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Haematological parameters of leiomyomatous Wistar rats administered a mechanically-engineered drug pellets doses of *Spondias mombin* and *Curcuma longa*

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ABSTRACT

Uterine leiomyoma is popularly known as fibroid. It is the most common myometrial tumour ravaging African women of child-bearing age and has enormous socio-economic and health consequences. The disease develops earlier and faster in black women than other races and scientifically this disparity is not understood. This work investigated effects of oral intake of pellets prepared as chemotherapy from aqueous extracts of *Spondias mombin* and *Curcuma longa* on female Wistar albino rats with monosodium glutamate-induced leiomyoma. Drug pellets from these samples were produced through a mechanically engineered process and the research objective was to investigate possible effects of these pelleted drugs on haematological parameters of leiomyomatous rats after oral administration. Twenty-four rats acclimatized for two weeks and divided into four groups of six rats each were used for this study. Leiomyoma was induced on rats in groups II to IV with the daily administration of 750mg/kgbw pellets of MSG for twenty-eight (28) days. Groups III and IV rats continued to receive formulated MSG pellets with the different extracts in the second twenty-eight (28) days. The neutrophil and eosinophil levels of control II rats increased significantly in weeks 3 (29.20±3.49, 3.00±1.00) and 4 (31.60±2.07, 5.60±0.55), compared to that of control I and group IV rats (25.20±2.28, 1.40±0.55; 25.00±2.12, 1.20±0.45). Lymphocyte levels of control II rats decreased significantly (P≤0.05) in weeks 3 (55.60±3.78) and 4 (50.60±3.21) when compared to group IV. The level of red blood cells decreased significantly (P≤0.05) in all groups except control I compared to white blood cell levels which decreased significantly (P≤0.05) in group IV. The 750mg/kgbw pellets prepared from *Curcuma longa* water extract alleviated MSG effect on fibroid compared to *Spondias mombin*.

Keywords: Uterine leiomyoma, pellets, fibroids, haematology, blood.

INTRODUCTION

In our world today, man-made chemicals have become part of our everyday life [1] and this influences hormones, which affect health, the development and progression of diseases including uterine leiomyoma. Uterine fibroid (UF) or uterine leiomyoma (UL) is the most common myometrial tumour ravaging African women of child-bearing age [2] with enormous socio-economic and health consequences. The disease develops earlier [3] and faster in black women than other races [4] and scientifically, this disparity is not completely understood [5]. Leiomyomas are benign tumours [6] composed of disordered smooth muscle cells and buried in abundant quantities of extracellular matrix (ECM) when compared to the surrounding uterine smooth muscles [7]. The ECM is well organized in normal tissues but highly disorganized in fibroid tissues [8]. ECM is a structural component of tissues that influences cellular and organ function [9], and involved in wound healing process [10]. In fibrotic tissues, the ECM would consist of matrix molecules which fill the wound (fibrotic) areas thereby not providing proper functional

integrity of the muscle cells and leading to loss of tissue function [10]

Hormonal factors are implicated in the development and progression of this disorder which is a major indicator of hysterectomy worldwide [11]. Hormones regulate human and animal cells. They modify biological processes and are therefore essential for reproduction, survival and biological performance [12]. Meanwhile, biological and cellular functions of these hormones are disrupted by chemicals (EDCs). These EDCs are often taken up in diet (like the MSG) or drinks (example bisphenol A) [13]. They can leak from polycarbonate plastics, bottles, and cans use to line food and water containers [14] or through inhalation of contaminated gases (e.g. cigarette smoke or dust) [15];[16]. Endocrine disruptors (EDs) with oestrogenic action have direct effects on genes where they cause DNA damage by promoting malignant differentiation of affected cells [16]. Results have shown that early puberty in girls, while contributed to by many factors including nutrition, stress, and ethnicity, may in part be due to exposures to oestrogenic EDCs [17] [18]. Such oestrogenic compounds are also associated with uterine fibroids, ovarian dysfunction, and subfertility in humans and in animal models [19]. Oestrogen has been implicated in the prevalence of uterine fibroid [20]. Factors that influence the overall exposure to oestrogen affect the incidence of developing uterine fibroids, consequently, exercise which decreases oestrogen concentration, slows its development [21]. Oestrogen is carcinogenic according to animal studies [22] and the International Agency for Research on Cancer [23]. It promotes cell proliferation, which may increase the likelihood of spontaneous mutations caused by errors during DNA replication. Once these mutations are present within the genome, continued replication would create clones of the mutations or perhaps more mutations. If these errors are uncorrected (either through repair or apoptosis) they can lead to a carcinogenic phenotype. Oestrogenic hormones exert their actions when bound to oestrogen receptors (ERs). Endocrine disruptors bind to these ERs altering the functions of normal oestrogen. And because EDCs are not natural hormones, a single EDC may have the ability to affect multiple hormonal signalling pathways. Thus, it is quite likely that one type of EDC can disrupt two, three, or more endocrine functions, with widespread consequences on the biological processes that are controlled by those vulnerable endocrine glands.

Medicinal plants, have contributed immensely to the management and treatment of diseases with *Spondias mombin* (Linn) known as the hog plum or yellow mombin and *Zingiber officinale* known as ginger not exceptions [1]. Yellow mombin or Hog plum (*Spondias mombin* Linn) is a fructiferous tree widely used in Nigeria for its medicinal and non-medicinal purposes as in cattle feed, house and boat building, etc [24]. It belongs to the family *Anacardiacae* and grows optimally up to the height of 15 – 22m in the rain forest and coastal areas [25]. It is known locally in Nigeria as *Ijikara* in Igbo, *tsadar masar* in Hausa, and *akika* in Yoruba [26]. *Spondias mombin* has been reported to have abortifacient and uterine muscle contraction effects [27]. The powdered bark of the tree is applied on wounds while a tea made from the flowers and leaves is taken to relieve stomach ache and throat infections [24].

This work was designed to directly target leiomyomatous cells of female albino Wistar rats with pelleted drugs prepared from water extracts of these plant samples. The aim was to pioneer the use of water extracts from these plants in the preparation of drugs and other forms of chemotherapy in the treatment of uterine leiomyoma.

Statement of the problem

Women of African descent have a high risk of developing fibroid, even at earlier age and with high morbidity rates [28] and non-invasive treatment of this disease is not known.

Justification of the study

Medicinal plants have provided a healthy alternative, from synthetic drugs, for new drug formulations [29]. The use of non-surgical approach in alleviating the burden of leiomyoma is gaining global attention because of lesser risks associated with it [2]. Recently, the WHO had directed the use of plants and herbs as alternative chemotherapy in the treatment and management of diseases and infections. In Nigeria, local health practitioners have always administered these herbs in their practices of treating uterine leiomyoma. Hence this research to formulate these drugs in pellets and administered to Wistar rats induced with leiomyoma.

Aim and Objective of the study

The aim of this research is to prepare and administer these drugs in solid form targeted at uterine leiomyoma in female albino Wistar rats while the objective was to assess the effect of these pelleted compounds on haematological parameters of leiomyomatous rats.

Materials and Methods

The study sites are Biochemistry Department of Enugu State University of Science and Technology (ESUT), Agbani, Enugu State and Haematology Laboratory of ESUT Teaching Hospital, Enugu, Enugu State.

Experimental Design

The work was divided into two (2) phases: the plant sample preparation and the animal studies.

Preparation of Extracts

Extracts from the leaves of *Spondias mombin* and *Curcuma longa* were prepared according to the method described by [30]. Leaves were plucked, destoned, washed and air-dried for four (4) weeks. They were then pulverised into coarse forms. The coarse forms were later macerated in distilled water and extracted using Soxhlet extractor. Aqueous extract of each dried samples was prepared in a Soxhlet extractor and the extracts were concentrated using the vacuum extractor. Samples were later prepared like drug tablets by pelleting them using a mechanical engineered pelleting machine. Pelleted drug samples were preserved to avoid microbial contaminations.

Animal Studies

Experimental animals for this research study were apparently healthy adult female Wistar albino rats between 6 and 8 weeks old with average weight of 150 to 250g. Rats were confirmed as adults following the method described by [31]. All the rats were obtained from the animal house of Applied Biochemistry Department of Enugu State University of Science and Technology. Respective solutions of the MSG given to the various animals were prepared following the dissolution of a calculated volume of MSG in a warm water (MSG is sparingly soluble in cold water/water at room temperature but readily soluble in hot water).

Twenty-four (24) adult female albino Wistar rats were acclimatized for two (2) weeks and divided into four (4) groups of six (6) rats each. Drug administration on all animals were done orally after dissolution on distilled water. Control I rats were the normal control, which received feed and water only. Control II rats were the negative control and received feed, water and 750mg/kgbw pellets of MSG daily for twenty-eight (28) days. Group III rats (MSG+SM) received feed, water and 750mg/kgbw pellets of MSG daily for twenty-eight (28) days and subsequently food, water, 750mg/kgbw pellets of MSG with 250mg/kgbw pellets of aqueous extract of *Spondias mombin* daily for another twenty-eight (28) days. Group IV rats (MSG+CL) received feed, water and 750mg/kgbw pellets of MSG daily for twenty-eight (28) days and subsequently food, water, 750mg/kgbw pellets of MSG with 250mg/kgbw pellets of aqueous extract of *Curcuma longa* daily for another twenty-eight (28) days. Leiomyoma was induced on rats in groups II to IV with the daily administration of 750mg/kgbw pellets of MSG for twenty-eight (28) days. Groups III and IV rats continued to receive formulated MSG pellets with the different extracts in the second twenty-

eight (28) days as specified above according to the method described by [32]. Blood were collected from laboratory animals through tail after anaesthetise and haematological parameters were examined using an automated haematology analyzer machine (Mindray BC 2300, USA).

RESULTS

Neutrophil and Eosinophil Levels

Tables 1 and 2 display the neutrophil and eosinophil levels of rat groups compared between weeks (28 days) of treatment and between rat groups, respectively. Control II had consistent slight increase, in rat's neutrophil level, in weeks 1-3 and an observable increase in week 4 compared to the eosinophil level of Groups III and IV which was constant in weeks 1-3 and slightly decreased in week 4.

Comparison by Groups

Control II group had the highest level of neutrophil in week 4 compared to Control I which had least neutrophil level for all weeks (Table 2). Control I and Group IV had the least neutrophil levels in weeks 1, 2, and 4 for all the groups compared to low eosinophil level observed in all weeks for Control II, Control I, Groups III, and IV. Highest neutrophil level occurred in week 1 for Control I and in week 3 for Group IV. Highest eosinophil level was observed from weeks 1-3 for Group IV (Table 1).

Comparison by Weeks

The neutrophil and eosinophil levels of the groups revealed a significant difference ($P \leq 0.05$) in Control II (Table 1) compared to all the Groups and Control I, which had no significant difference ($P \geq 0.05$) (Table 1). Comparison of the neutrophil and eosinophil levels for the different weeks gave a significant difference in weeks 3 and 4. Neutrophil level in week 3 for Control I differed significantly ($P \leq 0.05$) from the other groups compared to week 4 where Control II differed significantly ($P \leq 0.05$) from Group V (Table 2). There was no significant difference ($P \geq 0.05$) in the eosinophil level between groups in weeks 1 and 2 (Table 2).

Table 1: Comparison of neutrophil and eosinophil levels between weeks

Group	Neut-wk1	Eosi-wk1	Neut-wk2	Eosi-wk2
Group III	28.20±3.70^{a}	1.60±0.55^{a}	29.00±2.92^{a}	1.60±0.55^{a}
Group IV	30.20±1.30^{a}	1.60±0.89^{a}	30.00±1.00^{a}	1.60±0.55^{a}
Control I	25.40±3.51^{a}	1.20±0.45^{a}	24.80±2.68^{a}	1.00±0.00^{a}
Control II	27.60±3.97^{a}	1.40±0.55^{a}	28.60±2.97^{a}	1.60±0.89^{ab}

Group	Neut-wk3	Eosi-wk3	Neut-wk4	Eosi-wk4
Group III	29.00±2.35^{b}	1.20±0.45^{a}	29.20±1.92^{bc}	1.80±0.45^{a}
Group IV	30.40±0.55^{a}	1.60±0.55^{a}	29.80±0.45^{a}	1.40±0.89^{a}
Control I	25.20±2.28^{a}	1.40±0.55^{a}	25.00±2.12^{a}	1.20±0.45^{a}
Control II	29.20±3.49^{a}	3.00±1.00^{b}	31.60±2.07^{b}	5.60±0.55^{c}

KEY: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment. wk = week; Neut = Neutrophil; Eosi = Eosinophil. [Post Hoc Test (Fishers LSD)]

Table 2: Comparison of neutrophil and eosinophil levels between rat groups

Group	Neut-wk1	Eosi-wk1	Neut-wk2	Eosi-wk2
Group III	28.20±3.70[a]	1.60±0.55[a]	29.00±2.92[a]	1.60±0.55[a]
Group IV	30.20±1.30[a]	1.60±0.89[a]	30.00±1.00[a]	1.60±0.55[a]
Control I	25.40±3.51[a]	1.20±0.45[a]	24.80±2.68[a]	1.00±0.00[a]
Control II	27.60±3.97[a]	1.40±0.55[a]	28.60±2.97[a]	1.60±0.89[a]

Group	Neut-wk3	Eosi-wk3	Neut-wk4	Eosi-wk4
Group III	29.00±2.35[b]	1.20±0.45[a]	29.20±1.92[bc]	1.80±0.45[a]
Group IV	30.40±0.55[b]	1.60±0.55[a]	29.80±0.45[bc]	1.40±0.89[a]
Control I	25.20±2.28[a]	1.40±0.55[a]	25.00±2.12[a]	1.20±0.45[a]
Control II	29.20±3.49[b]	3.00±1.00[b]	31.60±2.07[c]	5.60±0.55[b]

Key: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment. wk = week; Neut = Neutrophil; Eosi = Eosinophil. [Post Hoc Test (Duncan Multiple Range Test)]

lymphocyte and monocyte levels

Tables 3 and 4 display the lymphocyte and monocyte levels of different rat groups compared between weeks (28 days) of treatment and between rat groups, respectively. Lymphocyte levels of all groups decreased in weeks 1-4 compared to Control I, which increased in weeks 1-2.

Comparison by Groups

Groups III and IV had their highest lymphocyte levels in week 1 compared to Control I with the highest level in week 2. Least lymphocyte level was observed in week 4 for all the groups compared to Control I with the least lymphocyte level in week 1. Groups IV and III had their highest monocyte levels in weeks 1 and 2, respectively, compared to Control II with the highest monocyte level in the same week. Also Group III and Control I had their highest monocyte levels in week 2 compared to Control II in which it was in week 4. Control II had the least lymphocyte level in weeks 3 and 4 compared to Control I with the least monocyte level in weeks 3 and 4.

Comparison by Weeks

Comparison of the monocyte levels between weeks for the rat groups revealed that no significant difference ($P \geq 0.05$) existed in all the groups (Table 3). This implies that for each group, the monocyte level between the weeks was statistically the same. There was a significant difference ($P \leq 0.05$) in the lymphocytes of all groups only in weeks 3 and 4 compared to no significant difference in their monocytes in all the weeks. This implies for each week, the monocyte level between the groups was statistically the same (Table 4). Control II differed significantly ($P \leq 0.05$) from other groups in weeks 3 and 4 compared to Control I which differed significantly ($P \leq 0.05$) from Group IV in week 4.

Table 3: Comparison of lymphocyte and monocyte levels between weeks

Group	Lymph-wk1	Mono-wk1	Lymph-wk2	Mono-wk2
Group III	65.40±3.21[b]	1.20±0.45[a]	64.00±2.55[b]	2.00±0.71[a]
Group IV	63.60±1.52[c]	1.80±0.84[a]	62.40±2.07[b]	1.80±0.84[a]
Control I	63.60±2.07[a]	1.60±0.89[a]	64.60±1.14[a]	1.80±0.84[a]
Control II	65.00±2.24[c]	1.60±0.55[a]	64.40±3.78[c]	1.40±0.89[a]

Group	Lymph-wk3	Mono-wk3	Lymph-wk4	Mono-wk4
Group III	63.00±2.24[ab]	1.60±0.55[a]	62.60±3.05[a]	1.60±0.55[a]
Group IV	61.00±1.41[ab]	1.40±0.89[a]	60.20±1.92[a]	1.80±0.45[a]
Control I	64.00±1.22[a]	1.20±0.45[a]	63.80±1.48[a]	1.40±0.55[a]
Control II	55.60±3.78[b]	1.80±0.84[a]	50.60±3.21[a]	2.80±1.48[a]

KEY: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment. wk = week; Lymph = Lymphocyte; Mono = Monocyte. [Post Hoc Test (Fishers LSD)]

Table 4: Comparison of lymphocyte and monocyte levels between rat groups

Group	Lymph-wk1	Mono-wk1	Lymph-wk2	Mono-wk2
Group III	65.40±3.21[a]	1.20±0.45[a]	64.00±2.55[a]	2.00±0.71[a]
Group IV	63.60±1.52[a]	1.80±0.84[a]	62.40±2.07[a]	1.80±0.84[a]
Control I	63.60±2.07[a]	1.60±0.89[a]	64.60±1.14[a]	1.80±0.84[a]
Control II	65.00±2.24[a]	1.60±0.55[a]	64.40±3.78[a]	1.40±0.89[a]

Group	Lymph-wk3	Mono-wk3	Lymph-wk4	Mono-wk4
Group III	63.00±2.24[b]	1.60±0.55[a]	62.60±3.05[bc]	1.60±0.55[a]
Group IV	61.00±1.41[b]	1.40±0.89[a]	60.20±1.92[b]	1.80±0.45[a]
Control I	64.00±1.22[b]	1.20±0.45[a]	63.80±1.48[c]	1.40±0.55[a]
Control II	55.60±3.78[a]	1.80±0.84[a]	50.60±3.21[a]	2.80±1.48[a]

Key: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment. wk = week; Lymph = Lymphocyte; Mono = Monocyte. [Post Hoc Test (Duncan Multiple Range Test)]

RBC and WBC Levels

Tables 5 and 6 display the RBC and WBC levels of different rat groups compared between weeks of treatment and between rat groups, respectively. The RBC levels of all groups decreased throughout the weeks compared to Control I, which increased. The WBC levels of Group III and Control I decreased compared to other groups which increased throughout the weeks.

Comparison by Groups

Control I had the highest RBC level in all weeks except in week 1. All groups had highest WBC levels in week 4 compared to Control I which had it in week 3. Group III had the highest WBC level in week 1 compared to Group IV which had it in weeks 2-4. Groups III and IV had the least RBC level in weeks 2 and 3, respectively.

Comparison by week

Comparison of the RBC levels between weeks for the rat groups showed that significant difference ($P \leq 0.05$) existed in all the groups except in Control I. Comparison of the WBC counts between weeks revealed a significant difference ($P \leq 0.05$) in Group IV. In week 1, the RBC count of Group IV differed significantly ($P \leq 0.05$) from weeks 2 and 4. In Control I, there was no significant difference ($P \geq 0.05$) in the RBC level between the weeks. In week 2, there was no significant RBC level difference between the rat groups. Comparison of the WBC counts between rat groups for the different weeks revealed no significant difference ($P \geq 0.05$) in all the weeks. This implies for each week, the WBC level between the groups was the same.

Table 5: Comparison of RBC and WBC levels between weeks

Group	RBC-wk1	WBC-wk1	RBC-wk2	WBC-wk2
Group III	7.98±0.78[b]	6.10±2.23[a]	7.33±0.40[ab]	5.97±2.07[a]
Group IV	8.14±0.39[b]	6.01±2.41[a]	7.42±0.30[a]	6.07±2.37[a]
Control I	8.27±0.76[a]	3.35±0.62[a]	8.11±0.73[a]	3.31±0.43[a]
Control II	7.97±0.11[b]		7.73±0.43[ab]	

Group	RBC-wk3	WBC-wk3	RBC-wk4	WBC-wk4
Group III	7.05±0.55[ab]	6.11±2.19[a]	6.78±0.43[a]	6.11±2.17[a]
Group IV	6.97±0.67[ab]	6.12±2.38[b]	6.82±0.61[a]	6.13±2.39[b]
Control I	8.36±0.43[b]	3.44±0.52[a]	8.21±0.42[a]	3.32±0.46[a]
Control II	7.60±0.32[a]		7.24±0.26[a]	

KEY: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment.
wk = week; RBC = Red blood cell; WBC = White blood cell. [Post Hoc Test (Fishers LSD)]

Table 6: Comparison of RBC and WBC levels between rat groups

Group	RBC-wk1	WBC-wk1	RBC-wk2	WBC-wk2
Group III	7.98±0.78[a]	6.10±2.23[a]	7.33±0.40[a]	5.97±2.07[a]
Group IV	8.14±0.39[a]	6.01±2.41[a]	7.42±0.30[a]	6.07±2.37[a]
Control I	8.27±0.76[a]	3.35±0.62[a]	8.11±0.73[a]	3.31±0.43[a]
Control II	7.97±0.11[a]		7.73±0.43[a]	

Group	RBC-wk3	WBC-wk3	RBC-wk4	WBC-wk4
Group III	7.05±0.55[a]	6.11±2.19[a]	6.78±0.43[a]	6.11±2.17[a]
Group IV	6.97±0.67[a]	6.12±2.38[a]	6.82±0.61[a]	6.13±2.39[a]
Control I	8.36±0.43[b]	3.44±0.52[a]	8.21±0.42[b]	3.32±0.46[a]
Control II	7.60±0.32[a]		7.24±0.26[a]	

Key: Group III = MSG + SM; Group IV = MSG + Turmeric; Control I = No MSG + No Treatment; Control II = MSG + No treatment. wk = week; RBC = Red blood cell; WBC = White blood cell. [(Duncan Multiple Range Test)]

DISCUSSION

Lethal dose of MSG in humans is 1500mg per 100g [33] compared to the effective dose (ED) of 750mg/kgbw in pellet form and administered to Wistar rats as seen in this study. At 750mg/kgbw pellets of MSG, there were haematological imbalances on test animals perhaps due to its oxidizing potentials. This is in agreement to the findings of [34] who reported hepatotoxic effects of MSG. The significant difference in weeks 3 and 4 for the neutrophil and eosinophil levels showed that the increased neutrophil was in response to autoimmune disease or inflammation common with the ingestion of MSG [35]. Therefore, ingestion of MSG is destructive to tissues and organs by influencing the production of oxidative radicals. These oxidative radicals attack self-cells initiating autoimmunity. When compared with the histology examinations, Control II group reported higher inflammatory cellular infiltrations than rat groups treated with the various extracts. All the extracts used have the potency of improving the neutrophil levels of the test animals. Eosinophils are specialised cells that play vital role in hypersensitivity reaction during which their blood levels are elevated in response to the causative factors [36]. This study shows that there was no significant difference in eosinophil level between the weeks in the rat groups treated with the various extracts when compared with the Control II rats as seen in Tables 1 and 2. However when compared between rat groups, the eosinophil level reported a significant difference only in weeks 3 and 4. It could be interpreted that the MSG destroys tissues through activation

of the complement pathways of the immunological reactions. This subsequently raised the levels of the eosinophil, as observed in the Control II group which showed a significant difference compared to the Control I group and the animals treated with different extracts.

CONCLUSION

This work indicates that formulated pellets of aqueous extracts of *Spondias mombin* leaves and rhizomes of *Curcuma longa* have anti-fibroid properties hence their usage among local inhabitants. However, *Curcuma longa* pellets at 250mg/kgbw gave higher anti-fibroid protection than *Spondias mombin* extract.

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