23) in day 14, while on 28, the RBC level was lowest in group 2 (5.40±0.25) followed by group 4 (6.26±0.70) and highest was in group 1 (6.90±0.21) followed by group 6 (6.78±0.50) . There is no significant increase in NT group compared to Control group. It was observed that there's a significant increase between TCL group and NT control group.

Table 3. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Red Blood Cell (RBC) (million cells/mcL) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	6.90±0.21	6.90±0.21
1ml of 20% NaCl	5.98±0.39	5.40±0.25
Vitamin C	6.57±0.21	6.71±0.61
250mg/kg Extract of TCL	6.10±0.38	6.26±0.70
500mg/kg Extract of TCL	6.80±0.23	6.55±0.31
1000mg/kg Extract of TCL	6.02±0.29	6.78±0.50

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

The Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on White Blood Cell (WBC) (million cells/mcL) levels in High salt-fed Wistar rats is shown in Table 4. It was observed that the value of WBC was lowest for group 1 (8.23±1.43) followed by group 6 (10.50±0.81) while WBC was highest for group 2 (20.34±3.53) followed by group 5 (13.85±2.26) in 14 days. While in 28 days group 1 has the lowest value (8.23±1.43) followed by group 6 (9.46±2.50). Group 2 (16.20±2.75) has the highest value followed by group 4 (13.78±2.25). There was a significant increase in WBC of NT control group compared to normal control group. It was also observed that there is no significant increase between the TCL group and the NT control group.

Table 4. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on White Blood Cell (WBC) (million cells/mcL) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	8.23±1.43[b]	8.23±1.43[b]
1ml of 20% NaCl	20.34±3.53[a]	16.20±2.75[a]
Vitamin C	13.65±0.94[a,b]	12.94±2.50[b]
250mg/kg Extract of TCL	13.30±0.72[a,b]	13.78±2.25[a]
500mg/kg Extract of TCL	13.85±2.26[a,b]	12.55±2.45[a]
1000mg/kg Extract of TCL	10.50±0.81[b]	9.46±2.50[b]

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

The Effect of Vitamin C and combined extract of Turmeric and Curry leaves on Platelet levels in High salt-fed Wistar rats is shown in Table 5. It was observed that the value for platelets was lowest for group 1 (470.00±91.2) followed by group 6 (505.4±107.83) while platelets was highest in group 2 (735.25±41.09) followed by group 4 (674.00±32.87) at 14 days. There is significant increase in NT control group compared to Control group, and there is significant increase in TCL group compared to NT control group.

Table 5. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Platelet (PLT) (million cells/mcL) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	470.00±91.23[b]	470.00±91.23[b]
1ml of 20% NaCl	737.25±41.09[a]	782.26±50.15[a]
Vitamin C	541.17±28.92[a,b]	526.45±45.20[a,b]
250mg/kg Extract of TCL	674.00±31.87[a,b]	610.17±40.35[a,b]
500mg/kg Extract of TCL	526.00±70.06[a,b]	496.89±62.45[b]
1000mg/kg Extract of TCL	505.40±107.83[a,b]	477.06±36.34[b]

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

The effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Mean Corpuscular Hemoglobin Concentration (MCHC) (g/L) levels in High salt-fed Wistar rats is shown in Table 6. It was observed that the value for MCHC was lowest for group 1 followed by group 3 and was highest in group 2 followed by group 6 in days 14. In day 28 the value was lowest in group 1 followed by group 6 and was highest in group 2 followed by group 3. There is a significant increase in NT control group compared the control group.

Table 6. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Mean Corpuscular Hemoglobin Concentration (MCHC) (g/L) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	32.00±5.28	32.00±5.28
1ml of 20% NaCl	35.50±4.95	35.65±5.25
Vitamin C	33.47±2.41	32.95±5.20
250mg/kg Extract of TCL	32.96±4.07	33.70±4.55
500mg/kg Extract of TCL	33.68±7.20	32.90±3.50
1000mg/kg Extract of TCL	32.82±6.32	32.76±3.64

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4.

The Effect of Vitamin C and combined extract of Turmeric and Curry leaves on MCH levels in High salt-fed Wistar rats is shown in Table 7. It was observed that group 2 has the lowest value (18.98±3.63) followed by group 4 and group 1 has the highest value followed by group 5 (19.28±3.92) in 14 days. While in 28 days group 2 has the lowest value (18.40±3.05) followed by group 5 (18.75±2.11) and group 1 has the highest value (20.51) followed by group 3(19.56±2.69). It was observed that there is no significant increase between the NT control group and the normal control group, and there is a significant increase between the TCL group and the NT control group.

Table 7. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Mean Corpuscular Haemoglobin (MCH) (g/L) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	20.51±3.23	20.51±3.23
1ml of 20% NaCl	18.98±3.63	18.40±3.05
Vitamin C	19.06±2.85	19.55±2.69
250mg/kg Extract of TCL	19.50±2.48	19.10±3.05
500mg/kg Extract of TCL	19.28±3.92	18.75±2.11
1000mg/kg Extract of TCL	19.06±2.27	19.45±4.05

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4.

The effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Mean Corpuscular Volume (MCV) (g/L) levels in High salt-fed Wistar rats is shown in Table 8. It was observed that group 2 has the lowest value (58.82±6.83) followed by group 6(60.40±5.73) and group 4 has the highest value (63.40±6.47) followed by group 5(60.52) in 14 days. While in 28 days, group 2(58.60±6.25) has the lowest value followed by group 3 (61.50±4.76) and 4(61.40±4.55) while group 1 has the highest value. It was observed that there is no significant increase between the NT control group compared with the normal control group. And there is a significant increase between the TCL group compared to the NT control group.

Table 8. Effect of Vitamin C and combined Extract of Turmeric and Curry leaves on Mean Corpuscular Volume (MCV) (g/L) levels in High salt-fed Wistar rats.

Group	Day 14	Day 28
Normal control	61.83±5.76	61.83±5.76
1ml of 20% NaCl	58.82±6.83	58.60±6.25
Vitamin C	60.85±9.85	61.50±4.76
250mg/kg Extract of TCL	63.40±6.47	61.50±4.55
500mg/kg Extract of TCL	60.51±5.80	61.25±5.05
1000mg/kg Extract of TCL	60.40±5.73	60.60±7.01

[a]value is significant when compared to normal control at $p<0.05$, [b]value is significant when compared to negative control (1ml of 20% NaCl) at $p<0.05$, n=4

Results Analysis of the Lungs

Group 1 showed normal architecture of the terminal bronchioles with Smooth columnar epithelial lining (Ep), smooth muscle, adventitia, lamina propria and mucosa fold (Plate 1A). The diagnosis is that the lungs tissue is with normal terminal bronchioles; Group 2 showed the effect of sodium chloride on the lungs for 14 days without treatment. It showed the lungs with its terminal wall infiltrated by acute and chronic inflammatory cells (Lymphocytic activities) (Plate 1B). It indicates severe inflammation of the lung tissue. Group 3 showed the effect of vitamin C on the lungs in Wistar rats after treatment. It showed the lungs with mild alveolar sac wall, infiltrated by acute and chronic inflammation cells (regressive Lymphocytic activities) (Plate 1C). It indicates mild inflammation of the lung tissue. Group 4 showed the effect of sodium chloride in the lungs with 250 mg/kg of combined extract of turmeric and curry leaves after 14 days. It showed the lungs

with thin alveolar (Plate 1D). It indicates reduced inflammation of the lung tissue. Group 5 showed the effect of sodium chloride in the lungs with 500 mg/kg of combined extract of tumeric and curry leaves after 14 days. It showed the lungs with mild congestion of alveolar sacs wall, with haemosiderin deposit (hyperamia of the lungs tissues) (Plate 1E). It indicates dysperamia of the lungs tissue. Group 6 showed the effect of sodium chloride in the lungs with 1000 mg/kg of combined extract of turmeric and curry leaves after 14 days. It showed the lungs with mild alveolar sac wall, infiltrated by acute and chronic inflammatory cells (regressive lymphocytic activities) (Plate 1F). This is called mild inflammation of the lung tissues. It indicates mild inflammation of the lung tissue.

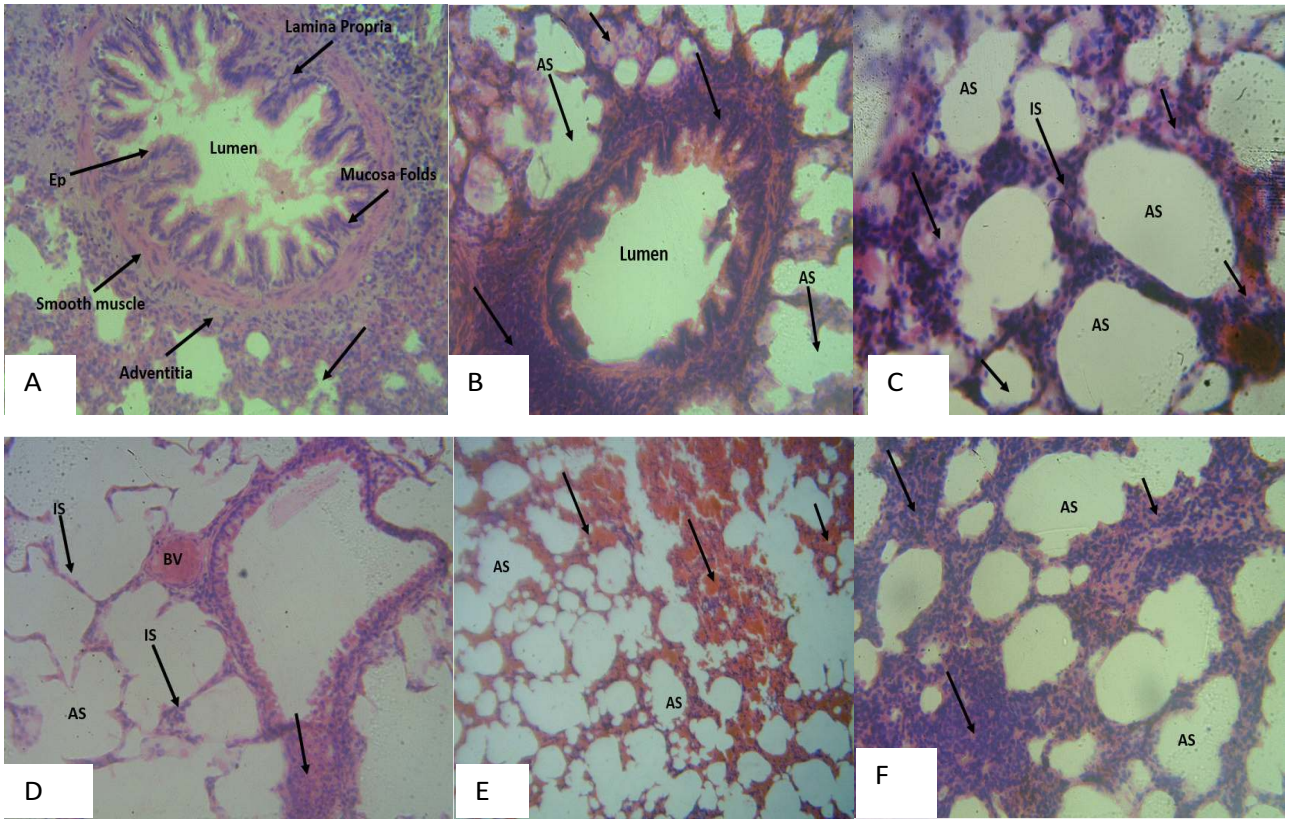

Plate 1. Different analysis of the lungs; A - Normal architecture of the terminal bronchioles with smooth columnar epithelial lining (EP) smooth muscle, adventitia ,lamina propria and mucosa fold; B - the lungs with its terminal wall infiltrated by acute and chronic inflammatory cells (lymphocytic activities) (arrows); C - lungs with mild alveolar sac wall, infiltrated by acute and chronic inflammatory cells (regressive lymphocytic activities) (arrows); D - lungs with thin alveolar sac wall, with reduced inflammatory cells activities (arrows); E - the lungs with mild congestion of the alveolar sac wall, with haemosiderin deposits (arrows); F - lungs with mild alveolar sac wall, infiltrated by acute and chronic inflammatory cells (regressive lymphocytic activities) (arrows). (H&E X400)

Group 1 showed the effect of sodium chloride in the lungs without treatment for 28 days. It showed the alveolar wall (arrows) with well differentiated alveolar sac (AS) and with interalveolar sept (IS) and also alveolar cells (type I and type II pneumocytes) with mild mononuclear activities (Plate 2A). This showed a lung tissue appearing normal with mild inflammation. Group 2 showed the effect of vitamin C in the lungs with 250 mg/kg of tumeric and curry leaves for 28 days. It shows the thickened bronchial wall, infiltrated by acute and chronic inflammatory cells (lymphocytic activities) (Plate 2B). It indicates severe inflammation of the lung tissue; Group 3 showed the effect of sodium chloride with 500 mg/kg of combined extract of turmeric and curry leaves on the lungs of Wistar rats after 28 days. It showed thickened and dilated alveolar sac wall, infiltrated by acute and chronic inflammatory cells (lymphocytic activities) (Plate 2C). It indicates

inflammation of the lung tissues. Group 4 showed the effect of sodium chloride in the lungs with 500 mg/kg of tumeric and curry leaves. It showed hemosiderin deposit clogging the alveolar sac causing congestion of the alveolar sac wall (Plate 2D). It indicates severe hyperamia of the lungs tissue. Group 5 showed regressive changes in the alveolar sac wall with visible alveolar sac (AS), interalveolar sept (IS) and alveolar cells (type I and type II pneumocytes) (Plate 2E). It indicates tissue appearing normal (Possible regeneration of the lung tissue).

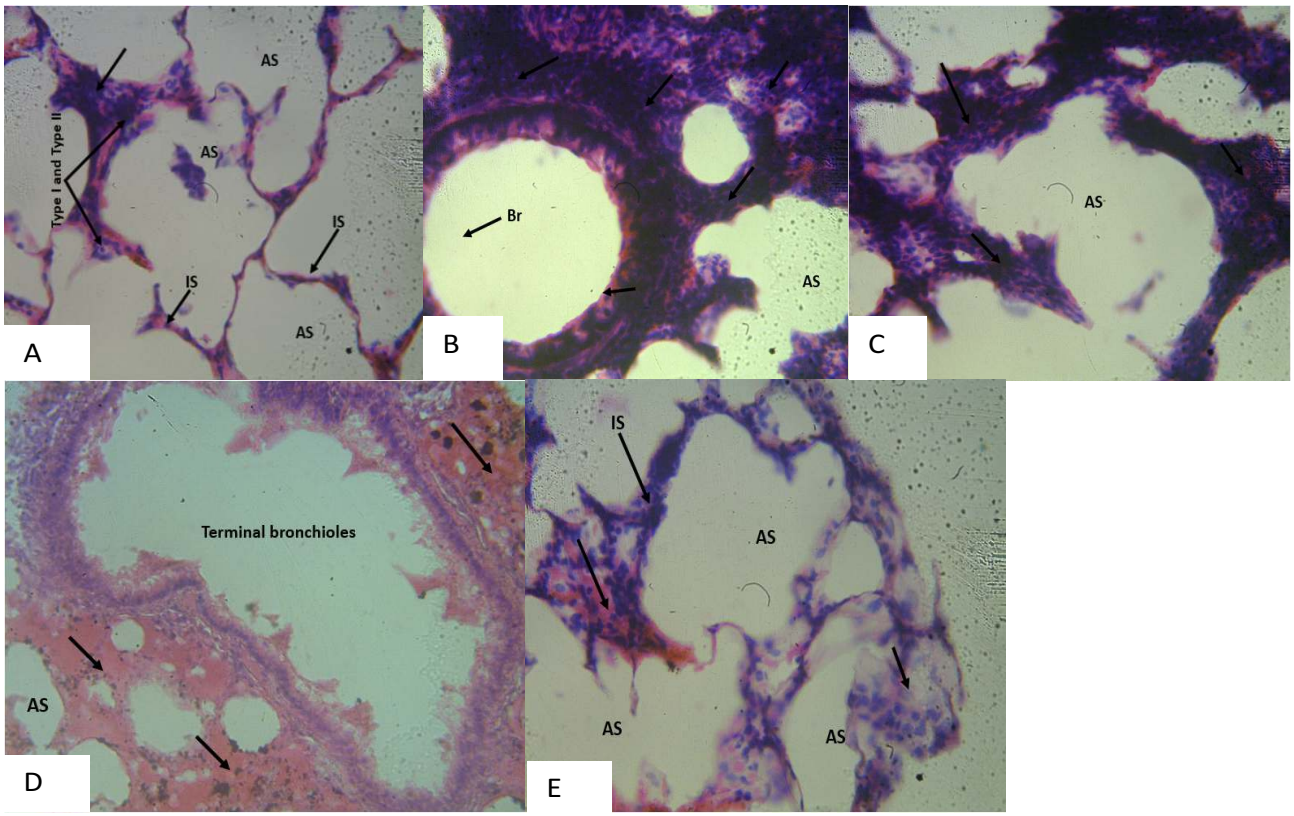

Plate 2. A - lungs with well differentiated alveolar sac (AS) and with interalveolar sept (IS) and also alveolar cells (type I and type II pneumocytes) with mild mononuclear activities (arrows); B - lungs with Thickened bronchial wall, infiltrated by acute and chronic inflammatory cells (lymphocytic activities) (arrows); C - lungs with thickened and dilated alveolar sac wall, infiltrated by acute and chronic inflammatory cells (lymphocytic activities) (arrows); D - lungs with hemosiderin deposit clogging the alveolar sac causing congestion of the alveolar sac wall (arrows); E - lungs with regressive changes in the alveolar sac wall with visible alveolar sac (AS), interalveolar sept (IS) and alveolar cells (type I and type II pneumocytes) (arrows). (H&E X400)

Discussion

In this study, the effects of combined extract of turmeric and curry leaf on RBC, PCV, Hb, absolute values (MCV, MCH, MCHC), platelet count and platelet indices were measured in high salt fed rats. Blood is a tissue fluid which consists of fluid portion or plasma that is suspended by some formed elements (erythrocytes, leucocytes and thrombocytes). Blood provides the basic connections between the various organs and cells of the body and to maintain a constant cellular environment by circulating through every tissue delivering nutrients to them and removing waste products (Ganong, 2003; Sembulingam and Sembulingam, 2006). The blood cells (erythrocytes, leucocytes and thrombocytes) are regulated so that excessive variation in their quality and quantity is prevented. This regulation is via some specialize feedback mechanism for the cells (Guyton and Hall, 2004; Sembulingam and Sembulingam, 2006). Findings revealed that the combined extract of tumeric and curry leaves had tremendous effect on the levels of the blood cells in high salt fed rats.

The high salt fed rats had significant increase in their total white blood cells, red blood cells and packed cell volume, the extract was observed to bring the elevated RBC, PCV and total WBC in the high salt-loaded rats to near control values. Therefore, with this stable level of red blood cells, is very likely that the blood viscosity was kept unchanged by the combined extract of turmeric and curry leaves. In other words, if the extract had caused an increase or decrease in RBC count, this could have led to a corresponding increase or decrease in viscosity. Combined extract of turmeric and curry leaves also reversed low platelet count induced by high salt loading. Platelets are very useful in blood clotting, their reduction is associated with bleeding tendencies, hence the ability of the extract to ameliorate bleeding tendencies associated with thrombocytopenia, especially when triggered by high salt load. Mean Platelet Volume (MPV) is the indicator for platelet function, including aggregation, release of thromboxane A2, platelet factor 4,beta-thromboglobulin (Sharp *et al.,* 1995) and expression of glycogen 1b and glycogen IIb/IIIa receptors (Tschoepe *et al.,* 1990; Giles *et al.,* 1994).

The MPV was significantly altered in this study following high salt loading. Mean platelet volume a determinant of platelet function; is a newly emerging risk factor for athero-thrombosis. Increase in MPV has been documented in patients with metabolic syndrome, stroke and Diabetes Mellitus (DM) (O□Malley *et al.,* 1995; Tavil *et al.,* 2007). Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischaemia and transient ischemic attacks (McCabe *et al.,* 2004; Nadar *et al.,* 2004; Kilicli-Camur *et al.,* 2005; Khandekar *et al.,* 2006) and chronic vascular disease (Endler *et al.,* 2002). Extract of turmeric and curry leaves reversed the increase in MPV in high salt loaded rats. Other platelet indices (platelet large cell ratio and platelet distribution width) also were reduced to near control level in high salt fed rats by combined extract of tumeric and curry leaves. The elevated lymphocyte and low neutrophil count in the high salt loaded rats were reversed to near control values by combined extract of tumeric and curry leaves. The increase in lymphocyte count in high salt loaded rats is a reflection of perturbation of the immune system.

The results of this study revealed that the lungs histo-architecture was normal in control animals. The effect of salt showed severe inflammation of the lungs tissues. These findings agree with animal□s experimental studies by (Mickleborough *et al.,* 2006) demonstrated that salt loading affected leukotriene metabolism involved in bronchial reactivity. In human experimental study, a high salt diet also induced a stronger inflammation response compared to a low salt diet among a group of men exposed to exercise (Mickleborough *et al.,* 2005). Another experimental study suggested that salt manipulation modified the induced sputum supernatant IL-Ib and IL-8 concentration after exercise (Hashimoto *et al*., 1999). These cytokines are associated with neutrophils inflammation, a typical feature of CB and rarer in asthma.

The effect of vitamin C combination with sodium chloride on the lungs showed mild inflammation of the lungs tissues. In a similar study, the relaxant effect on tracheal smooth and anti- inflammatory mechanism was shown. The effect of sodium chloride treated with low dose combined extract of turmeric and curry leaves showed thin alveolar sacs with reduced inflammation of the lungs tissues, sodium chloride treated with high dose showed mild inflammation of the lungs tissues. Curcumin is a potent immunomodulatory agent that can attenuate the activation of T cells, B cells macrophages, neutrophils natural killer cells and dendrites. Curcumin is also reported to down regulate the expression of pro-inflammatory cytokines including tumor necrosis factor.

Conclusion and Recommendation

We conclude that combined extract of turmeric and curry leaves prevents deleterious changes in total WBC, RBC count, packed cell volume (haematocrit), platelet count, lymphocytes, neutrophils, MPV, P-LCR and PDW in high salt fed rats, these parameters are determinants of the blood volume, haemostasis and the tendencies to excessive bleeding, cardiovascular diseases, hypertension and sudden death.

The results from the histology of the lungs have shown that antioxidant level present in tumeric,

curry leaves and vitamin C, are able to reduce the damaging effect (inflammation) of sodium chloride in the lungs. Turmeric and curry leaves extract at an appropriate dose play an important role on platelets; that is turmeric and curry leaves extract can be used as an anticoagulant. We recommended that combined extract of turmeric and curry leaves, vitamin C can be added to the administering of high salt diet for treatment of inflammation.

Declaration of Conflict of Interest

The authors declare that there was no conflict of interest

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