EVALUATION OF THE EFFECT OF TOTAL FLAVONOIDS RICH EXTRACT OF *Kigelia africana* ON THE IN VITRO AND IN VIVO INFLAMMATORY ACTIVITY IN WISTAR RATS

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PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

IN LINE WITH THE PARTIAL FULFILMENT OF THE REQIUREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (BS.C) IN BIOCHEMISTRY

APRIL, 2024

CERTIFICATION

This is to certify that this project was done and carried out by **EVANS MIRACLE ESOSA** with Matriculation number **LSC1906503** of the Department of Biochemistry, Faculty of Life sciences, University of Benin, in partial fulfilment for the award of Bachelor of Sciences, (B.Sc) in Biochemistry.

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DEDICATION

I’m delighted to pledge this project report to God Almighty for his love, kindness, his grace and mercy upon my life and for his sustenance throughout my academic pursuit, may glory and adoration be unto His Holy Name, and also to my parent and family for all their love and support.

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ABSTRACT

Kigelia africana, commonly known as the sausage tree, is a plant widely recognized for its medicinal properties in various traditional medicine systems across Africa. The aim of this study was to determine the effect of total flavonoid Rich Extract (FRE) on membrane stabilization and the hematological property of the rat. The study was carried out in Wister rats exposed to Carrageenan for 24 hours. The membrane stabilizing effect of the Flavonoid Rich Extract was evaluated using the Human Red Blood Cell (HRBC) membrane stabilizing method. The Flavonoids Rich Extract fraction in heated induced hemolysis and hypotonic solution of HRBC as against the standard (aspirin) in wastar rats. The total Flavonoids Rich Extract has shown protection against hemolysis of human red blood cells either through heat-induced or hypertonicity, that is it can be used for the management of in-vitro inflammation. The in vitro anti-inflammatory activity of the total flavonoids extract from the plant *Kigelia africana* was assessed using the in vivo hypoglycemic (anticancer) and antineoplastic (antitumor) activities of the extracts. The results showed a significant decrease ($p > 0.05$) in the White Blood Cells (WBC) of carrageenans only treated rat, carrageeenans and sodium diclofenac treated rats, and a significantly increase in it WBC of rats treated with Carrageenenan and Quercetin, Carrageenans and Flavonoids Rich Extract (100 mg/kg body wt.), and Carrageenans and Flavonoids Rich Extract (200 mg/kg body wt.), when compared to the control group.

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1.0 INTRODUCTION AND LITERATURE REVIEW

In the context of traditional medicine, the integration of plant-based remedies like *Kigelia africana* with naturally derived substances such as carrageenan highlight the potential for developing novel therapeutic agents. *Kigelia africana*, commonly known as the sausage tree, is a plant widely recognized for its medicinal properties in various traditional medicine systems across Africa. The fruit, leaves, and bark of *Kigelia africana* have been utilized in traditional medicine to treat a range of ailments due to their anti-inflammatory, antimicrobial, and antiviral properties (Islam *et al.,* 2022). This integration not only underscores the importance of preserving ethnobotanical knowledge but also points towards a multidisciplinary approach in drug discovery and development, leveraging the synergies between traditional medicine and modern scientific research. It is renowned for its diverse medicinal properties, including the treatment of skin diseases, and fungal infections, and as a general health tonic. The fruit, leaves, and bark of *Kigelia africana* are utilized in different preparations to address a wide range of health issues (Islam *et al.,* 2020; Nanjala *et al.,* 2022; Sharma *et al.,* 2022).

The pharmacological potential of *Kigelia africana* is attributed to its rich content of phytochemicals such as naphthoquinones, iridoids, flavonoids, and phenolic compounds, which exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Alami Merrouni Ilyass *et al.,* 2021).

Carrageenan, on the other hand, is a polysaccharide extracted from red seaweeds and is widely used in the food industry as a thickener and stabilizer. Beyond its industrial applications, carrageenan has been explored for its medicinal properties, including anti-inflammatory and immunomodulatory effects. It has been studied for its potential in treating gastrointestinal disorders and as a carrier for drug delivery systems (Antwi-Baffour *et al.,* 2014).

Carrageenan-induced inflammation in Wistar rats is a commonly used model to study the anti-inflammatory and analgesic effects of various compounds, including those derived from traditional medicinal plants. The carrageenan-induced paw edema model is a well-established method to evaluate the anti-inflammatory activity of substances. It involves the injection of carrageenan into the paw of rats, leading to localized inflammation. This model has been used to demonstrate the efficacy of various extracts and compounds in reducing inflammation, which is relevant for conditions like arthritis (Abdullahi, 2011).

The relationship between *Kigelia africana* and carrageenan in traditional medicine could be explored through their individual anti-inflammatory properties. Both have been recognized for their potential in treating conditions associated with inflammation, suggesting a complementary role in traditional medicine formulations. While *Kigelia africana* provides a direct link to traditional plant-based remedies, carrageenan represents a bridge between traditional knowledge and modern applications in medicine and pharmacology.

1.1 Aim of the study

The project work was carried out to determine the effect of total flavonoid (FRE) on membrane stabilization and the hematological property of the rat.

The major objective of this project includes:

1. This project is to determine the effect of the Flavonoids Rich Extract (FRE) which is the n-Hexane fraction of FRE, Ethyl-acetate fraction of FRE, and FRE of the *Kigelia africana* anti-inflammatory effect on membrane stabilization.
2. This study aims to assess the hematopoietic effect of flavonoid-rich extract obtained from *Kigelia africana* in Wister rats exposed to Carrageenan for 24 hours.

1.2 Literature review

1.2.1 *Kigelia africana*

Kigelia africana, is commonly referred to as the sausage tree, it is a tree native to continental Africa that is both ornamental and utilitarian. It has been introduced to tropical and subtropical regions as an ornamental attraction due to its large flowers and distinctive sausage-shaped fruit. Despite being widely cultivated beyond cultivation and becoming established in natural habitats.

The exception to this is Cuba, where it has been noted as naturalized and showing a propensity for expansion. The fruit, leaves, and bark of *Kigelia africana* have been utilized in traditional medicine to treat a range of ailments due to their anti-inflammatory, antimicrobial, and antiviral properties (Burkill, 1985).

This plant holds deep cultural significance in many African communities, revered as a sacred tree. It is often safeguarded from felling and preserved near dwellings for its medicinal and other beneficial properties. Various parts of the plant, particularly the fruits, are utilized in traditional African medicine to address diverse ailments, including digestive and respiratory issues, urinary tract infections, venereal diseases, infertility, malaria, and skin conditions, among others. Recent research has unveiled numerous bioactive compounds within the plant, exhibiting analgesic, anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Jackson and Beckett, 2012).

While the unripe fruit is toxic to humans, the seeds of ripe fruits are edible when roasted and reportedly contain substantial amounts of protein and lipids. Ripe fruits are also employed to aid fermentation and enrich the flavor of alcoholic beverages.

Additionally, the leaves are consumed as a nutritious vegetable, boasting essential amino acids and noteworthy levels of calcium, magnesium, and iron (Jackson and Beckett, 2012).

The wood of *Kigelia africana* is prized for its quality and finds application in crafting canoes, posts, stools, drums, boxes, toys, tool handles, and utensils. Furthermore, the tree's extensive root system renders it suitable for erosion control and stabilization of riverbanks (PROTA, 2020).

Kigelia africana is sometimes cultivated in parks, gardens, and roadside plantings for its ornamental appeal. Seeds and seedlings are available for purchase in nurseries and online platforms to cater to this demand.

In local African markets, the fruits and bark are traded for their medicinal and spiritual significance. Extracts from the fruit, along with various skincare and therapeutic products derived from it, are globally marketed by several cosmetic and pharmaceutical enterprises, giving it a good economic value (Burkill, 1985).

The local names of *Kigelia africana* vary throughout Africa due to the ethnic and cultural diversity on the continent. The many dialects reflect *Kigelia africana's* ethnobotanical significance among several African communities (Van Wyk, 2002). Some of K. *africana's* local African names include: Nufuten, Nanaberetee (Ashanti and Akwapem), Etua (Fante), Blimmo (Baule), Akpele (Ga), Lele (Adanme), Nyakpe (Ewe), Rawuya (Hausa), Jilahi (Fulani), Bulungu (Kanuri), Bechi (Nupe), Pandoro (Yoruba), Ugbongbon (Bini), Uturubein (Ibo), Abu Shutor, Abu Sidra, Um Shutur, Umm Hashatur (Arabic), Rangbarabgbo (Zande), muVeve (Tonga), muVumati (Ndau), muZunguru (KalanBa), mPolota (Lozi), umBvewe, iPfungwani, muBvee (Shona, Zezuru, Manyika), Mufungufungu (Bemba and Lozi), ~lunguli (Lozi) Muzungule (Lozi and Tonga), Kufungule (Kaonde) Ifungufungu, Mufunofuno (Lunda), Chizutu, Mvula, Mvunguti (Nyanja). Muratina, (Kikuyu and Meru), Muatini, Kiatine (Kamba), Hwasini, Mvongonia (Teita), Ol-Suguroi, Ol-Darpoi (Masai), Yago (Luo), Morabe (Kakamega), Mvungunya, Mvungavunga, Hwegea, Mwicha, Mranaa (Swahili),

Muratini (Gitiama), Mukisha (Taveta), Ratiunet (Nandi), Sifungu (Lugisu), Naizungwe (Lusoga), Omusa (Luganda), Roti (Pokot) and Bukuraal (Somalia).

Figure 1.1: *Kigelia africana* (sausage tree); Fruit habit. Lower Sabie, Mpumalanga, South Africa. (Olmstead *et al.,* 2009).

Figure 1.2: *Kigelia africana* habit captured in Equatorial Guinea, (Costa *et al.,* 2016)

1.2.1.1 Botanical Description and Distribution

Botanical Description

Kigelia africana (Lam.) Benth. belongs to the family Bignoniaceae and is the only species in the genus Kigelia (Nsubuga, 2018). The generic name Kigelia comes from the Mozambican name for sausage tree, "kigeli-keia". *Kigelia africana* is native to Africa, thus the derivation of the species name "africana". The tree is deciduous, with a rounded crown, thick trunk, dark-grey to light-brown, scaly slash creamy-white with a green edge, low-branching, branches and branchlets spreading and lenticellate (Dhungana, 2017). The tree reaches maturity within four to six years, with a height of up to 24 meters (Dhungana, 2017). The leaves are alternate, pinnate and stipules absent; rachis up to 50 cm long; leaflets three to six opposite pairs, usually with a terminal leaflet, elliptic to elongated lanceolate, 7–20 cm long, 4–12 cm wide, apex abruptly to gradually shortly acuminate, base slightly asymmetrical, rounded to cuneate, margins entire or sometimes slightly toothed, coriaceous or papyraceous, shiny green and usually scab rid above, dull green and glabrous to tomentose below; midrib impressed above, major lateral veins 7–12 pairs and prominent below (Dhungana, 2017). The flowers of K. *africana* are hermaphrodite, zygomorphic and five-merous. The calyx is campanulate approximately 1–4-cm-long, 1–2-cm-wide, fleshy, irregularly five-lobed, the lower lobes generally longer at maturity and the calyx mouth thus oblique. The corolla is greenish-yellow to purplish-red or bright claret, 5–12-cm-long, the throat rather abruptly expanded, limb 9–18 across with the two upper lobes smaller than the three lower and velvety insides; stamens four fertile and one staminode about half the fertile stamens. The ovary is conical, tapering into a slender style subequalling the stamens (Adam and Alhameed, 2013; Cragg and Newman, 2001; Sidjui *et al.,* 2014; Hussain *et al.,* 2016). They possess a very unpleasant scent, which is most notable at night, indicating their reliance on pollination by bats, which visit them for pollen and nectar (Hussain *et al.,* 2016).

The fruits are indehiscent, woody, greyish-brown, sausage-shaped and pendulous, up to 50-cm-long and 15 cm in diameter, with elongated pedicels. The seeds are numerous, unwinged, obovate and 1.25-cm-long (Adam and Alhameed, 2013; Cragg and Newman, 2001; Sidjui *et al.,* 2014; Burkill, 1985). The fruits usually weigh 10 kg (Priya *et al.,* 2013).

The mature fruits can be found on trees throughout the year (Singh, 2018; Hussain *et al.,* 2016). Although not eaten by humans, they find wide applications in traditional medicine (Dhungana, 2017). Due to the unusual fruits and large attractive flowers, K. *africana* is considered a striking ornamental plant, and the fruits are used as florists' materials. The thick stem is an attractive feature for bonsai. The tree is sometimes planted as a boundary marker but usually at roadsides and for shade. Due to its occurrence along watercourses, it is suitable for erosion control and riverbank stabilization.

Distribution

Kigelia africana, commonly known as the sausage tree, is indigenous to and widely dispersed across sub-Saharan Africa. It has been introduced to various African island such as Madeira, the Canary Island, Cape Verde, Reunion, and Mauritius, as well as to region beyond Africa, including the United State (California, Florida, and Hawaii), the Caribbean island, Center and South America (Mexico, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Peru and Brazil), Western Asia (Iraq and Israel), and Southeast Asia (Philippines, Indonesia, China, India, Laos, Vietnam, Sri Lanka, Pakistan, Malaysia, Myanmar, Maldives, Thailand), Pacific Island (Marquesas Island, New Caledonia, Guam) and Australia.

Kigelia africana has been cultivated beyond its native range since at least the 1800s. It was observed growing in the botanic Garden in Mauritius and in the Royal botanic Garden at Kew in the UK (Seeman, 1854). As of the early as 1888, it was already featured in seed catalogues of Florida (Royal Palm Nurseries, 1888).

The earliest record of Kigelia in Cuba dates back to 1914 (De la Maza and Roig,1914). Presently, the species is categorized as naturalized on the island with a tendency to spread (Oviedo-Prieto and Gonzalez-Oliva, 2015).

Fruit that are periodically removed from trees planted in the city's park to avoid damage when they fall, and place in sites where the seeds are able to germinate. The Sausage tree was mentioned in Britton's Flora of Bermuda (1918) as 'occasionally planted'. It was introduced to the Virgin Islands Agriculture Experiment Station in St.Croix in 1928 from the Panama Canal Zone (Thompson, 1930). *Kigelia africana*, was also recorded to be present in the island of Madeira in 1928 and in Hawaii in 1917 (Cockerell, 1928). The largest tree in Honolulu at that time was growing in the Queen's Hospital grounds (Rock, 1917). By the year 1948, *Kigelia africana*, had become common in Honolulu, and was been used as street and garden tree (Fosberg, 1948).

It was remarked that this species, was a bat-pollinated, obligate outcrosser, is infrequently pollinated in the new world due to the native bats seemingly being too small to effectively contact the anthers and stigma. However, in Cuba and other countries, trees often yield abundant fruit, indicating that other animals, possibly birds, may serve as pollinators in these areas (Gentry, 1974).

Means of movement and dispersal

Natural dispersal of *Kigelia africana* seeds is primarily facilitated by large vertebrates, including mega-herbivores, primates, and large rodents. Observations in Tanzania have documented the presence of seeds in elephant dung (Gonthier, 2009).

Baboons (Papio ursinus) consumed the mature fruits, which are specially adapted for dispersal by these primates, only baboons possess the ability to tear apart these fruit for seed dispersal (Hamilton *et al*., 1978).

However, studies conducted in the Keuger National Park revealed that the fruit of Kigelia were largely unattractive to potential dispersers, including elephants and were consequently ignored (Namah *et al*., 2019).

Additionally, it was observed that Kigelia seeds do not have the ability to float.

Reproductive Biology

Kigelia *africana* has large campanulate flower which are found below the crown on long on long dangling branches. These flowers typically open just before or at dusk, emitting an unpleasant odor and abundant nectar that attracts various vertebrates, including bats, birds, primates and small carnivores such as genets (Namah *et al.,* 2019). Despite their brief lifespan of just one day, these flowers play a crucial role in pollination. While megachiropteran bats are recognized as the primary pollinators (Gentry, 1974), diurnal birds also contribute significantly to this process (Namah *et al.,* 2019).

As an obligate outcrosser, Kigelia *africana* relies on animal vectors to transfer pollen between flowers on different individuals (Namah *et al*., 2019). Mature trees can yield up to 225 friuts,. Which may persist on the tree for up to six months before being dispersed by animals that consume them. In cases where native disperses are absent, the seeds are released when the fallen fruits decomposed on the ground.

Propagation of *Kigelia africana* is straightforward and can be achieved through both seeds and vegetative cuttings. Germination rates are typically high and positively correlated with temperature within the range of 11-31°C (Pritchard *et al.,* 2004). Seeds collected from freshly fallen fruits have been reported to exhibit a remarkable 97% germination rate without any pre-treatment (Namah *et al.,* 2019). Germination typically commences with 10-25 days.

Furthermore, *Kigelia africa*na seeds are characterized as orthodox and desiccation-tolerant, with the ability to maintain viability even at low moisture content (Pritchard *et al.,* 2004).

Seeds desiccated to 4-6% moisture content using silica gel at 26°C exhibited germination rates exceeding 80%. With proper storage conditions, including hermetic storage at ambient temperature with 11-15% moisture content, seeds can retain viability for three years.

Physiology and Phenology

Kigelia africana is an evergreen species in regions with abundant rainfall throughout the year but is deciduous in areas with a long dry season. Trees flower during the dry season, from November to February in West Africa (Oni *et al.,* 2014) and from June to November in Southern Africa. Fruits are borne from March to July in West Africa and from November to June in Southern Africa (Rønne and Jøker, 2005; Oni *et al.,* 2014).

In warm regions and under the right conditions, young plants can grow more than 1 m per year, but in colder climates vegetative growth is slower (PROTA, 2020). Trees bloom for the first time at 6 years of age (Rønne and Jøker, 2005). In Kruger National Park, South Africa, the minimum size of trees producing fruits was 16.7 cm diameter at breast height (DBH; Namah *et al.,* 2019).

1.2.1.2 Taxonomical Classification

Domain – *Eukaryota*
Kingdom – *Plantae*
Division – *Spermatophyta*
Sub-division – *Angiospermae*
Class – *Dicotyledonae*
Order – *Scrophulariales*
Family – *Bignoniaceae*
Genus – *Kigelia*
Species – *Kigelia africana*

Kigelia comprises a single species in the rumpet-creeper family (Bignoniaceae), widely distributed throughout Africa. Around ten species and several varities have been recognized in the past, but nowadays most authors regard these taxa as ecotypes of a single variable species (Diniz, 1988).

The genus is distinctive among the Bignoniaceae for its gaint, sausage-like, indehiscent fruit which can weigh up to 10kg. However, phylogenetic studies place it as sister to Stereospermum, an Old World genus previously assigned to Tecomeae *sensu lato* (Zjhra *et al.,* 2004; Olmstead *et al.,* 2009).

Kigelia africana is a multipurpose tree widely used by African communities and so it is known in different African countries by a large number of common names.

1.2.1.3 Pharmacology activity of *Kigelia africana*

Pharmacology activity

Anti-Atherosclerotic effects

Kigelia africana, also known as the sausage tree, has been used traditionally for various medicinal purposes. Finding that its methanol extract exhibits anti-atherosclerotic effects on Endothelial Cells without causing cytotoxicity at doses of 10 ~ 200 μg/ml. Atherosclerosis, characterized by the buildup of plaque in the arteries, is a significant risk factor for cardiovascular diseases. At a concentration of 50 μg / ml, it showed a significant inhibition. If the extract can inhibit this process without harming endothelial cells, it could indeed hold potential for therapeutic use in the treatment of atherosclerosis, without causing cytotoxicity (Ko *et al.,* 2019).

Anticonvulsant activity

The anticonvulsant activity of methanolic (KPM) and aqueous (KPA) bark extracts of *Kigelia africana* was studied using pentylene tetrazole and convulsions induced by maximum electroqueque in glycine rats.

The results showed that 250 mg/kg and 500 mg/kg of KPM and KPA had a significant anticonvulsant effect. They increased the onset of clonic convulsions and decreased the onset of tonic convulsions (Singh *et al.,* 2010).

Anti-Inflammatory Activity

Researchers investigated the anti-inflammatory effect of the methanolic extract obtained from the fruits of *Kigelia africana*. They conducted experiments on mice and rats using different inflammation models such as formaldehyde-induced leg edema, acetic acid-induced vascular permeability, cotton granules, estimation of plasma MDA levels, and models of carrageenan-induced peritonitis. The results showed that the doses of 100, 200, and 400 mg/kg of methanol extract of *Kigelia africana* exhibited an effect similar to that of standard drugs (Carey et al., 2008).

According to a study on wound healing and anti-inflammatory effects of *Kigelia africana* extract were explored in animal models. The findings of the study suggested that the extract could speed up wound healing and reduce inflammation, which supports the traditional use of this extract in treating various skin conditions (Abubakar *et al.,* 2018).

In another study, the inhibitory effects of *Kigelia africana* extract on inflammatory mediators such as prostaglandins and leukotrienes were investigated. The results of the study demonstrated significant inhibition of these mediators, indicating the potential of this extract as an anti-inflammatory agent by (Adesegun *et al.,* 2011).

Hypoglycemic Activity

An investigation of the in vivo hypoglycemic effects of aqueous and ethyl acetate extracts derived from *Kigelia africana* leaves, utilizing the Alloxan-induced diabetes model.

The research revealed that both aqueous and ethyl acetate extracts of *Kigelia african* leaves exhibited significant anti-diabetic activity when administered at therapeutic doses, either intraperitoneally or orally. These findings underscore the potential of *Kigelia africana* leaf extracts as therapeutic agents for diabetes management (Njogu *et al.,* 2018).

Antineoplastic Activity

In 2012, Momekov and his co-workers. conducted a study to assess the antineoplastic (anticancer) activity of a total methanolic extract from the stem bark of *Kigelia africana*. The study used in vitro testing which included cytotoxicity (MTT assay) and investigation of pro-apoptotic activity. The extract showed significant cytotoxicity against a panel of human tumor cell lines and exerted strong antineoplastic activity against Lewis lung carcinoma. The treated animals exhibited a significant increase in lifespan, and the extract inhibited tumor growth (Momekov *et al.,* 2012).

Antimicrobial, antioxidant and wound healing

The study of antimicrobial and antioxidant properties of methanol leaf extracts and the cortex of the *Kigelia africana* stem. The antimicrobial activities of the methanol extracts were determined against two Gram positive and two Gram negative bacteria and one fungus using micro and micro dilution diffusion methods. Antioxidant activity was determined using the 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) method. The influence of the extracts on the wound closure rate was studied using the excision injury model. The MIC of the extract of *Kigelia africana* leaves against the examined organisms was 2.5–7.5 mg / ml and the stem bark extract was 2.25-7.5 mg / ml.

Extracts of *Kigelia africana* (7.5% w/w) showed a significant wound contraction ($p < 0.05$) on day 7 with a wound closure of 72% while significant wound contractions ($p < 0.05$) (Agyare *et al.,* 2013).

1.2.1.4 Phytochemistry

Several compounds have been identified from *Kigelia africana*, but further studies are needed to fully understand its phytochemistry. According to Table 2, iridoids and quinones are present in all parts of the plant, with the stem bark containing a greater diversity of phytochemicals than other parts. Leaves contain alkanes, while very little research has been done on phytochemicals in Kigelia flowers, which are often used as ornamentals. *Kigelia africana* is unique in containing monoterpenoid naphthoquinones such as pinnatal, isopinnatal, kigelinol and isokigelinol. Table 2 summarizes the different classes of phytochemicals in *Kigelia africana*, the specific phytochemicals present, and the corresponding plant parts (Arkhipov *et al.,* 2014).

1.2.2 Carrageenan

Carrageenans are sulphated linear polysaccharides extracted from red seaweeds, with d-galactose and 3,6-anhydro-d-galactose as their main components (Campo, 2009). They are used in the food and pharmaceutical industries due to their gelling, thickening, and protein-suspending properties (Campo, 2009). The chemical structure and hydrodynamic properties of carrageenans play a significant role in their rheology (Kim *et al.,* 2019). κ-Carrageenans, in particular, have good gelling properties, with their strength influenced by factors such as concentration and potassium content (Kim *et al.,* 2019; Chan, 2016).

Additionally, it is used in pharmaceutical applications (Takamatsu and Tosa, 1993, as cited in Van de Velde *et al.,* 2002), and in experimental medicine, it is often used for testing anti-inflammatory agents (Zacharopoulos and Phillips, 1997).

1.2.2.1 Chemical structure, chemical composition of carrageenan

Carrageenan is a type of sulfated polygalactan that contains 15 to 40% of ester-sulfate content. Its average relative molecular mass is above 100 kDa.

Carrageenan is formed by combining alternate units of d-galactose and 3,6-anhydro-galactose (3,6-AG) using α-1,3 and β-1,4-glycosidic linkage. Carrageenan is classified into various types such as λ, κ, ι, ε, μ, all of which contain 22 to 35% sulfate groups. This classification is based on its solubility in potassium chloride. The differences in carrageenan types are primarily based on the number and position of ester sulfate groups and the content of 3.6-AG. These names do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer. Higher levels of ester sulfate mean lower solubility temperature and lower gel strength. The ester sulfate content of kappa-type carrageenan is about 25 to 30%, and the 3,6-AG content is about 28 to 35%. The ester sulfate content of iota-type carrageenan is about 28 to 30%, and the 3,6-AG content is about 25 to 30%. The ester sulfate content of lambda-type carrageenan is about 32 to 39%, and it does not contain any 3,6-AG (Soares *et al.,* 2015). The physicochemical properties of κ-carrageenan extracted from Kappaphycus alvarezii are comparable to those of commercial κ-carrageenans (Chan, 2016).

The chemical reactivity of carrageenans is mainly due to their half-ester sulfate groups, which are strongly anionic and comparable to inorganic sulfate in this respect. The free acid is unstable, and carrageenans are typically available commercially as stable sodium, potassium, or calcium salts, or as a mixture of these.

The physical properties of carrageenans are determined by the associated cations and the conformation of the sugar units in the polymer chain.

For instance, kappa- and iota-carrageenans can form gels in the presence of potassium or calcium ions, whereas lambda-carrageenans cannot (Perino *et al.,* 2019; Loureiro *et al.,* 2017). The rheological properties of carrageenans play a vital role in their functionality in various applications.

These linear, water-soluble polymers usually form highly viscous aqueous solutions, and the viscosity is dependent on the concentration, temperature, the presence of other solutes, and the type of carrageenan and its molecular weight (Jiao *et al*., 2011). As the concentration increases, the viscosity increases nearly exponentially, while the viscosity decreases with temperature.

Carrageenans are prone to depolymerization through acid-catalyzed hydrolysis. At high temperatures and low pH, the functionality of carrageenans can be quickly lost, which can lead to a complete loss of functionality (Wang, 2012).

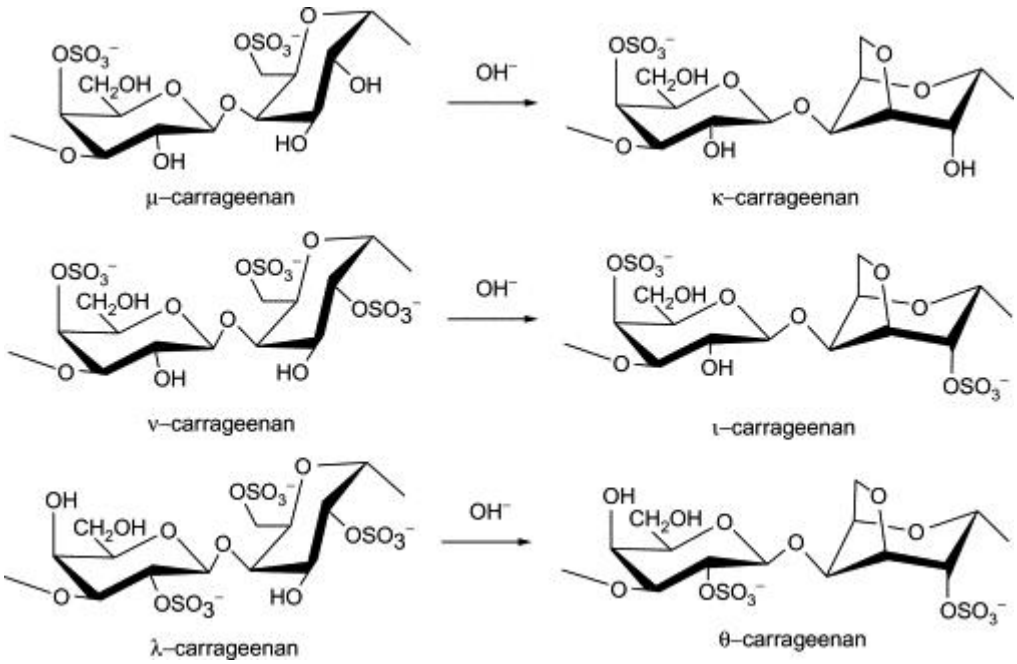

Figure 1.3; Chemical structures of carrageenan, (Levendosky *et al.,* 2015).

1.2.2.2 Biological and toxicological properties of carrageenan

Sulphated polysaccharides found in marine algae have various biological activities such as immunomodulation, anticoagulation, antithrombotic, antiviral and antitumor effects. It is believed that these negatively charged molecules, including sulphated polysaccharides, hinder the virus or cell surface's positive charges and thus prevent the virus from penetrating the host cells.

Carrageenan, a type of sulphated polysaccharide, doesn't affect virus attachment or penetration into host cells, but it inhibits the synthesis of viral proteins inside the cells. Although carrageenan has been reported to have anti-HIV activity, its strong anticoagulant activity is considered an adverse effect when used as a therapeutic drug for AIDS (Necas and Bartosikova, 2013; Wijesekara *et al.,* 2011; Levendosky *et al.,* 2015; Derby *et al.,* 2018).

Anticoagulant and antithrombotic activity

The blood coagulation system includes intrinsic and extrinsic pathways, with several factors involved in the mechanisms. Coagulation factors promote blood coagulation to stop the flow of blood through the injured vessel wall in cases of abnormal vascular conditions and exposure to non-endothelial surfaces at sites of vascular injury. As endogenous or exogenous anticoagulants interfere with coagulation factors by inactivating them or restricting their activity, blood coagulation can be prolonged or stopped (Wijesekara et al., 2011).

Carrageenan has several types, all containing 22–35% sulfate groups. Several reports exist on the anticoagulant activity of carrageenan. Among the different types of carrageenan, λ-carrageenan has approximately twice the activity of unfractionated carrageenan and four times the activity of κ-carrageenan. However, the most active carrageenan has approximately one-fifteenth the activity of heparin.

The principal basis of the anticoagulant activity of carrageenan appears to be its anti-thrombic property.

λ-Carrageenan showed greater anti-thrombic activity than κ-carrageenan probably due to its higher sulfate content, whereas the activity of the unfractionated material was somewhere between the two. λ-Carrageenan consistently prolonged the clotting time and was more toxic than κ-carrageenan. The difference in sulfate content between the two carrageenans did not correspond directly to differences in anticoagulation action and toxicity (Shanmugam and Mody, 2000).

The mechanism underlying the anticoagulant activity of carrageenan involves thrombin inhibition. Amidolytic studies initially indicated that the anti-thrombin activity might be mediated via AT-III (anti-thrombin-III), the major mechanism by which heparin acts. In these studies, carrageenans appeared to inhibit amidolysis of thrombin directly and via AT-III; however, only AT-III potentiated Xa amidolysis was observed. These interactions may be influenced by certain critical qualities of the polyanionic polymers, i.e., sulfation, size, pattern of ionic substitution, and polymer rigidity. However, subsequent studies using AT-III-depleted plasma showed residual anti-thrombin activity in the presence of carrageenans. λ-Carrageenan has been shown to potentiate the inactivation of thrombin by 'anti-thrombin BM' (Guan *et al.,* 2017, Liang *et al.,* 2014).

Anti-tumor and immunomodulatory activities

Several studies have shown that carrageenans have antitumor and immunomodulatory activities. These studies found that carrageenans have anti-proliferative activity in cancer cell lines in vitro, as well as inhibitory activity of tumor growth in mice.

Carrageenans have been found to possess anti-metastatic activity by blocking the interactions between cancer cells and the basement membrane.

They also inhibit tumor cell proliferation and tumor cell adhesion to various substrates (Zhou *et al.,* 2005; Zhou *et al.,* 2006; Liu *et al.,* 2019). However, the exact mechanisms of action are not yet completely understood.

(Yamamoto *et al.,* 1986) reported that the oral administration of several seaweeds can cause a significant decrease in the incidence of carcinogenesis in vivo. The modifying effects of carrageenan on colonic carcinogenesis in male rats and found that carrageenan does not possess any promoting activity at the highest dietary level of 5.0% for colorectal carcinogenesis under the present experimental conditions.

No treatment-related changes in clinical signs and body weights were found, and histopathological examination did not demonstrate any enhancement by carrageenan carcinogenesis (Luo *et al.,* 2015).

CHAPTER TWO

2.0 MATERIAL AND METHOD

2.1 Collection and preparation of FRE extracts *Kigelia africana*

Kigelia *africana* trees was located and spotted in Ondo State by a botanist. The fruit of the *Kigelia africana,* was identified by a taxonomist and given a voucher number (UBH-289). The various plant samples were sorted out, washed with tap water, and rinsed with distilled water. The plucked fruit (*Kigelia africana*) was then transported using a sack bag to the Department of Biochemistry Laboratory, Faculty of Life Sciences at the University of Benin. The fruit of *Kigelia africana* was washed and separately chopped into small pieces by the aid of a knife, the chopped pieces was then fill-dried at room temperature. Days later the properly dried sample was taken to the Department of Pharmacognosy, Faculty of Pharmacy at the University of Benin to ground the dried sample into fine smooth homogenous power via the use of an electric mill.

2.2 Extraction

300gram of the finely ground homogenous power was soaked in 2.5litres

2.3 Concentration

The various fractions of the Flavonoid Rich Extract (FRE) was collected after fraction and sent to the biochemistry research laboratory. The fractions are then place into a rotary evaporator where it is allowed to dry at a temperature of 60℃, one after the other. The rotary evaporator consist of a vacuum pump with supplies pressure to the fraction placed inside the conical flask, thus removing air from the system, and creating a reduced pressure environment that allows the liquid to accelerate distillation and evaporate more quickly, a running water source helps the cooling and condensation of the solvent.

The water bath helps in heating the fraction in the conical flask and its rotation allows for equal distribution of this heat. At the end of this process the concentrate was then poured out into a pettish dish and further sent to the oven for more drying. This process was done for all the extract of Flavonoids Rich Extract (FRE) fraction, n-Hexane fraction of Flavonoids Rich Extract (FRE), Ethyl acetate fraction of Flavonoids Rich Extract (FRE). The completed dried concentrate was taken out of the oven and stored in the refrigerator.

2.4 Experimental design

2.4.1 Laboratory animals used

Wistar Male Rats was the laboratory animals used for this experiment, this rats weighted between 169 – 299 grams. The laboratory animals were gotten from the Mr. Emmanuel, from the Department of Biochemistry, Faculty of Life Sciences, University of Benin. Prior to experimentation, the animals underwent a 24 hours acclimatization period in the animals housing facility at the Biochemistry Department, Faculty of Life Sciences, University of Benin. All through the period of this experiment the laboratory animals were kept in a standard cages under a controlled environment, maintaining an ambient temperature ranges between the 20ºC - 28ºC with a 12-hour light-dark cycle. The rats were provided with the standard rodent pellet feed and water, every 6hours. Ethical considerations and protocols for handling ethics guidelines established by the University of Benin, Benin City, Edo State.

2.4.1.1 Inducement with carrageenan

The male Wistar Rat, prior to the inducement of carrageenan, each of the rats were administered drugs such as sodium diclofenac, Dexamethasone, Plant extract Flavonoid Rich Extract (FRE), and 1hours be the inducement of carrageenan.

Using the carrageenan-induced right paw edema in wistar rat as this described by Winter *et al.,* (1962). 0.1ml of 1% Carrageenan in normal saline was used to induced via a sub-plantar injection, causing and acute inflammation in the rats. Using a digital vernier caliper to measure the diameter of the rat's paws before injection, after inflammation in the paw of the male Wistar Rats, a digital vernier caliper is used to measure the paws at 1, 2, 3, 4, 5, 6, and 24 hours after inflammation and the readings are record at each intervals.

2.4.1.2 Sacrifice

At the end of the inflammatory observation, the rats were euthanized in a chloroform saturated chamber, which induces rapid and painless loss of consciousness followed by ventrally dissected via the abdominal cavity.

2.4.1.3 Collection of sample and preparation

Immediately after sacrificing the blood sample was collected in a 5mL tubes that contains EDTA in order to prevent the coagulation and also in a 5mL plain tube containing no anti-coagulate. The blood sample for the Wistar Rats was collected using a 2mL syringe via vein-puncture. The blood sample place into the tube containing anti-coagulate was sent to the University of Benin Teaching Hospital (UBTH) Laboratory for full blood count to be ran on the various samples, and the blood in the red tube or the Non-EDTA, was placed into a centrifuge and centrifuged at 4000rpm of 5mins and the serum was taken out.

2.4.1.4 Hematology analysis

This is crucial for diagnosing and managing diseases related to blood cells and platelets. It helps in identifying conditions like anemia, infections, hemophilia, blood-clotting disorders, and leukemia (cancer of the white blood cells).

2.4.1.5 Complete blood count machine and it's principle (Hematology Analyzer)

Hematology analyzers are sophisticated automatic machines, and are commonly used in laboratories for Complete Blood Count (CBC) tests. This analyzer can rapidly process a large number of samples and provide accurate measurements of blood components, including detailed cell morphology (Zhang et al., 2014, Nezar *et al.,* 2014).

Hematology analysis plays a crucial role in diagnosing and monitoring various diseases, guiding treatment decisions, and evaluating patient response to therapy. With advancement in technology, the field of hematology continues to evolve, offering more precise and comprehensive diagnostic capabilities (Au *et al.,* 2012).

Principle (Hematology Analyzer)

The electrical impedance principle also called the Coulter principle, this method involves passing a blood sample through an aperture, they displace their volume, allowing for the counting and sizing of cells (Wang *et al.,* 2022).

The method is the Optical method, where light scatter measurement provide information on cell size, granularity, and complexity. This principle often complements electrical impedance in modern analyzer to enhance the differentiation and classification of various blood cells types (Wang *et al.,* 2022).

The Volumetric Absorptive Mirco-Sampling (VAMS) method is used, and this allows for accurate volume blood collection and analysis, minimizing the impact of hematocrit variability on the test results (Dvorak *et al.,* 2022).

2.4.2 Membrane stabilization

2.4.2.1 The Human Erythrocyte (HRBC) Membrane Stabilization Hypotonicity Process

A fresh whole blood (5mL) was collected from a healthy human volunteer, using syringe and transferred into and heparin or sodium citrate or EDTA tube to prevent it from clotting. The whole blood was gently mixed and with the anti-coagulant to avoid clotting. These test tube was then sent into the centrifuge, and was centrifuged at 3000rpm for a during of 10 minutes and was washed three times with equal volume of normal saline (isosaline) and 10% v/v suspension of the red blood cells was prepared and used for this study (Sadique *et al.,* 1989).

This test solution consist of 1mL of Phosphate buffer (pH 7.4, 0.15M), hypotonic saline (2mL), 0.5mL of *Kigelia africana* extracts, fraction and sodium Diclofenac at various concentration (100 µg/ml , 200 µg/ml , 300 µg/ml , 400 µg/ml , 500 µg/ml , 1000 µg/ml) respectively and 10 % HRBC (0.5mL). Control test comprised phosphate buffer (1 mL), distilled water (2 mL) and 10% HRBC (0.5 mL) in isotonic saline. These assays mixture are then incubated at 37°C for 30mins and centrifuged at 3000rpm for 20mins. The supernatant was decanted and hemoglobin content was estimated using a spectrophotometer at an absorbance of 560nm. Diclofenac was used as standard and control was prepared without extracts. The percentage membrane stabilization was projected using the below formula (Sakat *et al.,* 2010; Oyedapo *et al.,* 2004).

$$\% \text{ Hemolysis} = \left(\frac{\text{Optical Density of Test Sample}}{\text{Optical Density of Control}}\right) x\ 100$$

$$\% \text{ Protection} = 100 - \left[\left(\frac{\text{Optical density of Test sample}}{\text{Optical density of Control}}\right) x\ 100\right]$$

2.4.3 Statistical Analysis

Data gotten from this study was analyzed with the aid of a Graph-pad prism and SPSS software, a statistical software version 8.0.2 and software version 29. Using a one-way ANOVA to compare the means, followed by the prefer Turkey's test correction, values were considered significant at $p < 0.05$. All the data were expressed as mean $\pm$ SD (Standard Deviation).

CHAPTER 3

3.0 RESULT

3.1 Flavonoids Rich Extract fraction of *Kigelia africana* on Membrane Stabilization

The Human Red Blood Cell (HRBC) membrane stabilization method, the total Flavonoids extract from the plant *Kigelia africana* was assessed for it in vitro anti-inflammatory activity using the HRBC membrane stabilization method.

3.1.1 Hypotonicity Induced Hemolysis

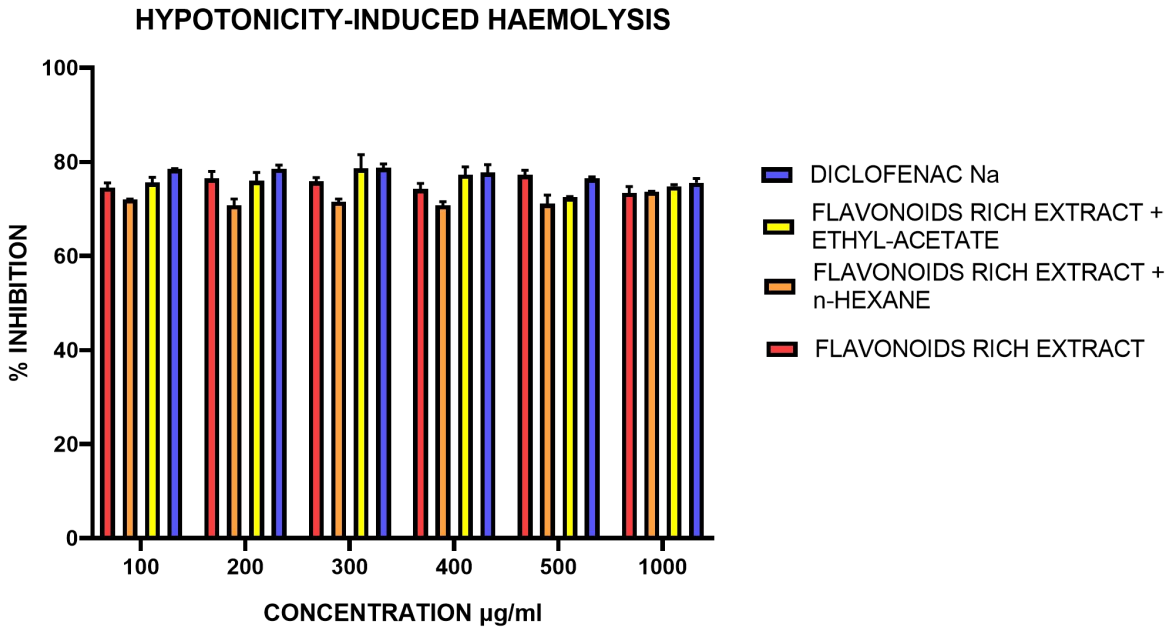

Figure 3.1: A statistical graph showing the concentrate of the Flavonoids Rich Extracts as against the %inhibition

3.1.2.1 Effect of Flavonoids Rich Extract (FRE) Hypotonicity induced hemolysis

The plant extracts FRE of *Kigelia africana* protection against the damaging effect of the hypotonic solution.

The result of FRE at the concentrations 100, 200, 300, 400, 500, 1000 µg/ml , at the concentration of 500 µg/ml FRE shows 77.245% maximum protection of human red blood cells in the hypotonic solution.

3.1.2.2 Effect of Ethyl acetate fraction of FRE on Hypotonicity induced hemolysis

The mechanism of flavonoids rich extracts with the ethyl acetate of *Kigelia africana* on human red blood cells induced by hypotonic hemolysis. Among all the concentrations 1000µg/ml shows significant anti-inflammatory activity and a 73.704% protection of HRBC in hypotonic solution.

3.1.2.3 Effect of n-Hexane fraction of FRE on Hypotonicity Induced hemolysis

The effect of n-Hexane fraction of FRE on the human red blood cells in hypotonic solution, shows that at concentration of 100 µg/ml is non-significant ($p > 0.05$) having 72.062% protection of HRBC in hypotonic solution, at the concentration 200 µg/ml non-significant ($p > 0.05$) and it having 70.803% protection of HRBC in hypotonic. At the n-Hexane fraction of FRE concentration at 300 µg/ml is considered extremely significant (**$p < 0.01$) and it protection is 71.533%. At 400µg/ml is considered significant (*$p < 0.05$) and has 70.78%, at the 500µg/ml show non-significant ($p > 0.05$) and 71.168% protection in hypotonic solution. At the 1000 concentration the n-Hexane fraction of FRE shows extremely significant (**$p < 0.01$) and a protection percentage of 73.704%.

3.1.2.4 Effect of Diclofenac sodium on Hypotonicity Induced hemolysis

The effect of the diclofenac sodium on the human red blood cells in a hypotonic solution in the concentration of the standard at 100 µg/ml is considered extremely significant and 78.504% protection of the diclofenac sodium, at a concentration of 200 µg/ml is contemplated significant and has 78.522% protection of the aspirin.

At the 300 µg/ml concentration has 78.777% protection of the diclofenac sodium in hypotonic solution. The diclofenac sodium at 400 µg/ml the percentage of protection was 77.774%, at the 500 µg/ml concentration 76.551% protection was found in the diclofenac sodium in a hypotonic solution and considered significant and lastly the diclofenac sodium at the 1000 µg/ml is significant and 75.602% protection of the diclofenac sodium. The diclofenac sodium is known to possess anti-inflammatory activities.

Table 3.1: Recorded values of the effect of flavonoids rich extracts of *Kigelia africana* fruit on hypotonicity hemolysis of RBC membrane

Solvents	**Concentrations**					
	100µg/ml	**200µg/ml**	**300µg/ml**	**400µg/ml**	**500µg/ml**	**1000µg/ml**
Diclofenac Sodium	78.504 ± 0.0063[a]	78.522 ± 0.453[b]	78.777 ± 0.005[a]	77.774 ± 0.948[b]	76.551 ± 0.158[b]	75.602 ± 0.516[b]
Flavonoid Rich Extract (FRE)	74.544 ± 1.004[c]	76.515 ± 0.854[b]	75.839 ± 0.485[b]	74.270 ± 0.674[b]	77.245 ± 0.559[b]	73.412 ± 0.790[b]
Ethyl acetate fraction of FRE	75.639 ± 1.077[c]	75.985 ± 1.033[c]	71.533 ± 0.358[b]	77.299 ± 0.948[b]	72.482 ± 0.084[b]	74.818 ± 0.210[b]
n-Hexane fraction of FRE	72.062 ± 0.053[b]	70.803 ± 0.779[b]	78.631 ± 1.675[c]	70.785 ± 0.453[b]	71.168 ± 1.032[c]	73.704 ± 0.053[a]

Values expressed as MEAN ± SEM (N = 3), where n represents number of absorbance values in triplate, the test sample were compared with the control. [a]$p < 0.01$, contemplated extremely significant; [c]$p > 0.05$, is non-significant (c); [b]$p < 0.05$ are contemplated significant.

3.2 HEMATOLOGY

Administration of total flavonoid extracts from *K. africana* mitigated the hematopoietic effects associated with carrageenan-induced inflammation in rats, shows a significant decrease ($p < 0.05$) in the White Blood Cells (WBC) of carrageenan only treated rat, carrageenan + sodium diclofenac treated rats, carrageenan + dexamethasone treated rats, and a significant increase in it WBC of rats treated with Carrageenan + Quercetin, Carrageenan + FRE (100mg/kg body wt) and Carrageenan + FRE (200mg/kg body wt) when compared to the control group (Figure 3.1).

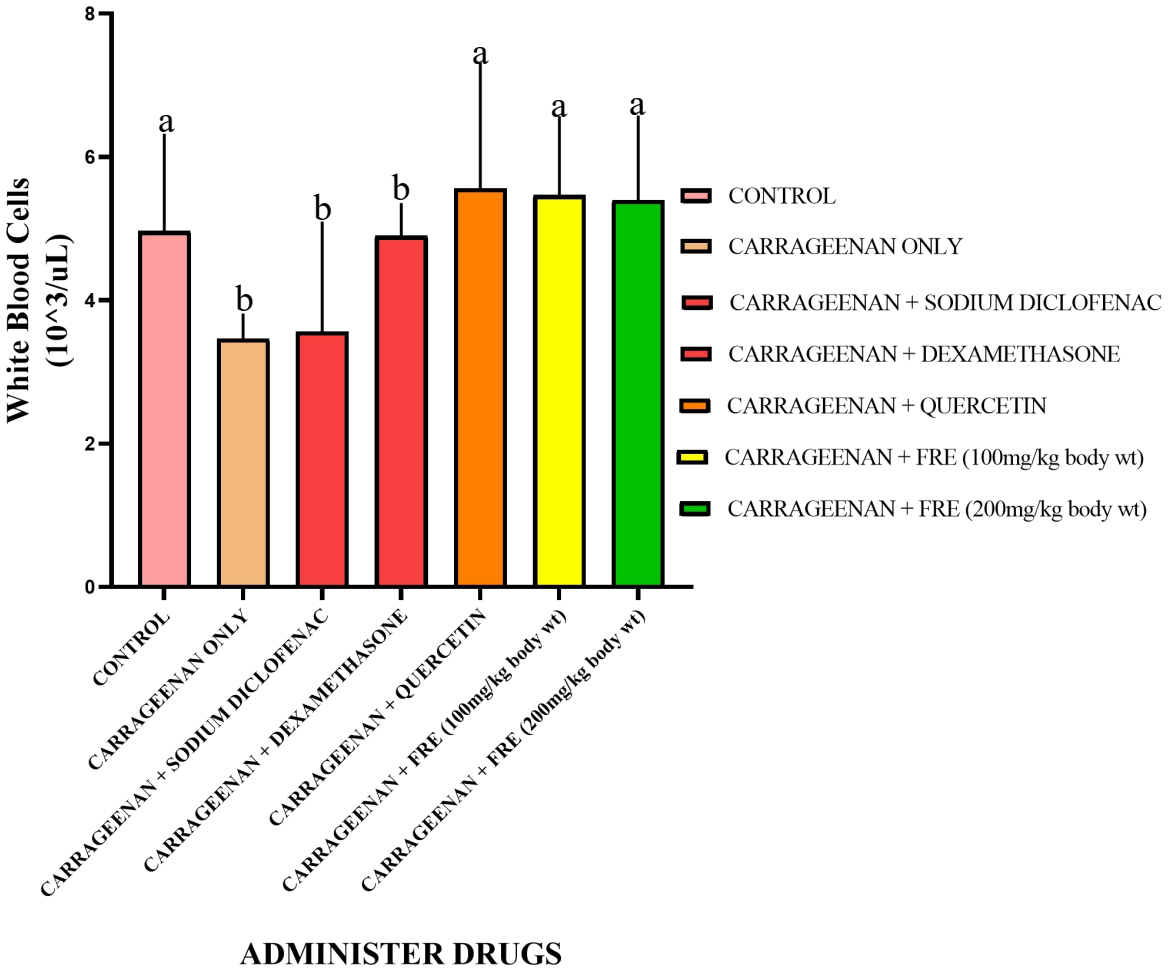

Figure 3.2: The White Blood Cells WBC expressed as Mean ± SD, $p < 0.05$ considered significant, this showing the graphical representation of the control rats group and the treated rats group.

3.2.1 Platelet (PLT), Red Blood Cells (RBCs), Hemoglobin (HGB) Effect of the treated rat hematology

There is a significant increase ($p < 0.05$) in Platelet (PLT), Red Blood Cells (RBCs) and Hemoglobin (HBG) of carrageenan only, carrageenan + sodium diclofenac, carrageenan + dexamethasone, carrageenan + quercetin, carrageenan + FRE (100mg/kg body wt) and Carrageenan + FRE (200mg/kg body wt).

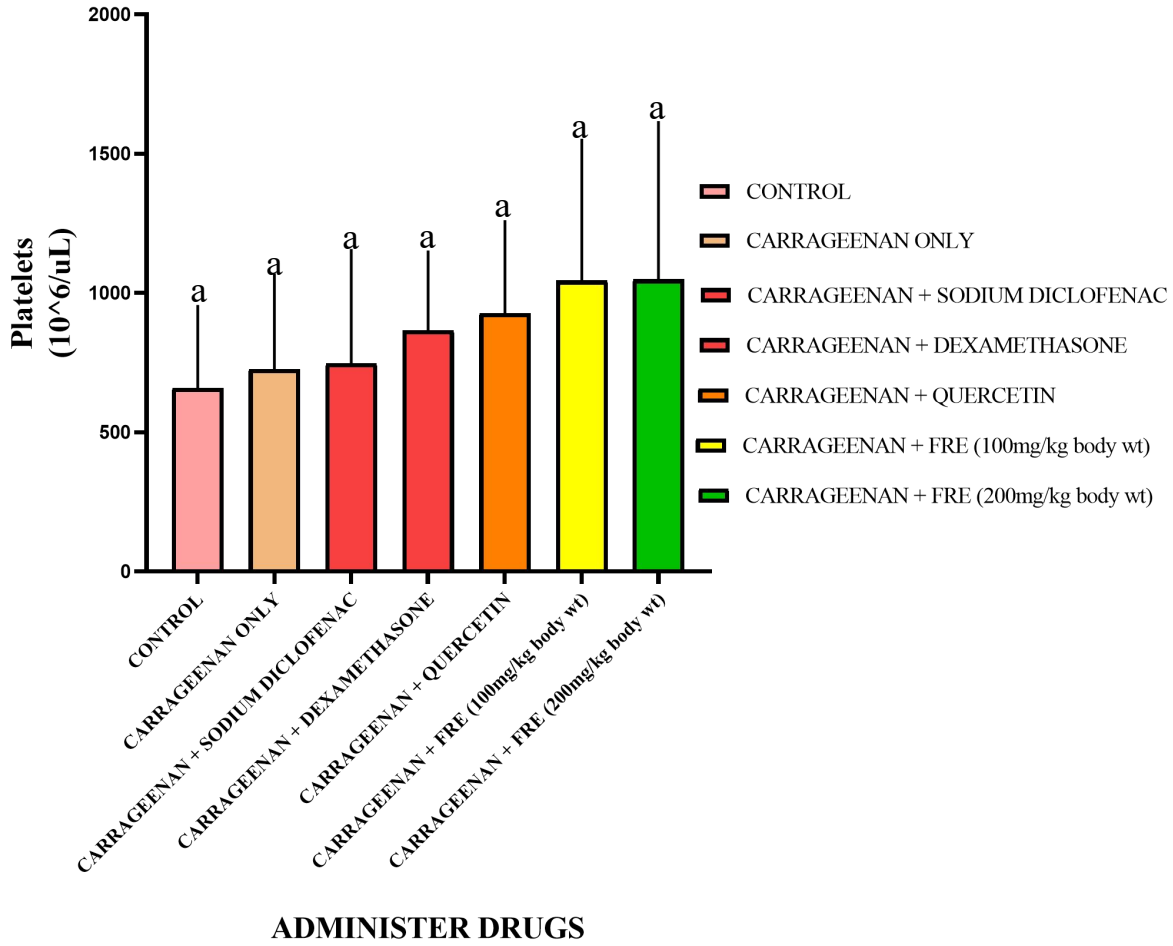

Figure 3.3: The Platelets (PLT) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats group and treated rats.

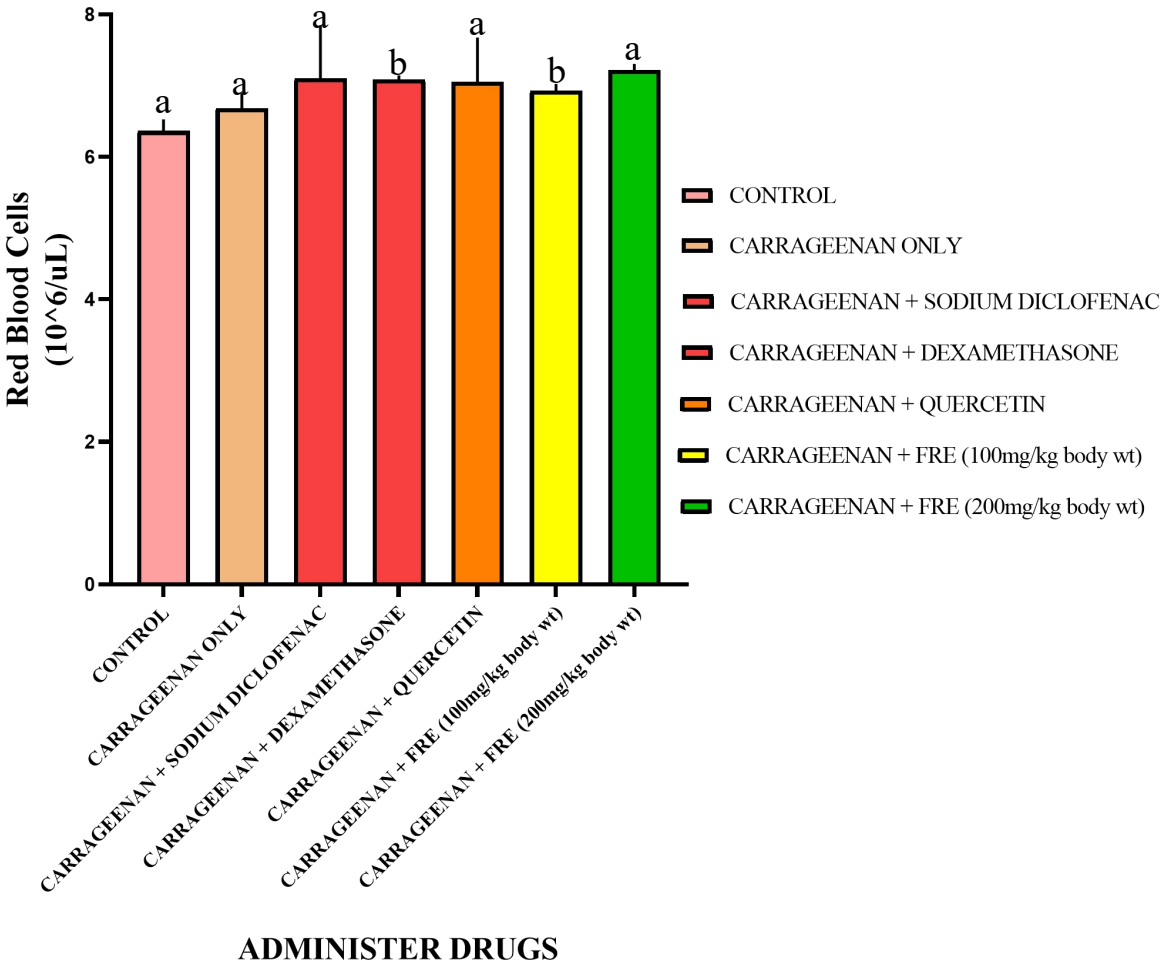

Figure 3.4: The Red Blood Cells (RBCs) expressed as Mean ± SD, $p < 0.05$ significant, showing it comparison between control rats and treated rats.

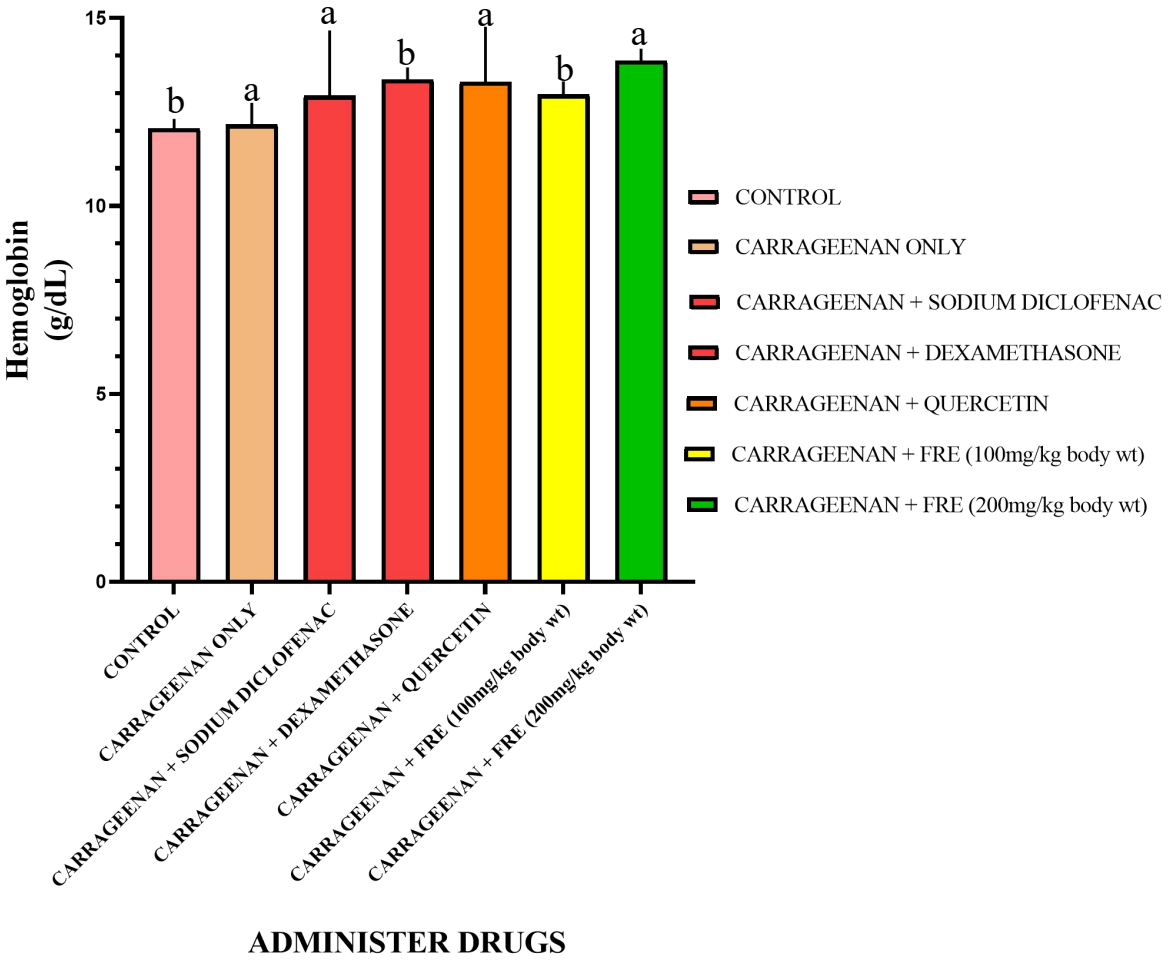

Figure 3.5: The Hemoglobin (HGB) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats and treated rats.

3.2.2 Lymphocytes (LYM) Effect of the treated rats hematology

There is a significant decrease ($p < 0.05$) in the Lymphocytes LYM of Carrageenan + sodium diclofenac, carrageenan + quercetin, and carrageenan + FRE (100mg/kg body wt) treated rats when been compared to the control group. There is a significant increase ($p < 0.05$) in the Lymphocytes (LYM) of Carrageenan only, carrageenan + dexamethasone, and carrageenan + FRE (200mg/kg body wt) when compare with control.

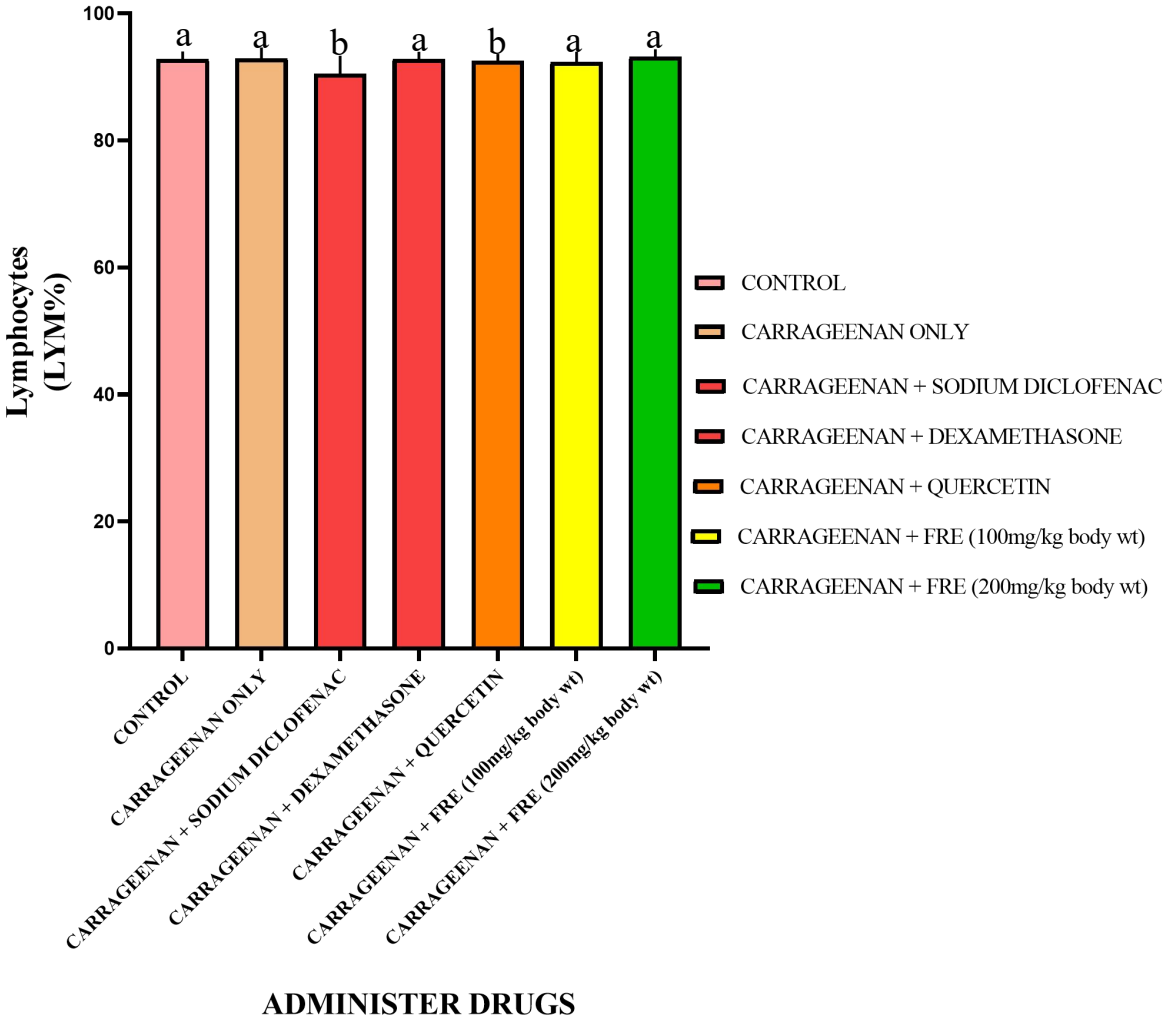

Figure 3.6: The Lymphocytes (LYM) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats and treated rats.

3.2.3 Monocytes (MXD) Effect of the treated rats hematology

There is a significant increase ($p < 0.05$) of the Monocytes (MXD) in carrageenan + sodium diclofenac, carrageenan + quercetin, carrageenan + FRE (100mg/kg body wt), carrageenan + FRE (200mg/kg body wt) treated rats, when compared to the control group. There is also a significant decrease of the monocytes (MXD) in carrageenan only treated rats. No significant change was noticed in the monocytes (MXD) of carrageenan + dexamethasone treated rats, as shown in Figure 3.6.

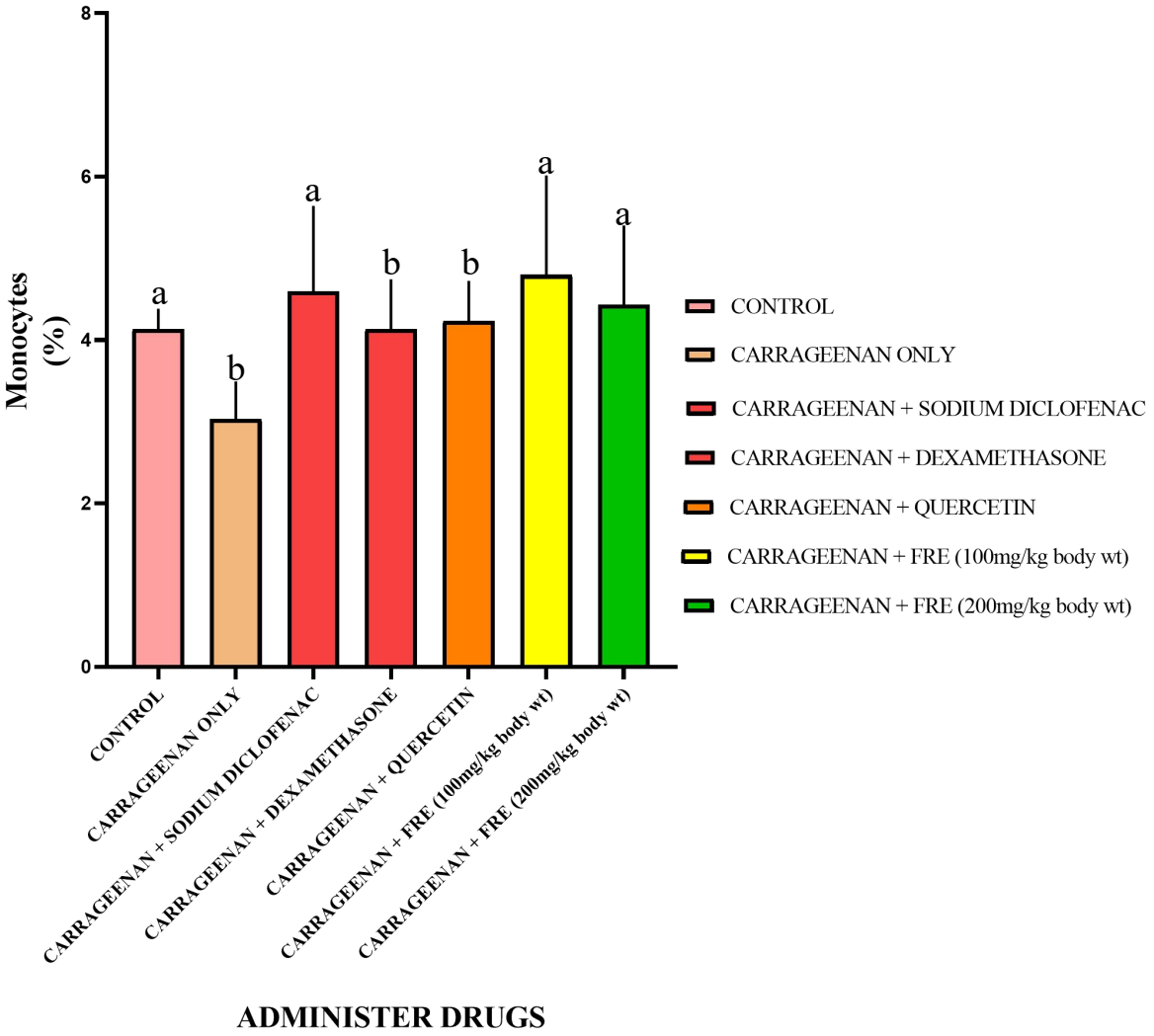

Figure 3.7: The Monocytes (MXD) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats group and treated rat.

3.2.4 Granulocytes (GRAN) Effect of the treated rats hematology

A significant increase ($p < 0.05$) of the Granulocytes (GRAN) was observed in carrageenan only, carrageenan + sodium diclofenac, and carrageenan + quercetin, treated rats when compared with the control groups. There is a significant decrease ($p < 0.05$) of the Granulocytes (GRAN) in rats treated with carrageenan + dexamethasone, carrageenan + FRE (100mg/kg body wt) and carrageenan + FRE (200mg/kg body wt) when compare to the control groups as shown in the Figure 3.7.

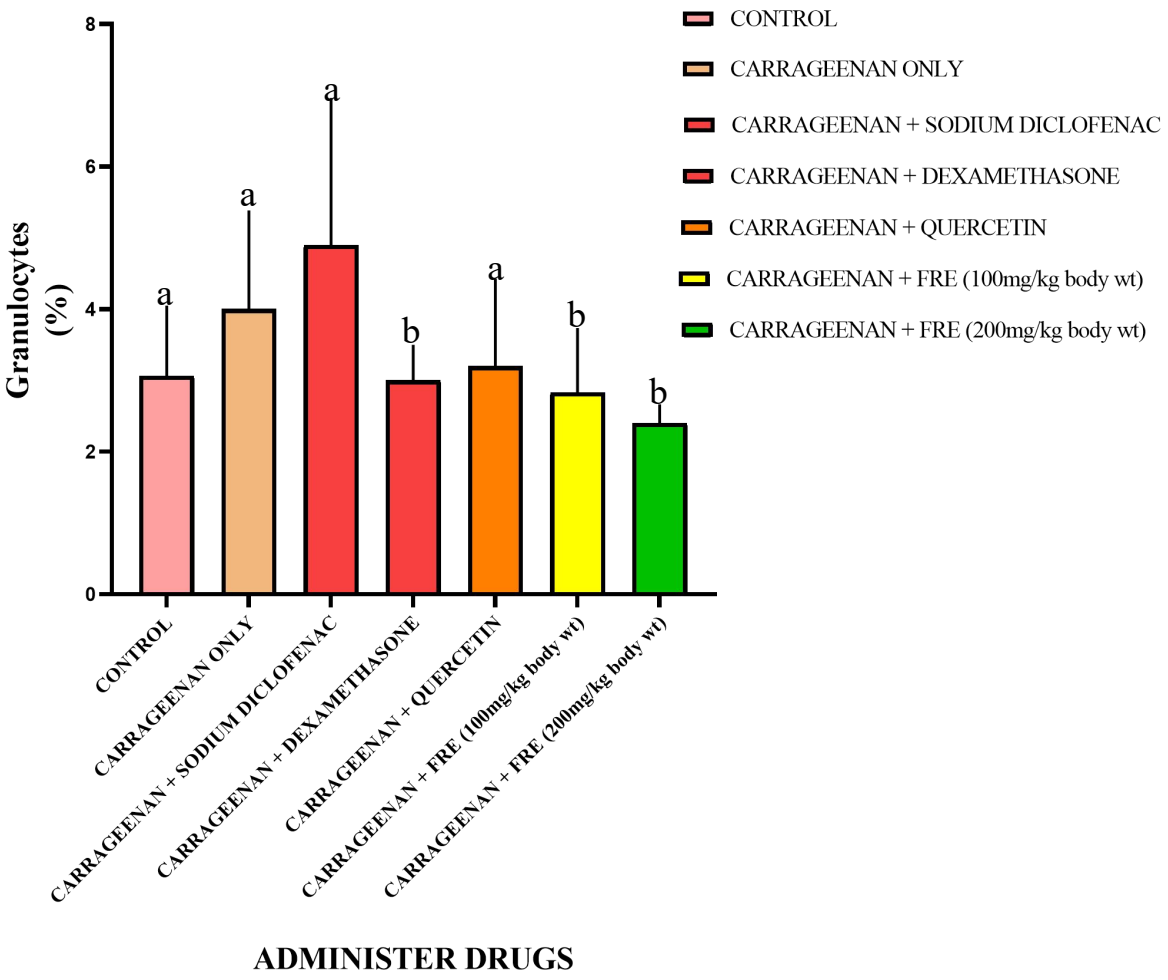

Figure 3.8: The Granulocytes (GRAN) expressed as Mean ± SD, $P < 0.05$ considered significant, showing it comparison between control rats and treated rats.

3.2.5 Hematocrit (HCT) Effect of the Treated Rats Hematology

There is a significant increase ($P < 0.05$) in the Hematocrit (HCT) level observe in rats treated with carrageenan + diclofenac, carrageenan + dexamethasone, carrageenan + quercetin, and carrageenan + FRE (200mg/kg) when compared to the control rats group. There is also a significant decrease in the Hematocrit (HCT) of carrageenan only, carrageenan + FRE (100mg/kg) when compared with the control sample (rats) as seen in Figure 3.8.

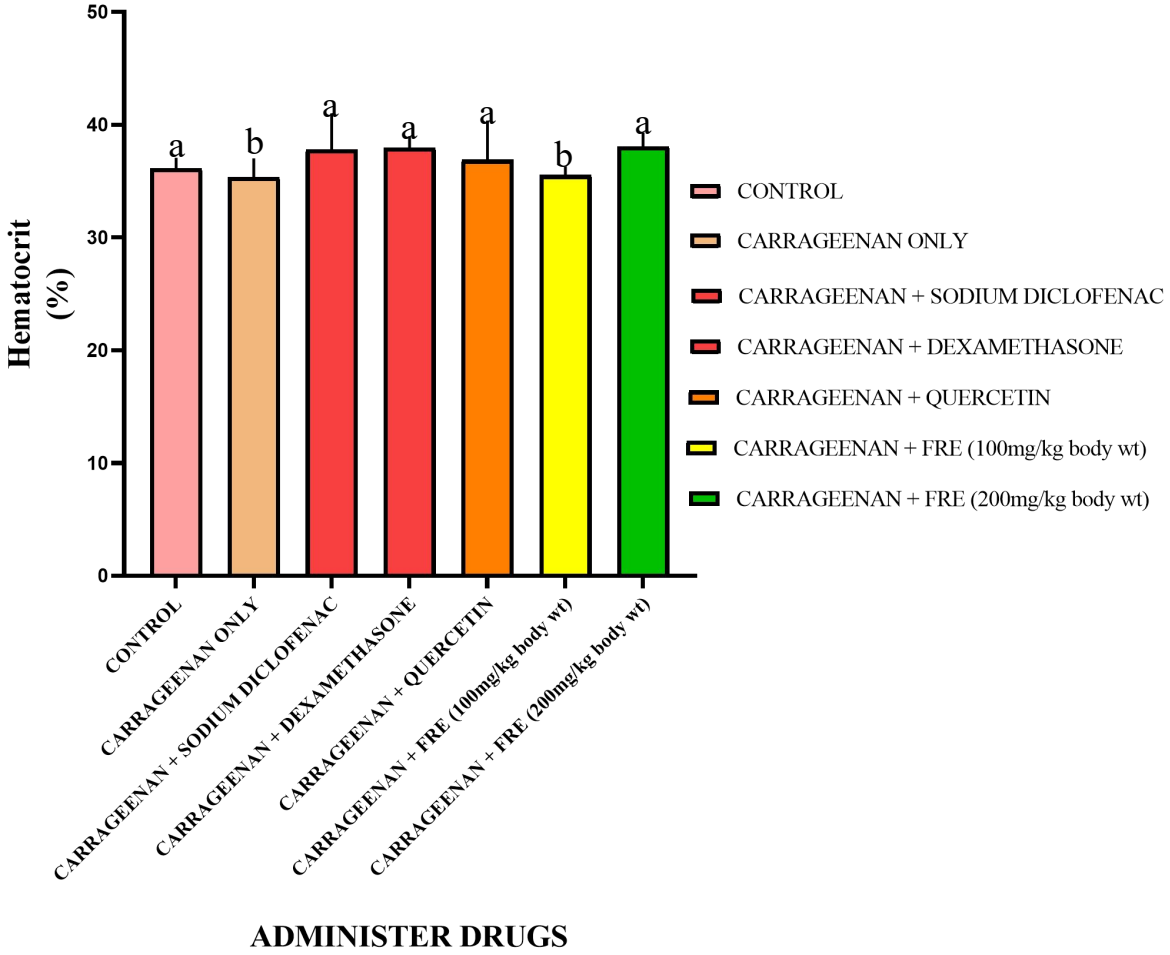

Figure 3.9: The Hematocrit (HCT) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats and treated rats.

3.2.6 Mean Corpuscular Volume (MCV) Effect of the treated rats hematology

There is a significant decrease ($p < 0.05$) in the Mean Corpuscular Volume (MCV) of carrageenan only, carrageenan + sodium diclofenac, carrageenan + dexamethasone, carrageenan + quercetin, carrageenan + FRE (100mg/kg body wt), carrageenan + FRE (200mg/kg body wt) treated rats when compared with control. As shown below in Figure 3.9.

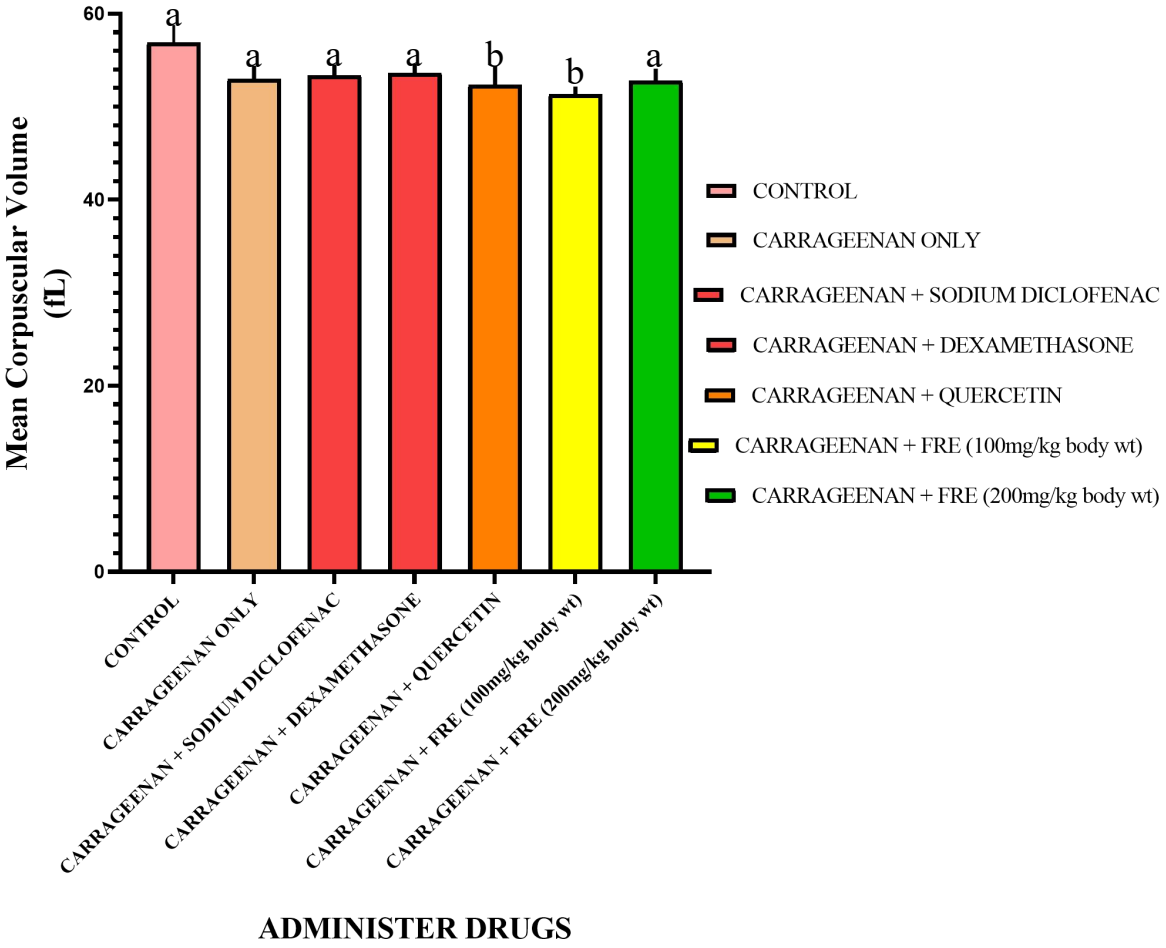

Figure 3.10: The Mean Corpuscular Volume (MCV) expressed as Mean ± SD, $p < 0.05$ considered significant, showing it comparison between control rats and treated rats.

3.2.7 Mean Corpuscular Hemoglobin (MCH) Effect of the treated rats hematology

There is a significant decrease ($p < 0.05$) in the Mean Corpuscular Hemoglobin (MCH) of carrageenan only, carrageenan + sodium diclofenac, carrageenan + dexamethasone, carrageenan + quercetin, carrageenan + FRE (100mg/kg body wt) treated rats when compared with control, and a significant decrease in the MCH of carrageenan + FRE (200mg/kg body wt) treated rats when compare with control group.

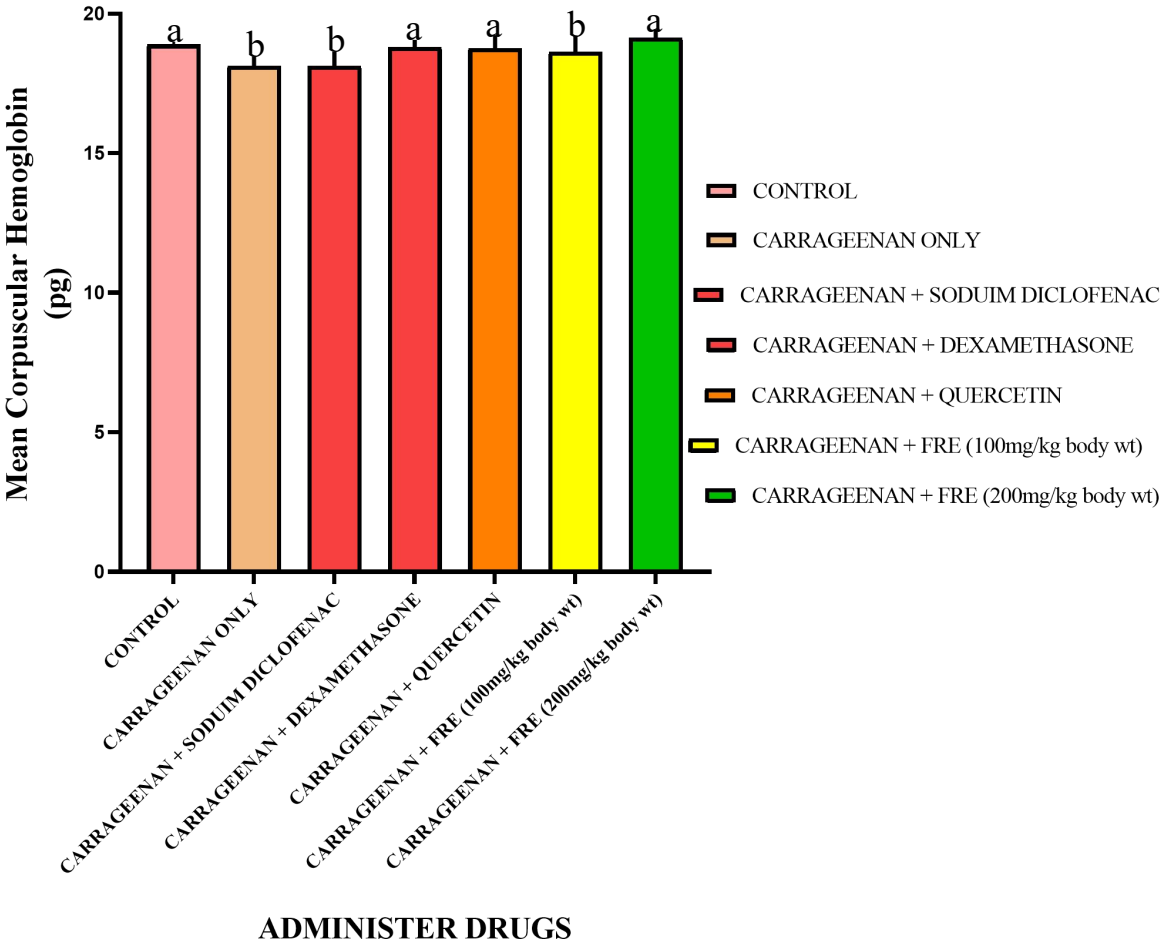

Figure 3.11: The Mean Corpuscular Hemoglobin (MCH) expressed as Mean $\pm$ SD, $p < 0.05$ considered significant, showing it comparison between control rats and treated rats.

CHAPTER 4

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

Kieglia africana is a medicinal plant that plays a key role in human health care system (Bonni *et al.,* 2018). Having a wide range of pharmacological activities been attributed to it, and this activities includes the anti-atherosclerotic effect (Kim *et al.,* 2018), hypoglycemia activity (Njogu *et al.,* 2018), anticonvulsant activity (Singh *et al.,* 2010), and anti-inflammatory activitiy (Abubakar *et al.,* 2018).

Inflammation is a complex biological vascular tissue to harmful stimuli. It is also a protective attempt by the organisms to remove the injurious stimuli and initiate the healing process (Ferrero-Miliani, 2007). These mediator molecule works collectively to cause increased vasodilation and permeability of blood vessels. Thus leading to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessel into the injured tissues (Chaitanya, 2011). Inflammation is classified into two Acute or Chronic inflammations (Okoli, 2007).

The Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) possess anti-inflammatory action for treating inflammatory diseases or inflammatory condition such as rheumatoid arthritis, osteoarthritis, the prolonged use of these non-steroidal anti-inflammatory drugs could lead to possible cause of vomiting, nausea, bleeding and gastric lesion (Abebe, 2002).

NSAIDs drugs like diclofenac sodium, ibuprofen, acetaminophen, naproxen, aspirin, dexamethasone, indomethacin are frequently employed in treating inflammation (Fiorucci *et al.,* 2000). These medication inhibits the enzyme cyclooxygenase (COX), thereby impeding the biosynthesis of PGE-2.

There are two isoforms of COX: COX-1 and COX-2. COX-2 generates prostaglandins that exacerbate inflammation, while COX-1 produces prostaglandins that aids platelets and safeguard the stomach (Mitchell *et al.,* 1993).

NSAIDs exert lesser inhibition on the initial stage of carrageenan-induced paw edema, primarily due to the release of histamine, serotonin, and bradykinin. Conversely, the second phase is linked to the induction of inducible COX-2 and can be counteracted using NSAIDs (Nantel *et al.,* 1999).

However, a number of studies have shown that flavonoids and a host of other plant compound exhibit anti-inflammatory effects as a result of their membrane stabilizing ability in various experimental models (David, 2007; Jorge *et al.,* 2004). It has also been shown in some experimental models that the *Kigelia africana* leaf extract contains flavonoids e.g quercetin (Caninia *et al.,* 2007).

The plant extracts FRE of *Kigelia africana* protection against the damaging effect of the hypotonic solution. The result of FRE at the concentrations 100, 200, 300, 400, 500, 1000µg/ml, at the concentration of 500µg/ml FRE shows 77.245% maximum protection of human red blood cells in the hypotonic solution.

The mechanism of flavonoids rich extracts with the ethyl acetate of *Kigelia africana* on human red blood cells induced by hypotonic hemolysis. Among all the concentrations 1000µg/ml shows significant anti-inflammatory activity and a 73.704% protection of HRBC in hypotonic solution.

The effect of n-Hexane fraction of FRE on the human red blood cells in hypotonic solution, shows that at concentration of 100 µg/ml is non-significant ($p > 0.05$) having 72.062% protection of HRBC in hypotonic solution, at the concentration 200 µg/ml non-significant ($p > 0.05$) and it having 70.803% protection of HRBC in hypotonic.

At the n-Hexane fraction of FRE concentration at 300 µg/ml is considered extremely significant (**$p < 0.01$) and it protection is 71.533%. At 400µg/ml is considered significant (*$p < 0.05$) and has 70.78%, at the 500µg/ml show non-significant ($p > 0.05$) and 71.168% protection in hypotonic solution. At the 1000 concentration the n-Hexane fraction of FRE shows extremely significant (**$p < 0.01$) and a protection percentage of 73.704%.

The effect of the diclofenac sodium on the human red blood cells in a hypotonic solution in the concentration of the standard at 100 µg/ml is considered extremely significant and 78.504% protection of the diclofenac sodium, at a concentration of 200 µg/ml is contemplated significant and has 78.522% protection of the diclofenac sodium.

At the 300 µg/ml concentration has 78.777% protection of the diclofenac sodium in hypotonic solution. The diclofenac sodium at 400 µg/ml the percentage of protection was 77.774%, at the 500 µg/ml concentration 76.551% protection was found in the diclofenac sodium in a hypotonic solution and considered significant and lastly the diclofenac sodium at the 1000µg/ml is significant and 75.602% protection of the diclofenac sodium. The aspirin is known to possess anti-inflammatory activities.

This is as shown that the plant extract possess anti-inflammatory activities due to the present of flavonoids as compared to the standard (diclofenac sodium).

The health status and physiological markers of experimental animals are closely linked to their hematopoietic systems. Additionally, these systems are particularly susceptible to the effect of these toxic substance in these models (Mukinda and Syce, 2007). In the carrageenan only induced rats there is a significant increase in the platelets (PLT), Red blood cells (RBC), hemoglobin and Granulocytes.

The finding from the previous study of Ogbe *et al.,* 2010 and Babayemi and Precious 2021, and a significant decrease in Monocytes, Hematocrit, Mean Corpuscular Volume and Mean Corpuscular Hemoglobin as backed with the study from Olatunde *et al.,* 2018.

Activity of Sodium diclofenac on carrageenan-induced edema in wastar rats significantly increase in Platelets, Red Blood Cells, Hemoglobin monocytes, Granulocytes, Hematocrit, and a significant decrease in the in white blood cells, Mean Corpuscular Hemoglobin and Mean Corpuscular Volume as in similar experiment of Farhangi *et al.,* 2013.

Activity of dexamethasone on carrageenan-induced edema in wastar rats shows a significant increases in Platelet, Red Blood Cells, Hemoglobin, Lymphocytes, and Hematocrit and a significant decrease was seen in the hematology results of White Blood Cells, Granulocytes, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, and no significant change in the monocytes. In line with the findings of Shobana *et al.,* 2017 experiment.

Activity of Quercetin on carrageenan-induced edema in wastar rats show a significant increase in the White Blood Cells Platelet, Red Blood Cells, Hemoglobin, Monocytes, Granulocytes, and Hematocrit. A significant decrease was seen in Lymphocytes, Mean Corpuscular Volume, and Mean Corpuscular Hemoglobin. Our result agrees with the finding and experiment of (Eyon *et al.,* 2004).

Activity of FRE (100mg/kg body wt) on carrageenan-induced edema in wastar ratsshow a significant increase in White Blood Cells, Platelet, Red Blood Cells, Hemoglobin, Monocytes, Mean Corpuscular Hemoglobin and a significant decrease in the Granulocytes, Lymphocytes, Hematocrit, Mean Corpuscular Volume just like the experiment of Abdul-Kalam *et al.*, 2018.

Activity of FRE (200mg/kg body wt) on carrageenan-induced edema in wastar rats is seen with a significant decrease in Granulocytes, Mean Corpuscular Hemoglobin and a significant

increases in White Blood Cells, Platelet, Red Blood Cells, Hemoglobin, Monocytes, and hematocrit, as seen in Amdekar *et al.,* 2012, findings.

4.2 Conclusion

It can be concluded from this study that *kigelia africana* possess anti-inflammatory properties, most especially the fractions of the Flavonoids Rich Extract has shown protection against hemolysis of human red blood cells in hypotonic solution, that is it can be used for the management of in-vitro inflammation. This plant extract has also shown therapeutic effect against the carrageenan-induced paw edema.

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APPENDICES

ONEWAY ANOVA

Ethyl acetate fraction of FRE						
CONCENTRATION	ABS 1	ABS 2	ABS 3	MEAN	Std. Error	
100	76.715	74.562	75.639	75.639	0.622	
200	77.774	74.197	75.985	75.985	1.033	
300	81.533	75.73	78.631	78.631	1.675	
400	75.657	78.942	77.299	77.299	0.948	

500	72.336	72.628	72.482	72.482	0.084	
1000	74.453	75.182	74.818	74.818	0.210	
n-Hexane fraction of FRE						
100	72.153	71.971	72.062	72.062	0.053	
200	69.453	72.153	70.803	70.803	0.779	
300	70.912	72.153	71.533	71.533	0.358	
400	70	71.569	70.785	70.785	0.453	
500	69.38	72.956	71.168	71.168	1.032	
1000	73.613	73.796	73.704	73.704	0.053	
FRE						
100	73.54	75.547	74.544	74.544	0.579	
200	75.036	77.993	76.515	76.515	0.854	
300	76.679	75	75.839	75.839	0.485	
400	75.438	73.102	74.27	74.270	0.674	
500	76.277	78.212	77.245	77.245	0.559	
1000	72.044	74.781	73.412	73.412	0.790	
				74.544	0.579	
DICLOFENAC SODIUM						
100	78.613	78.394	78.504	78.504	0.063	
200	79.307	77.737	78.522	78.522	0.453	
300	77.993	79.562	78.777	78.777	0.453	
400	76.131	79.416	77.774	77.774	0.948	
500	76.825	76.277	76.551	76.551	0.158	
1000	74.708	76.496	75.602	75.602	0.516	

MULTIPLE COMPARISON

TUKEY'S MULTIPLE COMPARISONS TEST	MEAN DIFF.	95.00% CI OF DIFF.	SIGNIFICANT?	SUMMARY	ADJUSTED P VALUE			
FLAVONOIDS RICH EXTRACT vs. N-hexane fraction of FRE	3.628	2.564 to 4.693	Yes	****	<0.0001			
FLAVONOIDS RICH EXTRACT vs. Ethyl acetate fraction of FRE	-0.5049	-1.569 to 0.5595	No	ns	0.5908			
FLAVONOIDS RICH EXTRACT vs. DICLOFENAC Na	-2.318	-3.382 to -1.253	Yes	****	<0.0001			
N-hexane fraction of FRE vs. Ethyl acetate fraction of FRE	-4.133	-5.198 to -3.069	Yes	****	<0.0001			
N-hexane fraction of FRE vs. DICLOFENAC Na	-5.946	-7.010 to -4.881	Yes	****	<0.0001			
Ethyl acetate fraction of FRE vs. DICLOFENAC Na	-1.813	-2.877 to -0.7481	Yes	***	0.0002			
TEST DETAILS	**MEAN 1**	**MEAN 2**	**MEAN DIFF.**	**SE OF DIFF.**	**N1**	**N2**	**Q**	**DF**
FLAVONOIDS RICH EXTRACT vs. N-hexane fraction of FRE	75.3	71.68	3.628	0.4	18	18	12.83	48
FLAVONOIDS RICH EXTRACT vs. Ethyl acetate fraction of FRE	75.3	75.81	-0.5049	0.4	18	18	1.785	48
FLAVONOIDS RICH EXTRACT vs. DICLOFENAC Na	75.3	77.62	-2.318	0.4	18	18	8.195	48
N-hexane fraction of FRE vs. Ethyl acetate fraction of FRE	71.68	75.81	-4.133	0.4	18	18	14.62	48

N-hexane fraction of FRE vs. DICLOFENAC Na	71.68	77.62	-5.946	0.4	18	18	21.02	48
Ethyl acetate fraction of FRE vs. DICLOFENAC Na	75.81	77.62	-1.813	0.4	18	18	6.409	48

HEMATOPOIETIC PROPERITIES	**EXTRACT AND DRUGS**						
	CONTROL	**CARRAGEENAN ONLY**	**CARRAGEENAN DICLOFENAC-Na**	**CARRAGEENAN + DEXAMETHASONE**	**CARRAGEENAN + QUERCETIN**	**CARRAGEENAN + FRE (100mg/kg)**	**CARRAGEENAN + FRE (200mg/kg)**
WBC	4.967	3.467	3.567	4.900	5.567	5.467	5.400
LYM	92.800	92.967	90.500	92.867	92.567	92.367	93.167
MXD	4.133	3.033	4.600	4.133	4.233	4.800	4.433
GRAN	3.067	4.000	4.900	3.000	3.200	2.833	2.400
RBC	6.363	6.683	7.103	7.087	7.057	6.930	7.217
HGB	12.067	12.167	12.933	13.367	13.300	12.967	13.867
HCT	36.133	35.367	37.800	37.967	36.900	35.533	38.067
MCV	56.900	52.967	53.400	53.633	52.367	51.333	52.800
MCH	18.900	18.133	18.133	18.800	18.767	18.633	19.133
PLT	660.000	727.333	747.667	865.333	928.667	1044.667	1049.333