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BIOLOGICAL SCREENING OF LEAF EXTRACTS OF PLANT HOLOPTELEA INTEGRIFOLIA ROXB.

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Abstracts:

All parts of Holoptelea integrifolia, a roadside plant, are suggested by Charaka Samhitha Sushrutha Samhita and other traditional systems for the treatment of inflammation, acid gastritis, dyspepsia, flatulence, colic, intestinal worms,vomiting, wounds, vitiligo, leprosy, filariasis, diabetes, haemorrhoids, dysmenorrhoea, and rheumatism.The present studywas aimed at macroscopic, microscopic, and chromatographic evaluation of Holopteleaintegrifolia leaves. The macroscopic,microscopic evaluation is carried out in terms of organoleptic, microscopic, and physical parameters like fluorescence analysis, treatment of plant drugs with different chemical reagents. The dried leaves were subjected to successive Soxhlet extraction by taking the increasing order of solvents like petroleum ether, chloroform, Acetone ,Benzene ethyl ,Water and methanol. These solvent extracts were subjected to a phytochemical evaluation to detect the different chemical principles present i.e. Protein, amino acids ,carbohydrates, steroids, alkaloids, glycosides, phenolic compound and tannis. And its find out a chromatographic evaluation to isolatethe chemical compounds. Macroscopic, microscopic, and physicochemical determination and chromatography screening canbe used as a diagnostic tool in the correct identification of plants. By the above studies, any adulterants present in these plants can be identified easily.The comparative and multidisciplinary approach to the study of HolopteleaintegrifoliaRoxb Roxb. Does help in

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understanding their identification taxonomical determination, and medicinal importance in depth. The adulterants in drugs obtain from HolopteleaintegrifoliaRoxb. Can be identified by this investigation. Adulterants if any can be easily identified using these parameters.

Keywords-Organoleptic, Fluorescence, Microscopic, Chromatography, Photochemistry

INTRODUCTION

1. Natural Product as anti-inflammatory activity A Drugs

Inflammation – A brief introduction

The host defense to tissue damage, infections, and oxidative stress is inflammation.In the response to the infection, damage, and ischemia, inflammatory processes are triggered. These are characterized in terms of cardiac features like swelling, redness, heat, and pain. Moreover, the definition of inflammation is also manifested by the invasion of immune cells (macrophages,lymphocytes, and neutrophils) to the injured site and by the local production of pro- inflammatory mediators, ultimately leading to the abatement of the invading agent and damaged tissue.15, in other words, it is said that inflammation (phlogesis) as a reaction of the body against injury.16 Inflammatory responses have their benefits for tissues and cells, at first glance, due to the timely balanced local activation and regulation of the immune system which is necessary to eliminate destructed cells and cell material[15]

Types of inflammation:- It can be classified into two types, either acute or chronic. Acute inflammation is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. It is also the initial response of the body to any kind of harmful stimuli. Chronic inflammation is also known as chronic inflammation. It leads to a progressive shift in the type of cells that are present at the site of inflammation. Simultaneous destruction and healing of the tissues from the inflammatory process characterize this.

Pathophysiology of inflammation: Inflammation occurs in 3 distinct phases—acute, sub- acute, and chronic (or proliferative). At the site of injury, the acute response occurs to the tissue injury in the form of microcirculation. Initially, there is a transient constriction of arterioles; however, within several minutes, chemical mediators released at the site cause relaxation of arteriolar smooth muscle, vasodilation, and increased capillary permeability. This fluid are contains the many of the compounds of plasma, including kinins ,fibrinogen ,

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and immunoglobulin's that mediate the inflammatory response.This is characterized (sub-acute phase) by the movement of phagocytic cells to the site of injury.In response to adhesion molecules released from activatedendothelial cells, leukocytes, platelets, and RBC in injured vessels become sticky and adhere to the endothelial cell surfaces.Polymorph nuclear leukocytes such as neutrophils are the first cells to infiltrate the site of injury. Basophils and eosinophilsare more prevalent in allergic reactions or parasitic infections. As the inflammatory process continues, macrophages predominate, actively removing damaged cells or tissue. If the cause of injury is eliminated then the acute inflammation may be followed by a period of tissue repair. Blood clots are removed by fibrinolysis. Damaged tissues are replaced with fibroblasts, collagen, or endothelial cells or they can be replaced. However, inflammation may become chronic, leading to further tissue destruction and fibrosis.[18]

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti- inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect thesystem. On **the other hand, some analgesics are commonly associated with anti- inflammatory drugs but do not have anti- inflammatory effects**.An example is a paracetamol, called acetaminophen in the U.S.and is sold under the brand name of Tylenol. As opposed to NSAIDs, which reduce pain and inflammation by inhibiting COX enzymes, paracetamol hasrecently been shown to block the reuptake of end cannabinoidswhich only reduces pain, likely explaining why it hasminimal effect on inflammation.[19]

Mechanism of action: The most important mechanism of action of anti- inflammatory action of NSAIDs is used for inhibit the synthesis of PG synthesis at the site of injury. The anti- inflammatory potency of different compounds roughly corresponds with their potency to inhibit COX. However,nimesulide is a potent anti- inflammatory but relatively weak COX inhibitor.PGs are only one of the mediators of inflammation; inhibition of COX dose not depress the production of other mediators like LTs,PAF, cytokine, etc. inflammation is the result of concerted participation of a large number of vasoactive, chemotactic, and proliferative factors at different stages, and there are many targets for anti- inflammatory action. Activated endothelial cells express adhesion molecules (ECAM- 1,ICAM- 1) on their surface. Italso plays a key role in directing circulating leukocytes to the site of inflammation.Similarly, inflammatory cells express selectins and integrins. Certain NSAIDs

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may act by an additional mechanism including inhibition of expression/activity of some of these molecules. Growth factors like GM- CSF, IL- 6, and lymphocytes transformation factors may also be affected. Stabilizing of leukocytes lysosomal membrane and antagonism of certain actions of kinin may be contributing to NSAIDs action.[20]

Diabetes – A brief introduction:-

Health and disease are coeval with life. From time immemorial man has been Interested in trying to control the disease. Themedicine man and herbalist under- - took in various ways to cures man's disease and bring relief to the sick. One of thechronic, worldwide heterogeneous, life-threatening diseases is Diabetes. The prevalence of it will be 5.4% by the year 2025,with the global Diabetic population reaching 300 million. Among the WHO region, the South East Asian region isthe highestaffected with a maximum global burden. Of the disease and by the year 2025 there will be nearly 80 million diabetics in Region.Diabetes is a lifelong disease marked by a high level of sugar in the blood. It develops due to the body's inability to makeappropriate use of food as a result of insufficient **insulin (a hormone produced by the pancreas to regulate blood Sugerlevel)**, insulin resistance, or both to understand diabetes,it is important To understand the normal process of foodmetabolism. Normally the food eaten is converted within the body into a form of sugar called glucose, which cells use as asource of energy fuel. Glucose cannot enter the cell alone they need insulin to aid in its transport into the cells.

Glucose causes an increase in blood glucose level which in turn signals the release of the hormone insulin. It is the role of insulin to move glucose from the bloodstream into muscle, fat, and liver cells where it can be used as a fuel. Without insulin, the cellbecomes starved of glucose energy despite the presence of abundant glucose in the blood. People with diabetes have highblood glucose levels, as their muscle, fat, and liver cells do not respond to insulin normally, or both. So, the cells in the ability toutilized glucose give rise to an ironic situation of starvation amid plenty. The abundant unutilized glucose is wastefully executed in the urine. Insulin is produced in the pancreas. **The pancreas has an islet of lengerhans, which produced different typesof hormones.**

Types of Diabetes:-

Diabetes can be categorized into two types:-

1. **Diabetes Insipidus (DI)**:- It is due to the deficiency of hormone vasopressin, produced by middle and intermediate lobe of the pituitary gland.

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2. Diabetes Mellitus (DM):- Diabetes mellitus is a group of metabolic disease characterized by high blood glucose level, which result from defects in insulin secretion or action. It is two types

Type I- Insulin dependent diabetes mellitus (IDDM)

Insulin dependent diabetes mellitus is characterized by β-cell destruction caused by an auto immune process usually leading to absolute insulin deficiency.

Type II – Non insulin dependent diabetes mellitus:-Non insulin dependent diabetes mellitus is characterized by insulin resistance in peripheral tissue and an insulin secretary defect of the β-cell.

1.2 plant profile

Botanical name – *Holopteleaintegrifolia*(Roxb.)

Common name – *Indian elm*

Synonym – *Ulmusintegrifolia*

Family – *Ulmaceae*

Ethno medicinal uses :(a) Young & fresh leavesof*Holopteleaintegrifolia* in the form of poultice effectivelycontrol skin disorder. (**b)** Root bark along with the root of plumbegozeylanica in equal proportion is crushed and the extract is a administered in dose of three spoonful twice a day for abortion of Pregnancy up to 3 mounth.**(c)** Stem bark of ***Holopteleaintegrifolia***is crushed with garlic and the extract is given two spoonfuls twice a day for asthmatic attack**.(d)** It is used to cure hydrocel, jaundice and skin disease.**(e)** 20 gm of the boiled bark mixed with the leaf juice of Nimbha (*Azadiractaindica)* is given once daily for 5-10 days.Alternatively, either bark or leaves may be Soacked in a cup of Luke warm water at night and the water is given in the morning for the treatment of Diabetes.**(f)** Stem bark Decoction with common salt 3:1 paste is applied the affected Areastill cures the wounds. **(g)** Bark boiled & juice is squeezed out and this is applied externally over the Affected Areas ofinflammation till cures.

Therapautics Activity:Leaves Wound boils, Jaundice, Fever, Blister**,** Anti-diarreal, antioxidant, Oedema,Antimicrobial, Leprosy, Sprue, Obesity, Intestinal disorder and other skin disease.**Bark**- Decoction of bark used in rheumatism, oral application used in intestinaltumer, dried bark used in pregnant ladies for post natal care, ringworm, Oxytocic,

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Scabies, Hydrocle,and piles. **Bark:** Asthma, fractured bone, fertility, dysmenorrhoea.**Seed** – Ringworm

3. OBJECTIVE AND PLAN OF WORK:-

3.1 Objective:

According to literature survey, the plant***Holopteleaintegrifolia***Roxb.exhibits a wide range of biological activities which have been reported by many workers. These all activities are subjected to further studies aimed at developing a promising pharmaceutical candidate for preclinical and clinical trials.These studies involve Phramacognosticinvestigation to determine its macro- and Microscopical characters and some of its physical constants, phytochemical investigation to determine its phytochemicals and pharmacological investigation for biological screening.In present study, the anti-diabetic and anti-inflammatory activity are to be selected for screening. This is because both the activities have been not reported in any literature survey but included in Ethanomedicinal and tribal uses of HolopteleaintegrifoliaRoxb.

4. MATERIALS AND METHODS

4.1Collection of Plant:-The specimen plant for the study was collected from Lalsot, District Dausa near Jaipur, Rajasthan. The taxonomic features of herbarium have Been Authentified by Mr.Vinod Sharma Botanist, "Rajasthan University Botany Department" Jaipur. (Authetification number: **RUBL20661**)

4.2 Anti-Inflammatory Screening

Materials and Methods

Preparation of drug solutions and reagents

- **Diclofenac Sodium** was purchased from Central drug house, New Delhi.
- **Normal saline** solution (9% NaCl),
- **Vehicle** (1% Carboxymethylcellulose, CMC, 10 ml/kg) were prepared and used.

Animals: Wistar albino rats (150-180 g) of both sexes were selected for the anti-inflammatory study. Wistar albino rats (150 gm to 180 gm) were selected (both sexes). Rats were given food and water ad libitum. Before performing the experiments, the rats were kept under laboratory conditions for an acclimatization period of 7 days. These studies were carried out in a group of 6 rats each. In a metabolic cage, each rat was housed

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Pharmacological study was approved by **Animal Ethical Committee of School of Pharmacy; Suresh GyanVihar University,** with **CPCSEA no1234/a.08**

Experimental Procedure:

Carrageenan induced paw oedema:

Carrageenan induced paw oedema model was used to determine the anti-inflammatory activity of the extracts by the method of Winter et al. (1962). Paw oedema was induced injecting 0.1 ml of 1% Carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat (Winter et al., 1962)[58]. 36 rats were allowed to fast for 18 h and divided into 6 groups of 6 animals each.

Group I served as Control received the vehicle (1% Carboxymethylcellulose, CMC, 10 ml/kg p.o.).

Group II served as Standard, received Diclofenac Sodium (10 mg/kg b.w).

Group III and IV served as test, received petroleum extract at doses of 100 and 200 mg/kg b.w. p.o. respectively

Whereas group V and VI received methanolic extract (100 and 200 mg/kg b.w. p. o).

The animals pretreated with extract or diclofenac sodium one hour before were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (UgoBasile, Italy) immediately after carrageenan application at 0, and 4 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. The results were expressed as a percentage of inhibition of oedema.

$$\text{Percentage inhibition of oedema} = \frac{V_c - V_t}{V_c} * 100$$

Where, V_c is the inflammatory increase in paw volume in control group of animals and V_t is the inflammatory increase in paw volume in drug-treated animals.

Observations and Results:-

The results were expressed as a percentage of inhibition of inflammation.

Statistical analysis

All the values are expressed as mean ± standard deviation and analyzed for ANOVA and post hoc Dunnet's t-test. Differences between groups were considered significant at $P < 0.01$ levels. The statistical analysis was carried out using Graph pad Instat 3.0 software.

Anti-diabetic - Screening

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Materials and Methods:

Preparation of drug solutions and reagents:

- **Alloxan** monohydrate was obtained from Sigma Chemical Company, St. Louis, Mo, USA.Alloxan monohydrate was dissolved in freshly prepared distilled water.
- **Glibenclamide** was used as the standard drug.

Animals

Test animals: Male wistar albino rats (150 – 200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet. (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water.

Pharmacological study was approved by **Animal Ethical Committee of School of Pharmacy; Suresh GyanVihar University,** with **CPCSEA no 1234/a.08**

Experimental Procedure:

Diabetes induction with Alloxan:

Diabetes was induced by a single dose of intraperitoneal injection of alloxan 150 mg/kg body weight (Vogel and Gang, 2002). Animals were maintained for four days in diabetic condition for well establishment of diabetes. After that the animals were divided into six groups (containing 6 animals in each) - Group I: served as normal control; Group II: received the standard drug; group III and IV received 100 mg/kg and 200mg/kg body weight of petroleum ether extract of *Holopteleaintegrifolia* leaves. Group V and VI received 100 mg/kg and 200mg/kg body weight of methanolic extract of *Holopteleaintegrifolia leaves*. Blood samples were collected from retro orbital plexus at zero time (before receiving the extract, gliibenclamide), 3, 6 and 9 days after treatment. Serum was separated by centrifugation of blood at 2000 rpm for 15 min and serum glucose level was determined by glucometer.[74-76]

Observations and Results:-

The results were expressed as in tables and figure below.

Statistical analysis

All the values of blood glucose estimations are expressed as mean ± SEM and analyzed for ANOVA and post hoc Dunnet's t-test. Differences between groups were considered significant at $P < 0.01$ levels. The statistical analysis was carried out using Graph pad Instat 3.0 software.

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5. RESULT AND DISCUSSION

Table 1: Effect of the Petroleum ether extract of *Holopteleaintegrifolia* leave (EPHI) against carrageenan induced paw oedema in rats

Group	Paw edema volume (ml)					% Inhibition
	0h	1h	2h	3h	4h	
Control (0.1 ml of 1% carrageenan)	**0.27±0.03**	**0.43±0.04**	**0.58±0.02**	**0.72±0.03**	**0.87±0.01**	-
Standard Diclofenac sodium (10 mg/kg)	**0.23±0.02**	**0.34±0.03**	**0.28±0.01**	**0.25±0.01**	**0.27±0.02**	**93.33**
Petroleum Ether Extract (100mg/kg) + (0.1 ml of 1% carrageenan)	**0.19±0.02**	**0.32±0.02**	**0.45±0.03**	**0.61±0.04**	**0.69±0.05**	**16.66**
Petroleum Ether Extract (200mg/kg) + (0.1 ml of 1% carrageenan)	**0.23±0.02**	**0.31±0.03**	**0.42±0.03**	**0.56±0.02**	**0.64±0.04**	**31.66**

Figure 1: Effect of the Petroleum ether extract of *Holopteleaintegrifolia* leave (EPHI) against carrageenan induced paw oedema in rats

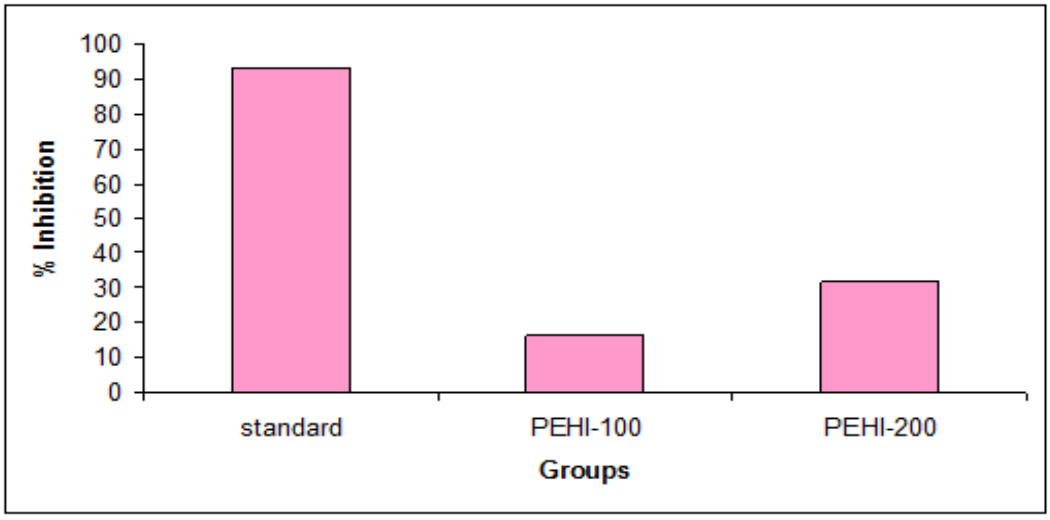

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Table 2: Effect of the Methanolic extract of *Holopteleaintegrifolia* leave (MEHI) against carrageenan induced paw oedema in rats

Group	Paw edema volume (ml)					% Inhibition
	0h	1h	2h	3h	4h	
Control (0.1 ml of 1% carrageenan)	**0.27± 0.03**	**0.43±0.04**	**0.58±0.02**	**0.72±0.03**	**0.87±0.01**	-
Standard Diclofenac sodium (10 mg/kg)	**0.23±0.02**	**0.34±0.03**	**0.28±0.01**	**0.25±0.01**	**0.24±0.02**	**93.33**
Methanolic Extract (100mg/kg) + (0.1 ml of 1% carrageenan)	**0.28±0.02**	**0.42±0.02**	**0.39±0.03**	**0.37±0.04**	**0.36±0.05**	**86.66**
Methanolic Extract (200mg/kg) + (0.1 ml of 1% carrageenan)	**0.24±0.02**	**0.39±0.03**	**0.35±0.01**	**0.32±0.02**	**0.31±0.02**	**88.33**

Figure 2: Effect of the Methanolic extract of *Holopteleaintegrifolia* leave (MEHI) against carrageenan induced paw oedema in rats

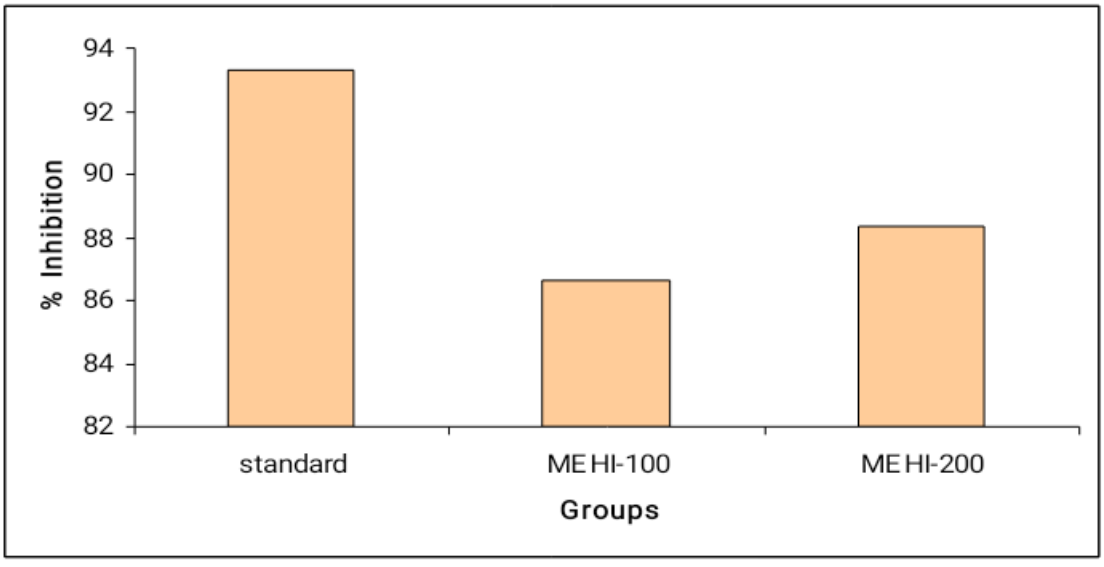

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Table 3 : Effect of the Petrolium ether and Methanolic extract of *Holopteleaintegrifolia* leave against carrageenan induced paw oedema in rats

Group	Paw edema volume (ml)					% Inhibition
	0h	1h	2h	3h	4h	
Control (0.1 ml of 1% carrageenan)	**0.27± 0.03**	**0.43±0.04**	**0.58±0.02**	**0.72±0.03**	**0.87±0.01**	-
Standard** Diclofenac sodium (10 mg/kg)	**0.23±0.02**	**0.34±0.03**	**0.28±0.01**	**0.25±0.01**	**0.27±0.02**	**93.33**
Petroleum Ether Extract (100mg/kg) + (0.1 ml of 1% carrageenan)	**0.19±0.02**	**0.32±0.02**	**0.45±0.03**	**0.61±0.04**	**0.69±0.05**	**16.66**
Petroleum Ether Extract (200mg/kg) + (0.1 ml of 1% carrageenan)	**0.23±0.02**	**0.31±0.03**	**0.42±0.03**	**0.56±0.02**	**0.64±0.04**	**31.66**
Methanolic extract** (100mg/kg) + (0.1 ml of 1% carrageenan)	**0.3±0.02**	**0.42±0.02**	**0.39±0.03**	**0.37±0.04**	**0.34±0.05**	**86.66**
Methanolic Extract** (200mg/kg) + (0.1 ml of 1% carrageenan)	**0.26±0.02**	**0.39±0.03**	**0.35±0.01**	**0.32±0.02**	**0.28±0.02**	**88.33**

**** Extremely significant (P<0.01), * Significant (p< 0.05), ns- Not significant (P> .05**

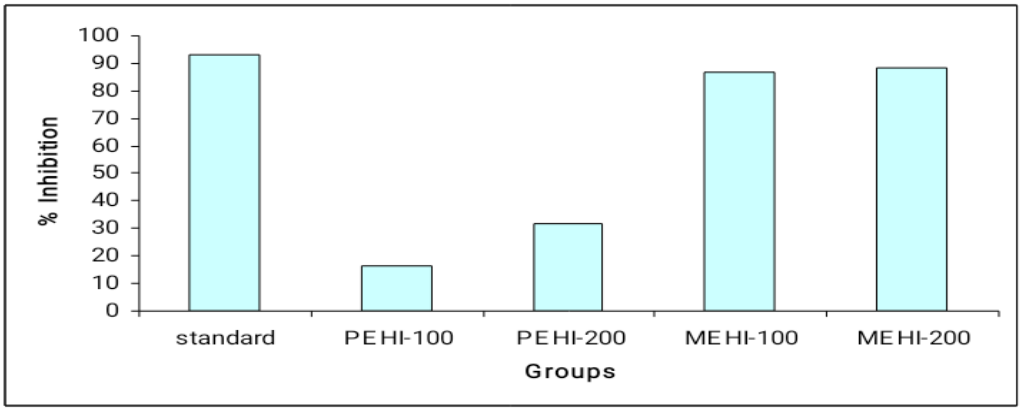

Figure 3 : Effect of the Petrolium ether and Methanolic extract of *Holopteleaintegrifolia* leave against carrageenan induced paw oedema in rats

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Table 4: Effect of Petroleum ether Extract of *Holopteleaintegrifolia* leaves (PEHI) on alloxan induced diabetic rats

Treatment and Dose (mg/kg p. o)	Serum glucose level (mg/dl)			
	0 day	3rd day	6th day	9th day
Control Group	235.72 ±3.81	233.17±2.56	236.68±2.09	235.18±2.44
Reference Group	242.93±2.51	226.03±3.76	214.33±1.89	187.08±2.66
Petroleum ether extract (100 mg/kg)	244.56±2.07	237.71±1.82	231.04±2.66	228.49±3.10
Petroleum ether extract (200 mg/kg)	253.14±3.42	241.79±3.18	228.91±2.32	217.33±1.99

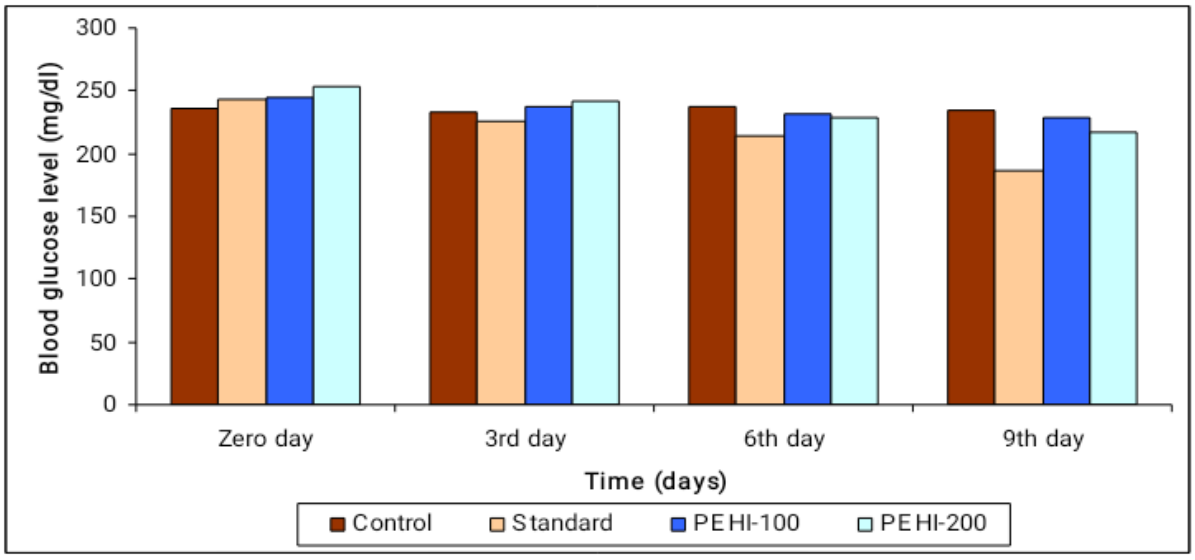

Figure 4: Effect of Petroleum ether Extract of *Holopteleaintegrifolia* leaves (PEHI) on alloxan induced diabetic rats

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Table 5: Effect of methanolic Extract of *Holopteleaintegrifolia* leaves (MEHI) on alloxan induced diabetic rats:

Treatment and Dose (mg/kg p. o)	Serum glucose level (mg/dl)			
	0 day	3rd day	6th day	9th day
Control Group	235.72±3.81	233.17±2.56	236.68±2.09	235.18±2.44
Reference Group	242.93±2.51	226.03±3.76	214.33±1.89	187.08±2.66
Methanolic extract (100 mg/kg)	265.74 ± 3.22	259.03 ± 3.35	254 ± 2.29	246.17 ± 3.48
Methanolic extract (200 mg/kg)	249.15 ± 3.08	241.72 ± 2.46	232.19 ± 3.08	223.79 ± 3.81

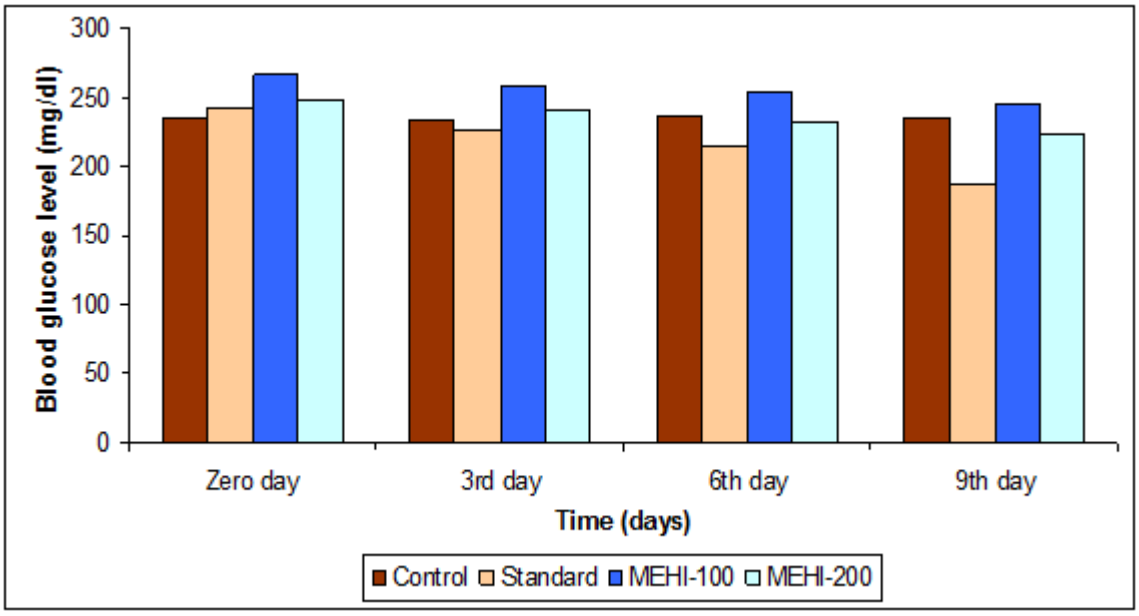

Figure 5: Effect of methanolic Extract of *Holopteleaintegrifolia* leaves (MEHI) on alloxan induced diabetic rats

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Table 6: Effect of Petroleum ether (PEHI)andmethanolic Extract of *Holopteleaintegrifolia* leaves (MEHI)

Treatment and Dose (mg/kg p. o)	Serum glucose level (mg/dl)			
	0 day	3rd day	6th day	9th day
Control Group	235.72±3.81	233.17±2.56	236.68±2.09	235.18±2.44
Reference Group**	242.93±2.51	226.03±3.76	214.33±1.89	187.08±2.66
Petroleum ether extract * (100 mg/kg)	244.56±2.07	237.71±1.82	231.04±2.66	228.49±3.10
Petroleum ether extract * (200 mg/kg)	253.14±3.42	241.79±3.18	228.91±2.32	217.33±1.99
Methanolic extract ns (100 mg/kg)	265.74 ± 3.22	259.03 ± 3.35	254 ± 2.29	246.17 ± 3.48
Methanolic extract* (200mg/kg)	249.15 ± 3.08	241.72 ± 2.46	232.19 ± 3.08	223.79 ± 3.81

**** Extremely sigmificant (P<0.01), * Significant (p< 0.05), ns- Not significant (P> 0.05)**

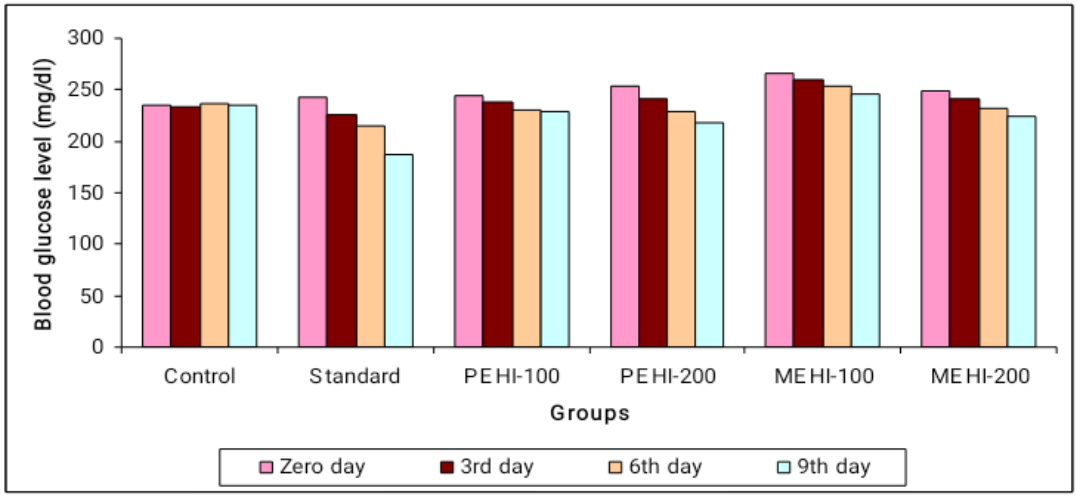

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Figure 6: Effect of Methanolic Extract and petroleum ether extract of *Holopteleaintegrifolia* leaves on alloxan induced diabetic rats

6. OBSEVATION AND INTERPRETATION

Anti-inflammatory activity

Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of nonsteroidal anti-inflammatory drugs and selective COX1-2 inhibitors. Several lines of evidence indicate that the COX-2-mediated increase in prostaglandin (PG) E2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. COX-2 is rapidly induced in the spinal cord and other regions of the CNS following carrageenan injection in the paw [59]. The administration of selective COX-2 inhibitors, but not COX-1 inhibitors, reduces the levels of PGE2 in the cerebrospinal fluid (CSF) and hyperalgesia[60-63]. In addition, it has been shown that the intrathecal administration of PGE2 potentiates carrageenan induced inflammation [64] and that the direct microinjection of PGE2 in the brain causes hyperalgesia[65.] Selective COX-2 inhibitors can also inhibit peripheral pain responses when given intrathecally[61, 66, 67] whereas a selective COX-1 inhibitor has no effect**.** [68] The central effects of PGE2 appear to be mediated via the EP3 receptor based on observations that the microinjection of an agonist of the EP3 receptor in the brain directly causes hyperalgesia[64],and the inflammatory responses are strongly reduced in the mice deficient in the EP3 receptor.[69] There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000).[70]Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti et al., 1995).[71] Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di and Willoughby, 1971)[72]whereas prostaglandins are detectable in the late phase of inflammation (Salvemini et al., 1996).[73]

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Anti- diabetic activity

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in

the pancreas when administered to rodents and many other animal species. This causes insulin-dependent diabetes mellitus (knows as "Alloxan Diabetes") in these animals. Its characteristics are similar to type 1diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells. This is because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates ROS (reactive oxygen species) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction.[77-78]The drug Gliibenclamide works by inhibiting ATP-sensitive potassium channelsin pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release.[79]

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