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RESEARCH ARTICLE
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COMPARATIVE EFFICACY OF ANTI-INFLAMMATORY POTENTIAL OF DIFFERENT AGAVE SPECIES IN RAT MODEL

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Abstract
Inflammation has been reported as an immune system regulator from the last two decades but cellular and molecular pathways involved in tissue damage has only been established in the recent era. Additionally, it has been involved in the morbidity of different diseases like cancer, diabetes, heart disease, and rheumatoid arthritis. The control of two key players of inflammation i.e., prostaglandins and cytokines activation must be provided with different therapeutic strategies to control all the diseases that have close relationship with inflammatory biomarkers. Use of several pharmaceutical treatments for inflammation is in practice however prolonged usage even with physician supervision may have threat to kidney and liver failure. Researchers from different regions of the world are working on the phytotherapy to lessen the risks that are associated with the use of synthetic drugs. For this purpose, the anti-inflammatory potential of different leaf extracts was investigated via in-vivo study on rat model. With regard to inflammation in carrageenan-induced paw edema in rats, we aim to contrast the leaf extracts of *Agave sisalana* and *Agave americana* with standard drug Aliskiren. Twenty-five albino rats were grouped as control, positive control, standard drug, *Agave sisalana* extract and *Agave americana* extract treated. To demonstrate acute phase protein activation and a rise in inflammatory biomarkers in the positive control group, serum levels of CRP, TNF-, COX-2, and IL-10 were assessed. Bilirubin levels remain unchanged before and after treatments, however the positive control group has a decline in ALT, AST, Albumin, and A/G ratio. Alkaline phosphatase, total protein, and globulin revealed significant improvements after therapy when compared to the positive control group ($p < 0.05$). Furthermore, histopathological analysis of paw skin of rats also revealed rete pegs and normal sub-epidermal structures. Edema and inflammatory cells diminished, and the epidermis became reddish. Agave species extract from *Agave sisalana* and *Agave americana* raised serum inflammatory indicators and promoted paw skin histology in groups that had been subjected to inflammation.

Keywords: Paw edema, *Agave sisalana*, *Agave americana*, phytochemicals, Aliskiren

1. Introduction

The immune system's physiological response to a particular stimulus is known as inflammation. A wide variety of chemical substances, infections, antibodies, irritants, and physical traumas can cause this syndrome to start (Chen et al., 2018). Inflammation may present as either acute or chronic depending on the underlying properties of the stimulus and the first response in removing the harmful tissue (Saha and Ahmed, 2009). Although it is essential for the healing process, inflammation also increases the risk of developing a number of illnesses, including rheumatoid arthritis, cancer, atherosclerosis, hay fever, periodontitis, neurologic disorders, autoimmune diseases, diabetes, and cardiovascular conditions (Aziz et al., 2020). There are several approaches that may be used to treat a wide range of diseases that are correlated with inflammation. During a certain stage of the illness, inflammatory cells like neutrophils and macrophages become active. The production of numerous anti-inflammatory mediators and inflammatory cytokines is caused by the activation of macrophages, which causes the expression of a cascade of genes linked to the host's capacity to fight infections (Yoon *et al*., 2009).

Plants that contain phytochemicals have advantages for human health. These substances fall under the category of secondary metabolites, which are produced through certain metabolic processes and act as building blocks for primary metabolites. Phytochemicals are essential for enhancing human health and warding off a variety of illnesses (De Silva et al 2017). Future medications based on plants have a great deal of potential to treat a variety of chronic diseases and inflammatory conditions (Begum *et al.,* 2015). As an alternate strategy for reducing inflammation, phytotherapy is used. Numerous medicinal plants and herbs have regulatory capabilities in terms of their anti-inflammatory potential against a variety of acute and chronic illnesses, including Turmeric (Curcumin), green tea, Maritime pine bark, black pepper, and various species from the Asparagaceae family (Dunder *et al*., 2010).

Furthermore, the Asparagaceae family includes *Agave sisalana* and *Agave americana*. On a worldwide level, these species are widely farmed. The plants under consideration have anti-inflammatory, analgesic, and insecticidal qualities (Viel, 2017; Shahzad et al., 2017). They have been evaluated for the hexanic fraction's reduced-toxicity anti-inflammatory and analgesic properties. (Tewari et al., 2014). *Agave sisalana, Agave americana*, and *Agave cupreata* have all been investigated for their potential medicinal uses in treating a variety of ailments and diseases. Prior study has also revealed the existence of potential anti-inflammatory chemicals in these plants (Pineda *et al.*, 2017). The medical industry uses a significant amount of the phytochemical substances found in agave sisalana. Additionally, it is distinguished by the inclusion of flavonoids and saponins in its makeup (Sarwar et al., 2020). The capacity of *Agave sisalana's* aqueous leaf extract to inhibit the enzyme cyclooxygenase, so preventing the production of prostaglandins, is the cause of its reported inhibitory activity. The release of vasoactive molecules is also thought to be constrained by this extract, adding to its inhibitory effects. According to Shahzad et al. (2017), Steroid saponins and diosgenin found in agave sisalana, such as tigogenin, sisalagenin, and neotigogenin, have been discovered to have anti-inflammatory characteristics and to be effective in treating wounds and gastrointestinal conditions. Additionally, *Agave americana* plants may be used therapeutically to cure a variety of illnesses. Different medicinal plants include phytochemical elements that are used for various pharmacological goals. The plant's leaves contain bioactive substances, including as alkaloids, flavonoids, and saponins, which have protective qualities and provide defense against a variety of diseases (Singh et al., 2018).

A particularly sensitive and accurate test that is frequently used in the assessment of new markers with anti-inflammatory activities is the production of paw edema in a rat model using carrageenan. Carrageenan, a high molecular weight sulfated polysaccharide produced from seaweeds, is used in experimental settings to cause inflammation in rats. According to Kim et al. (2020), it has been proven that, in the context of inflammation, it also causes the release of histamine, a mediator of vascular outflow. Carrageenan administration causes an instantaneous and localized inflammatory reaction that can be used to assess the effectiveness of orally active anti-inflammatory medications. Carrageenan

is therefore extremely important as an anti-inflammatory medication that works by aiming at mediators of severe inflammation (Dzoyem et al., 2017; Cordaro et al., 2020).
Ibuprofen and diclofenac are currently used commonly for the short-term treatment of inflammation. When taken without adequate medical supervision, nonsteroidal anti-inflammatory medications (NSAIDs) have been demonstrated to have negative consequences (Haley & Recum, 2019). Aspirin, Indomethacin, Phenylbutazone, and Aliskiren are some of the pharmaceuticals medications used to treat inflammation. One of the substances, Aliskiren, belongs to a family of oral anti-hypertensive drugs and works as a direct renin inhibitor (Oliveira et al., 2019). Pacurari et al. (2014) have documented the participation of the renin-angiotensin-aldosterone system (RAAS) in the inflammatory process according to recent research findings. It is well recognized that the renin-angiotensin-aldosterone system (RAAS) is essential for controlling and promoting inflammation. The renin-angiotensin-aldosterone system (RAAS) has been proven to have pro-inflammatory and pro-fibrotic effects at the cellular and molecular levels. Since aliskiren possesses anti-inflammatory properties, it has been widely used in animal models of inflammation (Patel et al., 2013).

2. Materials and Methods

2.1. Ethical Approval

The experimental research work on a rat model received ethical approval from the Ethical Committee of the Institute of Molecular Biology and Biotechnology at The University of Lahore. The approval number for the USM Animal Ethics is Approval/2009/ [45] [140].

2.2. Experimental Animal Model

A total of 25 healthy adult female Albino rats, ranging in age from 10 to 12 weeks, were acquired from the animal facility at the Institute of Molecular Biology and Biotechnology, The University of Lahore. Prior to conducting the experiment, the mean weight of each rat was documented to range between 180 and 200 grammes. The rats were housed in sanitary cages maintained at a temperature range of 23°C-25°C, and subjected to a natural light/dark cycle. The animals were provided with unrestricted access to pelleted rat food and water. The duration of the acclimatisation phase prior to the commencement of the experiment was two weeks, during which the animals were housed under controlled conditions in an animal facility (Dave *et al,* 2020).

2.3. Extract Preparation

2.3.1. Preparation of Agave sisalana Leaf Extract

Fresh leaves of *Agave sisalana* were taken from Jinnah Garden, Lahore, Pakistan and were afterwards validated by the Flora and Phyto-taxonomy Research Department of Botany at The University of Lahore. The leaves underwent a washing process including tap water and afterwards distilled water. The leaves were fragmented into smaller segments. Subsequently, fragments of foliage were pulverised into a granulated state. A total volume of 1500 millilitres of water was utilised in the process of percolation, specifically for the purpose of extracting compounds from 200 grammes of crushed leaves. The percolation procedure involved shaking the mixture intermittently over a duration of 48 hours. The mixture that has undergone percolation was subjected to filtration using a filter paper. The filter extract was subjected to vacuum concentration using a rotary evaporator at a temperature of 40 °C. The specimen will be stored in a refrigerated environment at a temperature of 4 °C until it is ready for utilization . (Dave et al., 2020).

2.3.2. Preparation of *Agave americana* Leaf Extract

Fresh leaves of *Agave americana* were additionally procured from Jinnah Garden, located near Lahore, Pakistan. The verification of plant species identification was conducted by referring to the Flora and Phyto-taxonomy research Department of Botany at The University of Lahore. The leaves underwent a washing process using both tap water and distilled water. The leaves were fragmented into little pieces and afterwards pulverised into a crushed state. The plant material was subjected to maceration in a solvent composed of 70% ethanol for a duration of 24 hours. The extract was subjected

to filtration using filter paper. The hydroalcoholic extract was prepared using a percolation technique. The combination will thereafter undergo a process of percolation, followed by drying in petri plates, resulting in the acquisition of the extract in powdered form. Prior to conducting the experiment, a new solution of *Agave americana* leaf extract will be made by dissolving it in distilled water. Subsequently, the experimental animal will be administered the leaf extract orally (Misra et al., 2018).

2.4. Test for Saponins and Flavonoids

The botanical extract is diluted using distilled water. The plant material is agitated within a graduated cylinder for a duration of 15 minutes to assess the production of a dense foam, which serves as an indicator for the presence of saponins. The presence of flavonoids can be detected by an alkaline reagent test. In order to achieve the intended objective, minute quantities of sodium hydroxide were incrementally introduced into the filtrate of the plant extract. The presence of flavonoids in a plant extract can be confirmed if the deep yellow colour of the solution turns colourless upon the addition of dilute acid, as demonstrated by Saxena et al. (2013).

2.5. Standard Drug

In this experimental investigation, the standard medicine utilised to combat inflammation was Aliskiren, obtained from Pacific Pharma under the brand name Rasilez at a dosage of 150 mg. The medicine was provided to a group of rats treated with Aliskiren in order to alleviate inflammation in the rat models (Aziz et al., 2020).

2.6. Induction of Inflammation and Measurement of Paw

To initiate an inflammatory response, carrageenan obtained from a local market was utilised. All rat groups, with the exception of the control group, were treated to induction through intraperitoneal injection into their paws. A total of 0.2 ml of a 1% carrageenan suspension was applied to the foot pads of the left hind paws of all the animals for a duration of 7 consecutive days. The measurement of paw thickness was conducted and documented four hours following the administration of the dose using vernier callipers, as outlined in the study by Kim et al. (2021).

2.7. Experimental Design

The animals have been categorised into five distinct experimental groups, namely Control, Positive control, Aliskiren treated, *Agave sisalana* treated, and *Agave americana* treated groups, respectively. Each group consisting of six animals was treated in accordance with the appropriate protocol. In the control group, the animals were administered a daily dose of normal saline solution at a volume of 1 ml/kg. The positive control group consisted of rats that were proven to have inflammation induced by the administration of a 0.2 ml suspension of 1% carrageenan into the left hind paw (Kim et al., 2020). In the Aliskiren treated group, the animals received a standard drug dosage of 20 mg/kg/day for a duration of seven consecutive days following the initiation of inflammation (Aziz et al., 2020). On the other hand, Group IV (treated with *Agave sisalana* extract) and Group V (treated with *Agave americana* extract) were administered an oral dose of 400 mg/kg (equivalent to 80 mg per 200 g) of the respective extracts for a period of seven days in order to investigate their potential anti-inflammatory effects (Tewari et al., 2014).

2.8. Toxicity Test

The acute toxicity experiments were conducted using the methodology outlined by Sawadogo et al. in 2006. The rats in group IV were administered a single dosage of approximately 80 mg/200g of *A.sisalana* extract, while the rats in group V were administered the same dose of *A.americana* extract. The rats were subjected to regular observation at a frequency of every 3 hours during a period of approximately 7 days. This finding encompasses several aspects such as mortality, behavioural and physiological alterations, which are characterised by disruptions in skin, eyes, feeding behaviour, and stress, as noted by Misra et al. (2018).

2.9. Biochemical Analysis

2.9.1. Sample Collection

Following the conclusion of the experimental trial, the rats that had received the designated treatment were subjected to a process of weighing, subsequent to which they were anaesthetized through the administration of chloroform. The collection of blood samples was conducted using 5cc syringes through heart puncture, and afterwards transferred into tubes coated with EDTA. The blood sample was held at a temperature of 4°C within a refrigeration unit prior to undergoing further processing.

2.9.2. Serum Preparation

Following the acquisition of blood samples from all the rats, the blood specimens were permitted to undergo coagulation at ambient temperature for around 15-20 minutes. The blood samples underwent centrifugation at a speed of 3000 revolutions per minute (rpm) for a duration of 15 minutes. The serum necessary for the biochemical assay was isolated and collected in Eppendorf tubes, after which it was stored at a temperature of -20°C until subsequent processing.

2.9.3. Inflammatory Biomarkers Analysis

The study will analyse inflammatory biomarkers, such as LFT, IL-10, COX-2, CRP, and TNF-α, utilising the sandwich approach with commercially available enzyme-linked immunosorbent assay (ELISA) kits, as described by Aziz et al. (2020).

2.9.4. Histopathological Analysis

Histopathological analysis will be conducted on liver and paw skin specimens. Glass slides were made for histological investigation. A 10% solution of formalin will be employed for the purpose of fixing all tissue samples. The tissue samples will undergo embedding in paraffin wax, followed by sectioning into thick slices, and subsequent staining with eosin and hematoxylin. The skin samples will be examined using a light microscope, as described by Khedir et al. (2016).

3. Results

3.1. Paw Measurement

Before Treatment

Paw edema was induced in all experimental rat groups, with the exception of the control group, through the administration of a thickening agent known as Carrageenan for a duration of 7 days. After the delivery of Carrageenan, it was noted that there was inflammation and redness in the area, as seen in Figure 1. However, after a period of 2 hours, a decrease in inflammation was documented in all the groups.

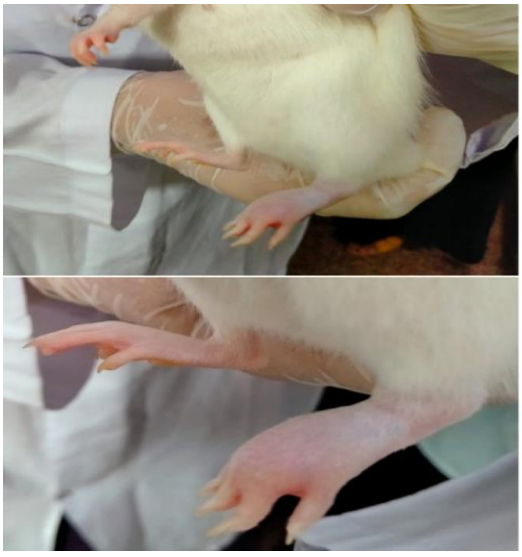

Figure 1: Edema induced in rat paw

After Treatment

In the present study, a decrease in inflammation was seen in the paw. The anti-inflammatory benefits of *Agave sisalana* and *Agave americana* extracts were detected in our study. However, the most significant results were noticed in the group treated with Aliskiren, surpassing the outcomes of all other treatment groups, as well as the control and positive control groups, on the seventh day following treatment, as seen in Figure 2.

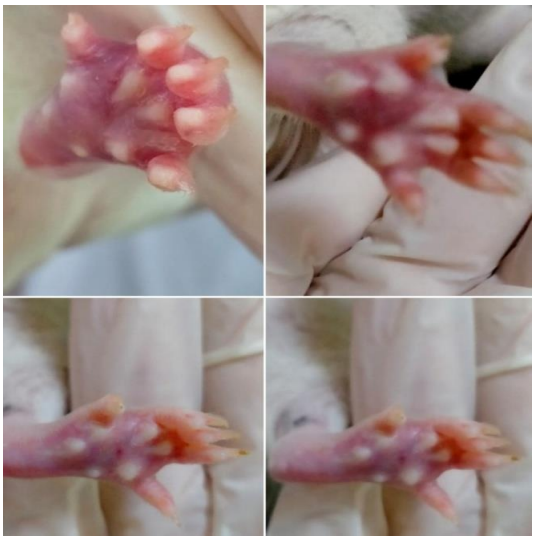

Figure 2: Inflammation reduction after treatment A: Positive control, B: Aliskiren Treated, C: *Agave sisalana* extract treated and D: *Agave americana* extract treated

The graph as illustrated in Figure 3 shows that the rat paw experienced swelling when inflammation was produced. In all experimental groups, the administration of carrageenan resulted in the observation of inflammation, with the exception of the control group. The therapy protocol commences with the administration of an extract derived from species of Agave plant, alongside a standard pharmaceutical medicine. These therapeutic interventions have notable efficacy in mitigating inflammatory processes, demonstrating a remarkable degree of similarity in their respective outcomes.

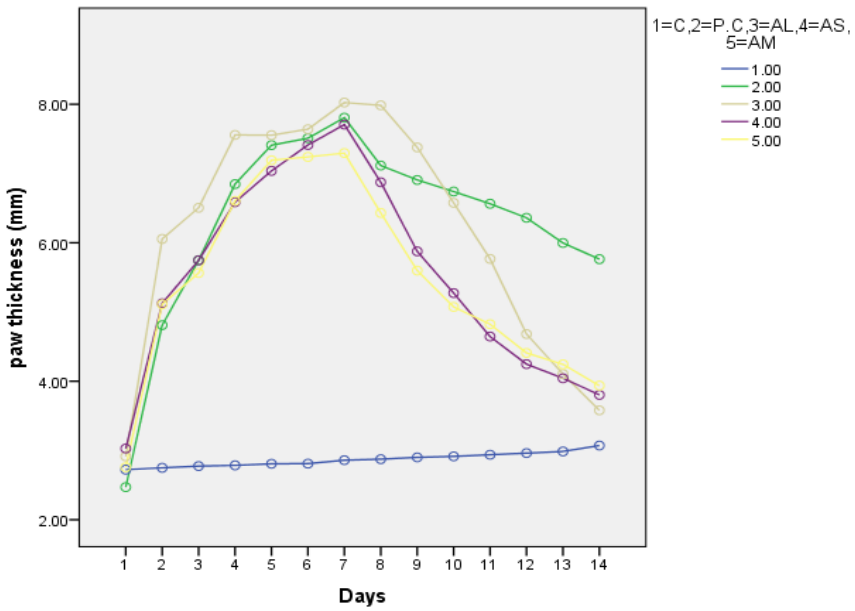

Figure 3: Paw thickness at induction of inflammation and treatment with extract and drug

3.2. Toxicity Test

The acute toxicity experiments were conducted using the protocol outlined by Sawadogo et al. in 2006. The rats in group IV were administered a single dosage of approximately 80 mg/200g of *A.sisalana* extract, while the rats in group V got the same quantity of *A.americana* extract. The initial body weights of the rats were recorded. The rats were subjected to observation at regular intervals of 3 hours for a period of approximately 7 days. The rats exhibited behavioural abnormalities, specifically laziness and stress, which were observed and documented as potential markers of harmful effects caused by the extracts. According to Misra et al. (2018), the utilisation of extracts can result in alterations in behaviour and physiology, which manifest as disruptions in skin, ocular function, food patterns, and stress levels. The control group exhibited typical behaviour throughout the duration of the investigation. In the positive control group, the rat paws exhibited swelling as a result of the production of inflammation. The group receiving the standard medication treatment had skin disturbances and disruptions in their feeding behaviour. The groups that received the extract exhibited signs of lethargy, with some individuals displaying hyperactivity, which may be indicative of underlying stress.

3.3. Biochemical Analysis

3.3.1. Bilirubin

The findings from the biochemical examination of Bilirubin, as well as the calculated mean values, indicated that there was no statistically significant alteration in the concentration of Bilirubin across all experimental groups, which encompassed the control group, positive control group, Aliskiren group, *Agave sisalana* group, and *Agave americana* group as shown in Figure 4.

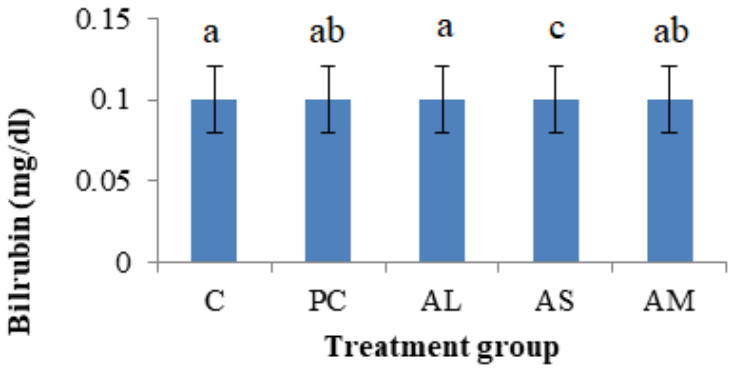

Figure 4: Mean serum level of Bilirubin Total (mg/dl) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group.

3.3.2. ALT

The biochemical examination of alanine aminotransferase (ALT) in the positive control group exhibited a decrease in comparison to the control group. Nevertheless, the treatment groups exhibited a noticeable upward trajectory in alanine aminotransferase (ALT) levels for Aliskiren, *A.americana*, and *A.sisalana* as shown in Figure 5.

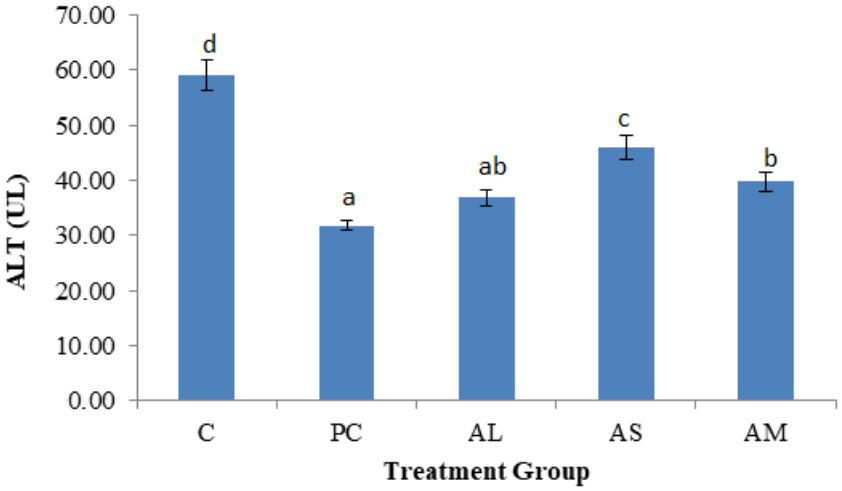

Figure 5: Mean serum level of ALT (UL) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group.

3.3.3. AST

The mean values of aspartate aminotransferase (AST) in the positive control group exhibited a decrease in comparison to the control group. Nevertheless, the treatment groups exhibited a rising inclination in aspartate aminotransferase (AST) levels in the Aliskiren, *A.sisalana*, and *A.americana* specimens as shown in Figure 6.

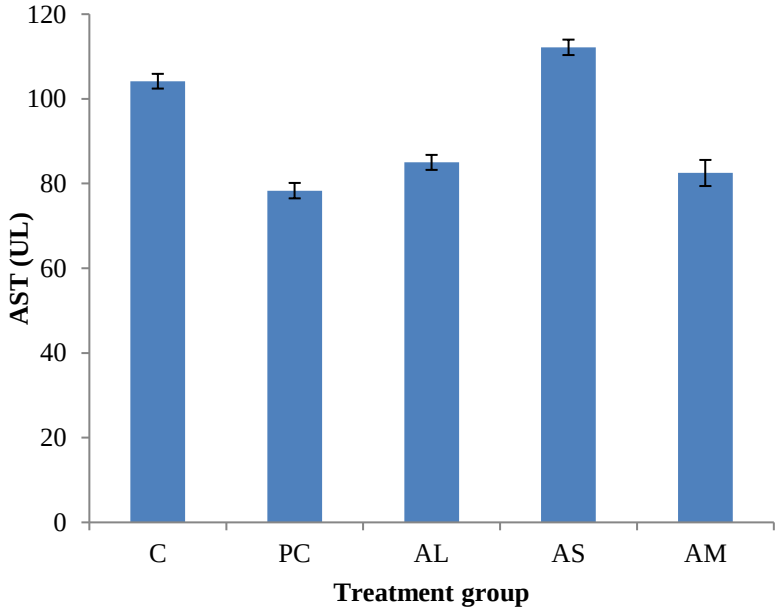

Figure 6: Mean serum level of AST (UL) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group.

3.3.4. Alkaline Phosphatase

The mean values of Alkaline Phosphatase in the positive control group exhibited a significant rise in comparison to the control group. Nevertheless, the treatment groups exhibited a noticeable upward trajectory in the levels of Alkaline Phosphatase within both the Aliskiren and *A.americana* groups. The *A.sisalana* group had the greatest quantity of Alkaline Phosphatase as shown in Figure 7.

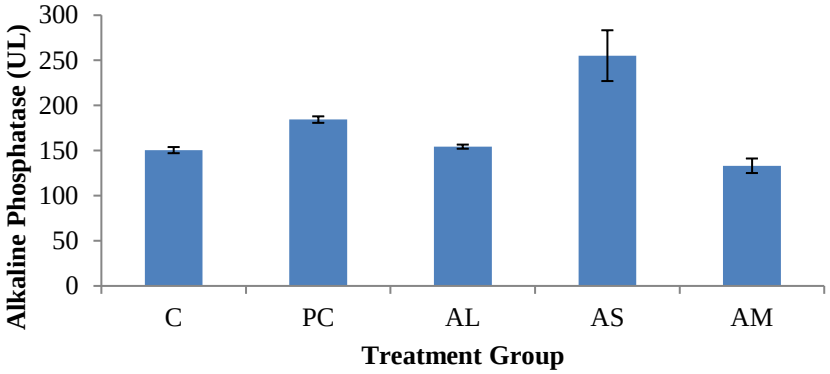

Figure 7: Mean serum level of Alkaline Phosphatase (UL) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.5. Total Proteins

The mean values of total proteins in the positive control group exhibited a significant increase in comparison to the control group. Nevertheless, the treatment groups exhibited a noticeable upward trajectory in the levels of total proteins within the *Agave americana* and *Agave sisalana* groups. The group treated with Aliskiren exhibited the lowest level of total proteins as shown in Figure 8.

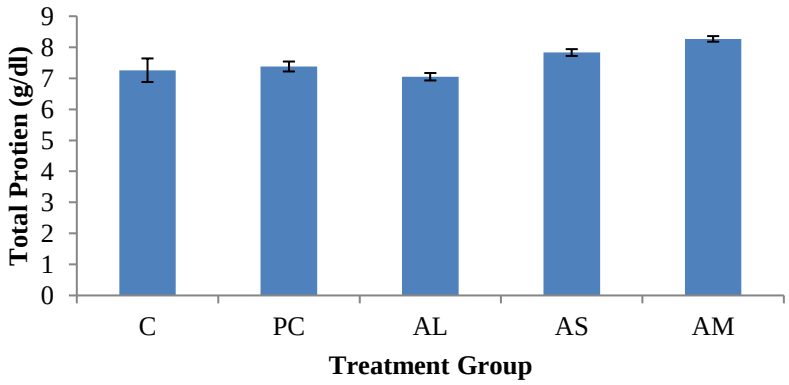

Figure 8: Mean serum level of Total Protein (g/dl) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.6. Albumin

The average value of Albumin in the positive control group has exhibited a decrease in comparison to the control group. However, the treatment groups exhibit a noticeable upward trend in the levels of Albumin when exposed to Aliskiren, *Agave sisalana*, and *Agave Americana* as shown in Figure 9.

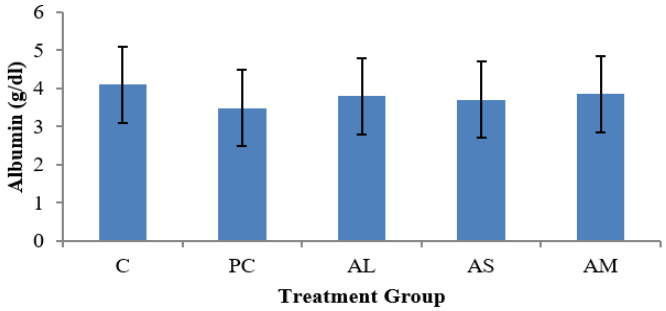

Figure 9: Mean serum level of Albumin (g/dl) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.7. Globulin

The average globulin levels in the positive control group exhibited a significant rise in comparison to the control group. Nevertheless, the treatment group exhibited a progressive decline in globulin levels in both the Aliskiren and *Agave sisalana* groups. The group that received treatment with *Agave americana* exhibited a higher level of globulin as shown in Figure 10.

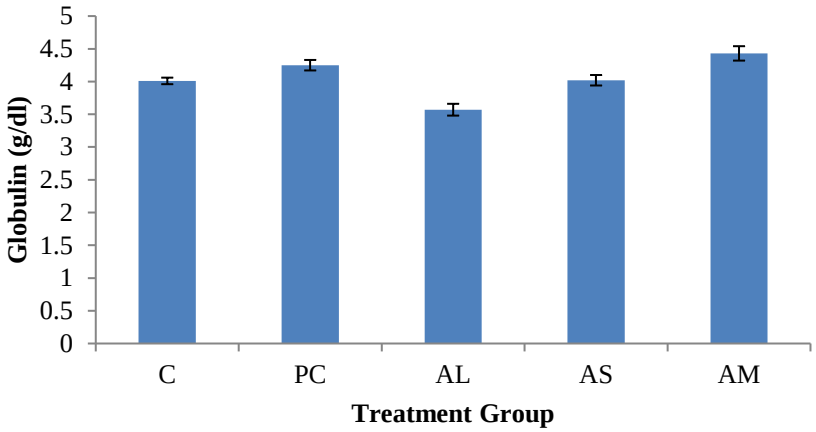

Figure 10: Mean serum level of Globulin (g/dl) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.8. A/G Ratio

The positive control group exhibited a decrease in the mean value of the A/G ratio in comparison to the control group. However, the treatment group exhibited a noticeable upward trend in the A/G Ratio when administered with Aliskiren, *Agave sisalana*, and *Agave Americana* as shown in Figure 11.

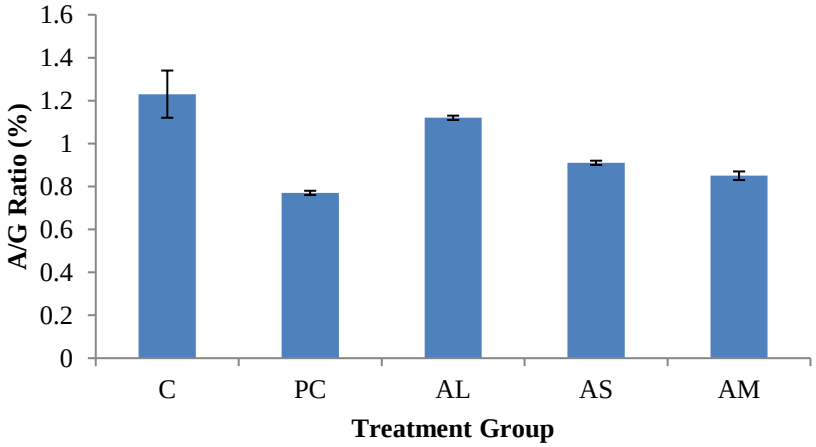

Figure 11: Mean serum level of A/G Ratio (%) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.9. C-Reactive Proteins

The average CRP value in the positive control group exhibited a significant increase in comparison to the control group. However, the experimental group exhibited a progressive decline in C-reactive protein (CRP) levels when administered Aliskiren, *Agave sisalana*, and *Agave Americana* as shown in Figure 12.

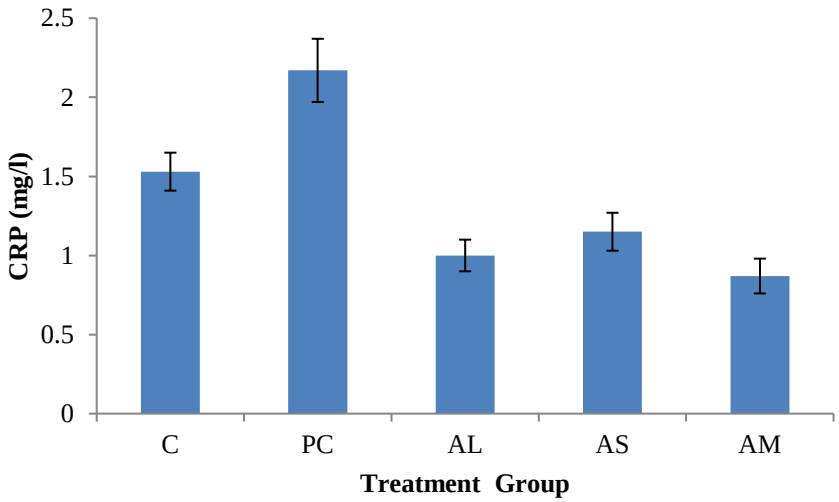

Figure 12: Mean serum level of C-Reactive Protein (mg/l) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.10. Tumor Necrosis Factor-alpha

The average TNF-α value in the positive control group exhibited a significant rise in comparison to the control group. Nevertheless, the group receiving therapy exhibited a declining pattern in the levels of Aliskiren and *Agave americana*. However, there has been an observed rise in the concentration of TNF-α in the group of *Agave sisalana*, as shown in Figure 13.

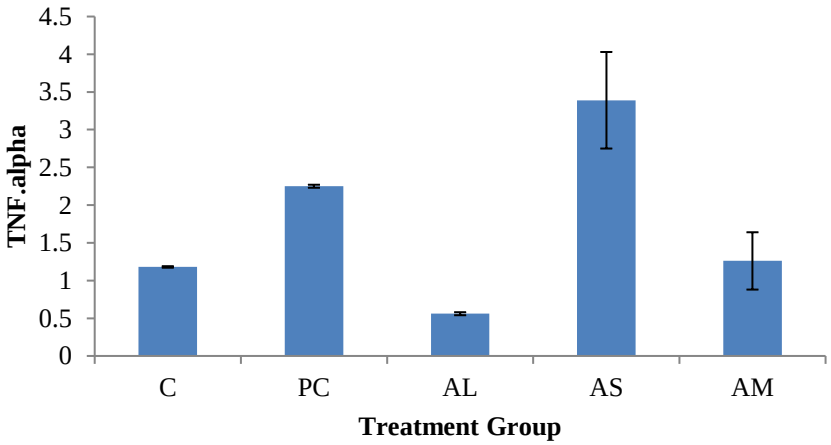

Figure 13: Mean serum level of Tumor Necrosis Factor-Alpha (g/L) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.11. Cyclooxygenase-2

The average COX-2 score in the positive control group exhibited a significant rise in comparison to the control group. Nevertheless, the treatment group exhibited a declining pattern of COX-2 expression in response to both Aliskiren and *Agave americana*. However, there has been an observed rise in the level of COX-2 in the group of *Agave sisalana*, as shown in Figure 14.

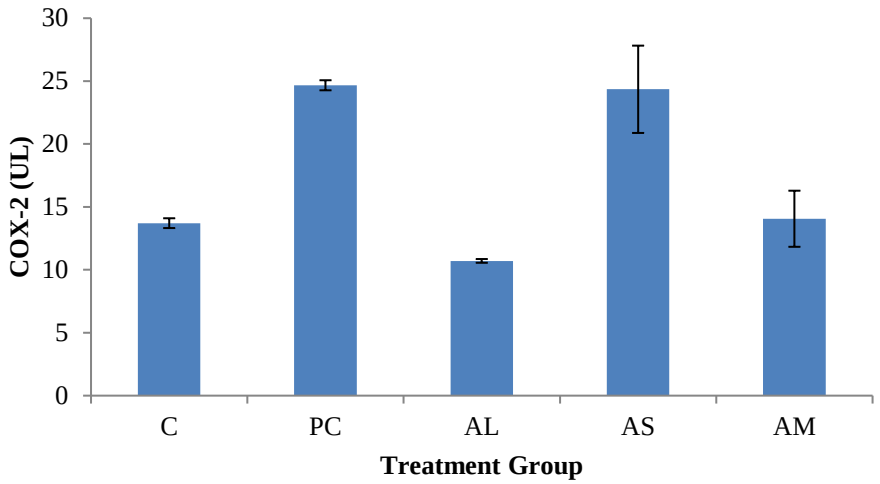

Figure 14: Mean serum level of Cyclooxigenase-2 (UL) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.3.12. Interleukin-10

The average IL-10 value in the positive control group exhibited a significant rise when compared to the control group. In contrast, the treatment group exhibited a progressive decline in IL-10 levels following administration of Aliskiren and *Agave americana*. However, there has been an observed increase in the level of IL-10 in the group exposed to *Agave sisalana*,as shown in Figure 15.

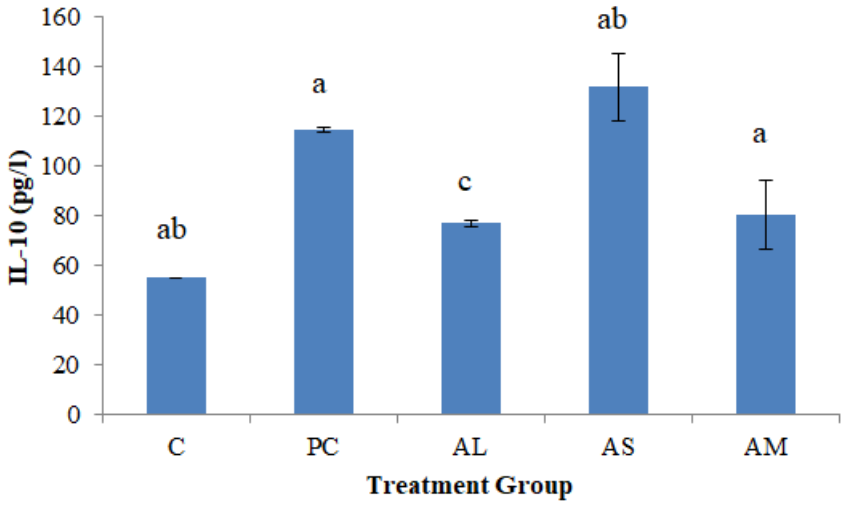

Figure 15: Mean serum level of Interleukin-10 (Pg/l) in control group, positive control group, Aliskiren treated group, *Agave sisalana* extract treated group and *Agave americana* extract treated group

3.4. Histopathological Analysis

During the histological analysis of the control group, it was observed that the epidermis had normal characteristics across all layers. The control group exhibits the presence of typical rete pegs, which are epithelial extensions. The sub-epidermal layers and dermis exhibit the presence of collagen, elastic fibres, and dermal glands in their respective tissues. The control group is a standard group in which the absence of tissue necrosis, malignancy, and granuloma is seen. During the histological inspection of the positive control group, it was noted that there was partial ulceration of the lining, with the nuclei in the stratum corneum being retained. The granular layer is not present. The interface between the epidermal and dermal layers exhibits localised tissue necrosis, tissue swelling, and the presence of

dispersed inflammatory cells. The absence of calcification and malignancy is not detected within this cohort. In the group receiving Aliskiren treatment, the histological investigation revealed the presence of a reddish epidermis. The group under investigation exhibits a reduction in the presence of rete pegs, which are extensions of the epithelial tissue. There is a modest presence of tissue edema observed in the sub-epidermal tissue and dermis. The absence of focal tissue necrosis is not found within this cohort. Based on the histological analysis conducted on the treated group of *Agave sisalana*, it was revealed that the rete pegs had normal characteristics, whereas the epidermis displayed a reddish hue. The tissues located in the sub-epidermal layer exhibit collagen, dermal glands, and elastic fibres that closely resemble their normal composition. No instances of calcification or cancer were detected within this cohort. In the group subjected to *Agave americana* treatment, our histological study revealed abnormal epidermal characteristics. The rete pegs have no abnormalities. The presence of extensive fibrosis is noticed. Mild edema and sporadic presence of inflammatory cells are observed in the sub-epidermal and dermal tissues. The presence of inflammatory cells and the observation of tissue necrosis are apparent in this cohort, as shown in Figure 16.

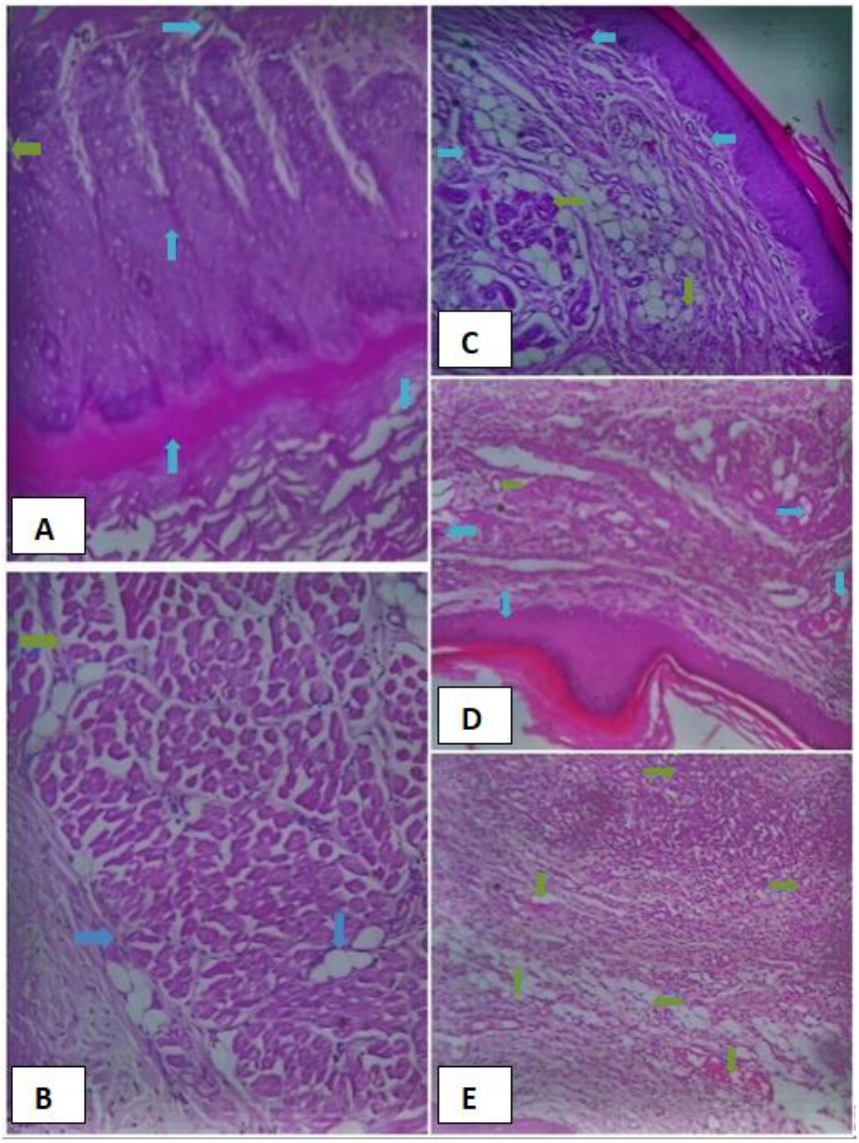

Figure 16: Photomicrograph of paw histology of control group, Positive control group, Aliskiren treated group, *Agave sisalana* and *Agave americana* treated group. Staining is done with (H & E) stain. (A) Low magnification, (B) High magnification. A) Normal epidermis and Normal inflammatory cells (green arrow and blue arrow respectively) in control group. B) Epidermis shows edema and increased inflammatory cells (green arrow and blue arrow respectively) in positive control group. C) Reddish epidermis and reduced inflammatory cells

(green arrow and blue arrow respectively) in Aliskiren treated group. D) Epidermis is normal as reddish (blue arrow), inflammatory cells decreases to normal (green arrow) in *Agave sisalana* treated group. E) Epidermis is not normal and shows mild edema and scattered inflammatory cells.

4. Discussion

A variety of chemical substances, viruses, antibodies, irritants, and physical traumas may all trigger inflammation, which is an immune system's physiological response (Chen et al., 2018). Inflammation can take the form of either acute or chronic inflammation depending on the underlying properties of the stimulus and the first inflammatory response (Saha & Ahmed, 2009). Although inflammation is an essential part of the healing process, it has also been shown to increase the risk of a wide range of illnesses, including rheumatoid arthritis, cancer, atherosclerosis, hay fever, periodontitis, neurologic disorders, autoimmune diseases, diabetes, and cardiovascular conditions (Aziz et al., 2020).

Due to its emulsifying and thickening abilities, carrageenan is used in the culinary industry. Carrageenan has been used as a substance with inflammatory qualities. (Necas and Bartosikova, 2013), is known for its capacity to stimulate histamine release as a modulator of vascular outflow in an inflammatory context. (Kim et al., 2020). In the course of our investigation, we used carrageenan to make albino rats' paws swell. Thirty rats were divided into five different groups, and after carrageenan-induced edema, three of the groups were treated with Aliskiren, *Agave sisalana* extract, and *Agave americana* extract, respectively.

During the course of our study, we were able to successfully produce edema by injecting 0.2 ml of a 1% carrageenan solution. Using a Vernier calliper, the volume of the paws was measured. A considerable increase in paw volume was noticed after the initial dosage of carrageenan was injected. To cause inflammation, the aforementioned process was repeated seven days in a row. Following the introduction of carrageenan for a period of two hours, the amount of inflammation shows a rise, which is then followed by a subsequent decline over time. According to the findings of Mwale et al. (2012), it was observed that the administration of carrageenan can induce paw edema.

After the medication was administered, the paw's inflammation significantly decreased. In our investigation, the extracts of *Agave sisalana* (3.80 ± 0.10) and *Agave americana* (3.9 ± 0.13) shown anti-inflammatory properties. However, the group who received Aliskiren (3.58 ± 0.13) saw the best results. A 14-day trial experiment was subjected to a repeated measures ANOVA. The graph shows that, in terms of lowering inflammation or paw edema, the therapy using extract and the conventional drug both had equivalent results.

The acute toxicity experiments were conducted using the protocol outlined by Sawadogo et al. in 2006. Rats in group IV received a single dose of *A.sisalana* extract at a concentration of around 80 mg/200g, whereas rats in group V received the same quantity of *A.americana* extract. The rats' original body weights were noted. For around 7 days, the rats were exposed to repeated inspections at a predetermined 3-hour interval. The rats displayed anomalous behavior, particularly sleepiness and tension, which were observed and recorded as potential indicators of negative consequences brought on by the supplied extracts. According to Misra et al. (2018), The extracts may cause behavioral and physiological changes that show up as abnormalities in stress, skin, eye, appetite, and other responses. Throughout the course of the inquiry, the control group behaved normally. The rats in the positive control group showed paw edema as a result of the inflammation being produced. The group getting the usual pharmaceutical therapy had changes in their food habits as well as skin problems. Lethargy and certain cases of hyperactivity, which may be indicators of stress, were seen in the groups who received the extract.

In this study, the blood levels of several biomarkers linked to inflammation were analyzed. This study detailed a liver function test, also known as an ALFT, that measures a variety of factors, such as total bilirubin, ALT, AST, alkaline phosphatase, total protein, albumin, globulin, and A/G ratio. The study also looked at biomarkers of inflammation as CRP, TNF- α, COX-2, and IL-6.

Red blood cell hemolysis causes bilirubin's concentration to increase, making it a key indicator for toxicity. In the current study, it was shown that none of the rats' blood bilirubin levels significantly

changed before or after treatment (meanstandard deviation: 0.1±6.2063). The information reported in this study supports the safety of the leaf extract used in the experimental methods, which was obtained from *Agave sisalana* and *Agave americana*. Furthermore, it is important to understand that paw edema has no effect on the amount of bilirubin. According to a prior study conducted by Ekam and Udosen (2012), it was observed that the level of Bilirubin remained stable in the majority of cases, with little fluctuations.

The enzyme alanine transaminase (ALT), whose levels tend to grow in the presence of liver damage, is used as an additional indication in liver function tests. In the most recent trial, the positive control group's serum levels of alanine aminotransferase (ALT) were shown to be significantly lower (31.83±0.95) than those of the control group (59.17±2.83). Although the administration of conventional pharmaceutical medications did not have the same normalizing effect, it has been shown that the use of *Agave sisalana* leaf extract and Agave americana leaf extract helps to normalize liver function. The results of this study show that the treatment group's alanine aminotransferase (ALT) serum level was elevated. Our findings align with the study conducted by Mostafa et al. (2020), which documented an elevation in the serum level of alanine aminotransferase (ALT).

Aspartate transaminase (AST) elevation is a marker for liver damage or hepatic stress. In the current study, after the onset of inflammation, there was a decrease in the blood level of AST in the positive control group (78.33±1.82) compared to the control group (104.17±1.74). The blood concentration of AST was restored after the administration of aliskiren and *Agave americana* leaf extract, with values of 85.001.77 and 82.503.08, respectively. The administration of our drug has no negative effects on the liver and does not cause hepatotoxicity, indicating that its use is probably safe. Mostafa et al. (2020) have found similar findings, noting an elevation in serum levels of AST.

Further liver function test called alkaline phosphatase (ALP) can be performed to evaluate the condition of the liver. ALP levels that are elevated are a sign of liver disorders. In comparison to the control group, the positive control group showed a considerable increase in blood levels of alkaline phosphatase (184.33±3.60) after the injection of carrageenan. While the levels of alkaline phosphatase in the groups treated with Aliskiren and *Agave americana* fluctuated somewhat, the levels in the group treated with Agave sisalana remained constant. This shows that the therapy did not make a big difference.

The Total Protein test calculates the body's overall globulin and albumin content. Following the delivery of carrageenan, the positive control group's total protein levels did not significantly alter.

An additional sign used in liver function tests is albumin. In the current study, the positive control group's serum albumin levels decreased (3.48±0.09) in comparison to the control group (4.10±0.09). In the groups given treatments with aliskiren and *Agave americana,* the level of [certain variable] persisted at an increased level. Comparing the *Agave sisalana* group to the positive control group, the former showed only slight variances. This shows that when compared to the group receiving *Agave sisalana* therapy, the other treatment groups are more successful. The information provided raises the possibility of a connection between paw edema and changes in albumin levels.

Another protein that is present in the liver is globulin; autoimmune disorders are frequently associated with low levels of this protein. In the current investigation, levels in the positive control group rose (4.250.08). All treatment groups showed minor fluctuations, proving that the medication had no effect on globulin levels. A prior study by Ekam and Udosen (2012) found that the globulin concentration dropped.

The A/G ratio is derived by dividing the total protein concentration by the sum of albumin and globulin concentrations. The positive control group exhibited a decrease in the A/G ratio (0.77±0.01) as compared to the control group. The extract did not demonstrate efficacy in normalising the A/G ratio. The conventional medication effectively restores the A/G ratio to its normal levels. The occurrence of paw edema has been noted to have an impact on the A/G ratio.

The liver produces the hepatocyte-derived protein known as C-reactive protein (CRP). The aforementioned biomarker is crucial in the context of inflammation since an increase in its concentration indicates the existence of inflammation (Aziz et al., 2020). The positive control group exhibited a statistically significant increase in C-reactive protein (CRP) levels (mean±standard

deviation: 2.17±0.20) following the administration of Carrageenan. The administration of the standard medicine aliskiren, as well as the extracts from *Agave sisalana* and *Agave americana*, exhibited minor differences when compared to the positive control group. The data presented suggests a potential association between paw edema and the concentration of C-reactive protein (CRP). Aziz et al. (2020) have previously documented the ability of Aliskiren to reduce C-reactive protein (CRP) levels.

One of the many inflammatory indicators is tumor necrosis factor-alpha (TNF-alpha). Tumor necrosis factor-alpha (TNF-alpha) levels above normal indicate the presence of inflammation. In the current study, the positive control group had considerably more TNF-alpha (2.25 ± 0.02) than the rest of the subjects. Numerous authors have discussed numerous inflammatory-related diseases and conditions, such as diabetes and rheumatoid arthritis, among others. The study conducted by Aziz et al. (2020). The groups treated with aliskiren and *Agave americana* showed a better degree of effectiveness compared to the group treated with *Agave sisalana* after the administration of the usual medication and extract. TNF-alpha levels did not return to normal after the use of ***Agave sisalana*.** This finding implies that a higher than normal level of TNF-alpha may have an impact on how paw edema develops. In a study conducted by Aziz et al. (2020), it was noted that the administration of Aliskiren resulted in a decrease in TNF-alpha levels.

The important biomarker for inflammation is the COX-2 enzyme. Prostaglandins and thromboxane are produced in the setting of inflammation as a result of the process. In a recent research, the injection of carrageenan was shown to have elevated COX-2 expression in the positive control group by an average of 24.67±0.40. According to the experiment's findings, both conventional medication and the administration of Agave species extract efficiently control COX-2 expression to normal levels.The efficacy of the *Agave sisalana* extract was found to be limited. The results presented suggests a potential correlation between paw edema and the modulation of COX-2 expression.

IL-10 is anti-inflammatory cytokine it reduces inflammation in various diseases described by Aziz et al., (2020). In present study its level increased (114.52±1.10) in positive control group. After treatment with extract and standard drug it is observed that all treatment works well in normalizing the level of IL-10. From previous study it is observed that Aliskiren is able to increased IL-10 in models of inflammation described by Aziz et al., (2020).

The epidermis in the control group exhibited normal features in all of the layers, according to the histological study. Typical rete pegs, which are epithelial extensions, may be seen in the control group. Collagen, elastic fibers, and dermal glands are visible in the dermis and sub-epidermal layers when they are in a healthy state. The lack of tissue necrosis, malignancy, and granuloma is observed in the control group, which is a standardized group. In their study, Ou et al. (2019) noted the absence of inflammatory cell infiltration in the control group.

The positive control group's histological evaluation revealed partial ulceration of the lining with the nuclei still present in the stratum corneum. There isn't a granular layer. Localized tissue necrosis, tissue edema, and the presence of scattered inflammatory cells can all be seen at the epidermal-dermal interface. Malignancy and lack of calcification are not found in this group. Based on the prior investigation conducted by Ou et al. in 2019, The presence of a significant influx of neutrophils in the dermal and subepidermal tissues was found in the group stimulated with carrageenan.

A reddish epidermis was seen in the group that received Aliskiren treatment, according to the results of the histological analysis. Rete pegs, which are epithelial extensions, are less frequent in the group under research. The dermis and sub-epidermal tissue both have a slight amount of tissue edema. Within this group, localized tissue necrosis is not absent. In their study, Aziz et al. (2020) documented the observation that the administration of the standard medicine Aliskiren resulted in a decrease in the quantity of inflammatory cells within the cohort of rats subjected to Aliskiren treatment. Based on histological examination of the *Agave sisalana*-treated group, it was discovered that the rete pegs revealed normal features whereas the epidermis had a reddish tint. Elastic fibers, dermal glands, and collagen in the sub-epidermal layer nearly match their natural makeup. Within this group, neither calcification nor malignancy are absent. According to a prior study conducted by Mwale et al. (2012), it was noted that the administration of *Agave sisalana* extract resulted in a reduction in inflammation and restoration of normal epidermal conditions.

The group treated with *Agave americana* had aberrant epidermal features, according to the histological study. There are no anomalies in the rete pegs. There is obvious substantial fibrosis present. The sub-epidermal and dermal tissues show occasional inflammatory cell presence and mild edema. In this cohort, tissue necrosis and the presence of inflammatory cells are evident. In a study conducted by Silva et al. (2017), it was observed that the phytochemicals present in *Agave americana* shown potential in reducing edema and causing limited dispersion of inflammatory cells.

In our experimental study, On the effectiveness of traditional pharmaceutical therapies, we made observations. It is crucial to remember that these medications might have some negative side effects, such as tiredness, anxiety, and hypertension. We have included the use of medicinal plant extracts, which are renowned for their inherent safety and efficacy in treating a wide range of diseases, in order to reduce these adverse results. To treat paw edema brought on by carrageenan, the current study uses extracts from Agave species, notably *Agave americana* and *Agave sisalana*. Comparing the effects of these extracts to those of the common drug Aliskiren is the goal.

After therapy, the amount of bilirubin is unaffected. With regard to ALT, the treatment groups had negligible variations from one another that were not comparable to the control group. The group given *Agave sisalana* treatment consistently showed increased AST levels, whereas the other two groups showed only slight variations from one another. The study's findings show that when compared to the group receiving extract treatment, the group getting normal pharmaceutical therapy with alkaline phosphatase had better outcomes. Both the extract-treated group and the group receiving standard treatment had the same level of total protein. The albumin levels exhibited minor variations within each treatment group, consistently differing from those observed in the control group. Globulin extracts have demonstrated efficacy in normalising globulin levels, surpassing the effectiveness of the conventional medication. The standard medicine had superior outcomes in terms of the A/G ratio compared to the extracts, as the group treated with extracts did not show any significant changes.

Based on the findings the level of C-reactive protein (CRP) decreased in both the conventional drug-treated group and the group treated with *Agave americana*, according to the data on inflammatory biomarkers. However, compared to the other groups, the *Agave sisalana* treatment group showed relatively more favorable results. The TNF-alpha standard medication and extract of *Agave americana* treatment group demonstrate advantages over the *Agave sisalana* treated group. The expression of COX-2 in the group treated with *Agave sisalana* remained unchanged, but the administration of the conventional medication and the extract from *Agave americana* demonstrated the most effective results in normalising COX-2 levels. When considering IL-10, all treatments effectively normalise the level of IL-10. The current research findings indicate that both the extract and the conventional medicine yielded comparable outcomes.

5. Conclusion

The primary focus of this investigation was to delve into the anti-inflammatory attributes inherent in extracts sourced from *Agave sisalana* and *Agave americana* within rat models exhibiting carrageenan-induced paw edema. Concurrently, the study aimed to evaluate levels of inflammatory biomarkers correlated with oxidative stress. This experimental inquiry was undertaken with the intention of observing the impact stemming from phytochemicals extracted from *Agave sisalana* and *Agave americana L*., in combination with the conventional medication Aliskiren.The application of extracts from Agave species, namely *Agave sisalana* and Agave americana, alongside a standard pharmaceutical intervention, yielded parallel outcomes. These interventions prominently displayed an elevation in inflammatory biomarker concentrations within the bloodstream, accompanied by enhancements in the histological integrity of paw skin samples within the inflammation-induced groups. In summation, this research underscores the potential anti-inflammatory benefits of Agave-derived phytochemicals, both independently and in conjunction with Aliskiren. The observed improvements in inflammatory biomarkers and tissue histology highlight a promising avenue for further exploration of these natural remedies in alleviating inflammation-related conditions.

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