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Research Article

In-vitro Anti-inflammatory Perspectives of Hydroalcoholic Extract of *Diospyros celebica*

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A B S T R A C T

Inflammation is a complex phenomenon that is most synonymous with pain that requires activities such as increased artery permeability, increased denaturation of proteins and modification of the membrane. This current research explores the *In-vitro* Anti-inflammatory perspectives of hydroalcoholic leaves extract of *Diospyros celebica* via the human red blood cell membrane lysis method at a single concentration. The *In-vitro* anti-inflammatory activity revealed that, relative to normal diclofenac sodium, the plant extract showed substantial activity. The percentage of stabilization/protection of the HRBC membrane provided was found to be 55.63% at an extract concentration of 1000 µg/ mL as compared to the standard drug diclofenac sodium which showed 73.21% protection.The inhibition of red blood cell membrane lysis caused by hypotonicity and heat was taken as an indicator of the mechanism of anti-inflammatory activity. These effects may be attributed to the existence of strong antioxidant properties in phenolic and flavonoid products. This study gives on the idea that the compound of the plant *Diospyros celebica* can be used as a lead compound for designing a potent anti-inflammatory drug that can be used for the treatment of various diseases such as aging, neurological disorder, cancer, inflammation, etc.

Keywords: *Diospyros celebica*, *In-vitro* Anti-inflammatory, Extract, Inflammation, HRBC, Phytoconstituents

Introduction

Inflammation is a complex phenomenon that is most synonymous with pain that requires activities such as increased artery permeability, increased denaturation of proteins, and modification of the membrane. When bacteria, physical agents, or chemical agents harm cells in the body, the injury is in the form of stress. Inflammation of tissue is caused by a stress reaction. It is a protective reaction that is defined in the wounded region by redness, discomfort, heat, swelling, and loss of function. Depending on the location and degree of damage, loss of control occurs.[1]

Since inflammation is one of the non-specific internal defense mechanisms of the body, a tissue's reaction to an accidental cut is identical to a reaction resulting from other forms of tissue damage induced by fire, radiation, bacterial or viral invasion burns. Specific kinins, prostaglandins, and histamine are released as tissue cells are damaged. These components act together to induce increased vasodilation (widening of capillaries in the blood) and capillary permeability. This results in an increased supply of blood to the wounded site. These compounds also serve as chemical messengers, attracting a process known as chemotaxis to some of the body's natural defensive cells.[2]

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The initial reaction of the body to harmful stimuli is acute inflammation, which is accomplished by increased migration of plasma and leukocytes (particularly granulocytes) from the blood into the wounded tissues. The inflammatory response, affecting the local vascular system, the immune system, and numerous cells inside the damaged tissue, is propagated and matured through a cascade of biochemical events. Prolonged inflammation, referred to as chronic inflammation, results in a steady change in the form of cells present at the inflammation site and is marked by tissue damage and regeneration from the inflammatory phase at the same time.In the rural environment, the treatment of inflammation-related diseases is a real issue; many substitute medicines are used by the people of these regions, such as substances derived from medicinal plants.[3]

This current research explores the *In-vitro* Anti-inflammatory perspectives of hydroalcoholic leaves extract of *Diospyros celebica* via the human red blood cell membrane lysis method at a single concentration.

Material and Method

Instrumentation

The spectroscopic measurements were conducted using a double-beam Shimadzu® Ultraviolet-Visible Spectrophotometer (Model UV-1800, Kyoto, Japan) linked to a device with a spectral bandwidth of 1 nm and a wavelength precision of ±0.3 nm with a pair of matched quartz cells with a 10 mm path range. All weighing was achieved using the Shimadzu® electronic balance (Model AUW220D, Kyoto, Japan).

Chemicals

Sigma-Aldrich® Ltd. (Germany) and HiMedia® Ltd. (India) provided all the analytical grade reagents, consumables, solvents, and chemicals from a local retailer in Nagpur. For the experiment, a double-distilled water apparatus (Borosil®, India) was used.

Collection of Leaves and Preparation of Extract

The *Diospyros celebica* leaves were obtained from the commercial sources through proper channel and were suitably authenticated in Department of Botany, RTM Nagpur University, Nagpur, Maharashtra, India. The leaves were harvested from the tree, dried for a specified time in the shade, and suitably powdered. Continuous hot Soxhlet extraction with 50 mL purified water and 50 mL alcohol (ethanol 90 percent) at a temperature of 55-65°C over 32 cycles was performed in dried powder (100 g, separated into many smaller amounts). The solvent was extracted by means of a rotary vacuum evaporator under decreased pressure and controlled temperature. The yield of extracts was found to be 11.8 percent w/w.

In-vitro Anti-inflammatory Activity

Plant extract anti-inflammatory function has been investigated using the approach defined by Mahapatra et al., 2017. This technique is based on the fact that any disturbance is induced by lysosomal enzymes produced during inflammation. Acute inflammation is the main consequence of diseases due to their extracellular role. The ability of experimental substances is determined by inhibiting these chemical mediators or by stabilizing the lysosomal membrane. Since, human red blood cell membranes are identical to those of the portion of the lysosomal membrane, the prevention of Human Red Blood Cell membrane lysis (HRBC) caused by hypotonicity was used as a componentindetermining the anti-inflammatory properties.Blood was properly obtained during this procedure from a stable person who had not taken any anti-inflammatory medications for the past 15 days. An equivalent amount (2 percent dextrose, 0.8 percent sodium citrate, 0.5 percent citric acid, and 0.42 percent sodium chloride) of Alsever's solution was blended and the material was centrifuged at 3000 rpm. The plasma was isolated and stored carefully. Saline solution (0.9 percent) washed the packed blood corpuscles and a 10 percent suspension was prepared. Aliquots of the plant extract with a concentration of 1000 µg/mL were prepared using distilled water. 0.18 percent hyposaline (2 mL), phosphate buffer (1 mL), and HRBC suspension (0.5 mL) were applied to each concentration. The contents above were incubated for 30 min at 37±1°C and further centrifuged for 20 min at 3000 rpm. Using diclofenac sodium as the reference norm, the hemoglobin content contained in the supernatant solution was spectrophotometrically measured at 560 nm. A control that exempted the extract was also prepared. On the presumption that the test group will demonstrate 100% hemolysis, the percentage hemolysis was determined.[4] By way of the formula, the % HRBC membrane stabilization by plant extract was determined:

$$\% \text{ Protection} = 100 - \frac{\text{OD of Drug treated sample}}{\text{OD of Control}} \times 100$$

Statistical Analysis

The tests were performed in a triplicate way. The information obtained was expressed as a mean ± standard deviation (SD). Minitab® v.17 was used for mathematical calculations. The unpaired Student t-test (two-tailed) was used for pharmacological tasks to assess the discrepancy between control groups and the studied groups.

Result and Discussion

The *In-vitro* Anti-inflammatory activity revealed that, relative to normal diclofenac sodium, the plant extract showed substantial activity. The percentage of stabilization/ protection of the HRBC membrane provided was found to

be 55.63% at an extract concentration of 1000 µg/mL as compared to the standard drug diclofenac sodium which showed 73.21% protection (Table 1). The inhibition of red blood cell membrane lysis caused by hypotonicity and heat was taken as an indicator of the mechanism of anti-inflammatory activity. These effects may be attributed to the existence of strong antioxidant properties in phenolic and flavonoid products.

Table 1.In-*vitro* anti-inflammatory activity of hydroalcoholic extract of *Diospyroscelebica*

Treatment	Concentration (µg/mL)	Absorbance	% Protection
Control	-	0.489 ± 0.004	-
D. celebica extract	1000	0.217 ± 0.006***	55.63
Diclofenac sodium	100	0.131 ± 0.002***	73.21

All values represent mean ± SD of n = 3;***p<0.001 with respect to control group. Determined as compared with the control group (solution of 0.9% sodium chloride).

During inflammation, lysis of lysosomal components results in the discharge of enzymes that aggravate a large number of disorders. Anti-inflammatory agents exert their beneficial effects by either inhibiting lysosomal enzyme release or by stabilizing the lysosomal membranes.[5] Since human red blood cell membranes are very much similar to that of lysosomalmembrane, the potential of thorn extract in preventing hypotonicity-induced HRBC membrane lysis was taken as a parameter for estimating anti-inflammatory property. The extract containing rich phenolic and flavonoid contents exhibited dose-dependent stabilization of the HRBC membrane. The phytoconstituents like flavonoid glycosides are believed to play a key role in combating inflammation.[6]

The specific active constituents present in this plant such as 1,2,3-trimethoxy-5-(2-propenyl)-benzene, 2,3-dimethoxy-naphthalene, 2-acetyl-3,5-dimethylbenzo(b) thiophene, 5,6-dimethoxy-4-methyl-quinolin-8-amine, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2*H*-1-benzopyran-8-yl)-ethanone, 3-*O*-methyl-*d*-glucose, 2-hydroxy-1,2-bis(4-methoxyphenyl)-ethanone, 6a,12*a*-dihydro-6*H*-(1,3)dioxolo(5,6)benzofuro(3,2-c)chromen-3-ol, *p*-cresol, 2,3,5,6-tetrahydro-3,3,4,5,5,8-hexamethyl-*S*-indacene-1,7-dione, 10,11-dihydro-10-hydroxy-2,3,6-trimethoxydibenz (*b,f*) oxepin, methyl ester-9(*Z*)-octadecenoic acid, 5-(4-methylphenyl) furan-2-carboxylic acid, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1a*R*-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-1*H*-Cycloprop[e]azulene, methyleugenol, 4-methoxybenzene-1,2-diol, 2-(2-butoxyethoxy)-acetate, 4-methyl-2-[5-(2-thienyl)pyrazol-3-yl]-phenol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-[2R-(2.alpha.,4a.alpha.,8a.beta.)]-2-naphthalenemethanol, levoglucosenone, 4-oxo-pentanoic acid, 5-methyl-2-furancarboxaldehyde, 5-methyl-2(3*H*)-furanone, 5-hydroxymethylfurfural, 1-(4-hydroxy-3-methoxyphenyl)-2-propanone, and 1,4:3,6-dianhydro-a`-*d*-glucopyranose have been found to play key roles in exhibiting anti-inflammatory effects.[7]

Conclusion

The results in the present analysis show that the hydroalcoholic extract of *Diospyros celebica* has anti-inflammatory effects with a concentration of 1000 µg/mL. The high presence of polyphenolic compounds, such as polyphenols and flavonoids, may be due to these behaviors. The percentages of the extract function as free radical inhibitors or scavengers or can act as key oxidants, stabilizing the RBC membrane. This study gives on the idea that the compound of the plant *Diospyros celebica* can be used as a lead compound for designing a potent anti-inflammatory drug that can be used for the treatment of various diseases such as aging, neurological disorder, cancer, inflammation etc.

Conflict of Interest: None

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