Section A-Research paper
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PHYTOCHEMICAL SCREENING OF CAJANUS CAJAN LEAVES EXTRACT AND EVALUATION OF ANTI-PYRETIC POTENTIAL AGAINST SUITABLE ANIMAL MODEL

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A B S T R A C T

Many herbal plants have been identified to have antipyretic properties. The current study examines the antipyretic efficacy of acetone, ethanol and methanol extracts of *Cajanus Cajan* leaves on experimental rats' fevers that were generated by brewer's yeast. We utilised 30 wistar rats, which ranged in weight from 140 to 180g. They were randomly developed into five groups of six rats each. Group one served as the control group and received 5ml/kg of distilled water; group two served as the negative conrrol group and received only brewer's yeast; group three served as the standard group and received 150 mg/kg of paracetamol; and groups four and five served as the test groups and received 200 mg/kg and 400 mg/kg of pigeon pea leaves extract, respectively. All of the test animals received a subcutaneous injection of a 20% w/v suspension of 10ml/kg brewer's yeast to cause fever. Except group one after 18 hours, the rats were given oral doses of paracetamol (standard group, 150 mg/kg) and *Cajanus Cajan* leaves extract (200 mg/kg, 400 mg/kg) orally. The rats' body temperatures were transrectally measured till 8 hours. Comparing group two (10ml/kg, brewer's yeast) to group three and group four (200mg/kg and 400mg/kg), *Cajanus Cajan* leaves extract significantly decreased yeast-induced pyrexia. When compared to group two (10ml/kg, brewer yeast), group three (paracetamol, 150mg/kg) similarly shows a sizable decrease. This experiment therefore demonstrates that the antipyretic action of *Cajanus cajan* leaves extract is dosage dependent and that the effect is brought on by the flavonoid component of the extract. These findings imply that *Cajanus Cajan* extract has potent antipyretic properties, and that its mechanism may include a reduction in the production of prostaglandins and inflammatory mediators.

KEYWORDS: Anti-pyretic activity, Cajanus Cajan leaves, Paracetamol, Brewer's Yeast Powder.

1. INTRODUCTION

The term "fever" refers to an increase in core body temperature above normal; the usual oral temperature in healthy persons is 37°C (98.6°F) [1]. Fever is a initial medical symptom rather than an illness, and it can be brought to by infection, cancer, or other sick states as a side effect. The body's thermostat, which is located in the hypothalamus and anterior pituitary, resets at a higher temperature when there is a fever, usually in reaction to an illness.

From the recent scientific discovery, most of the antipyretic drugs have been developed to reduce elevated body temperature, of which many acts by the mechanism of inhibition of the COX-2 expression to reduce PGE2 biosynthesis.

Many drugs on chronic usage can result a several side effects including gastrointestinal, renal, hepatic, central nervous system, and dermatological effects. It is currently accepted that prostaglandin E2 (PGE2) is the final fever mediator in the brain, specifically in the pre-optic area of the anterior hypothalamus. In the current scenario paracetamol is the most frequent using medicine for the treatment of hyperthermia while paracetamol is potent hepatotonic drug on higher dose. To over one there insure related to adverse effects of allopathic medicines, the trends of herbal use is increasing day by day [2].

Ancient scholastic writings provided a rich history of information on both preventative and therapeutic treatments. The mixtures of secondary products found in plant materials are often what give them their positive therapeutic benefits [3]. Many different antipyretic substances, including medicinal herbs, have been utilised for a long-time effect. Therefore, using natural plants as fever relievers can be useful in many ways, such as being readily available, affordable, and having little to no adverse effects.

1.2 MATERIAL AND METHODS

1.2.1 Collection of plant

Following a thorough analysis of the medical literature and taking into account bio-conservation issues, fresh leaves of pigeon pea were collected from the north region (Hapur), Utter Pradesh, on 16th of March in the year 2023.

1.2.2 Authentification

The plant was authenticated by National Institute of Science Communication and Policy Research (NIScPR), with the authorization number NIScPR/RHMD/Consult/2023/4390-91.

1.2.3 Drugs and reagents

Normal saline, Paracetamol, Brewer's yeast and were used in the study.

1.2.4 Extraction of plant leaves

Fresh leaves of cajanus cajan were collected and air-dried. Plant material was dried under shade at comfortable room temperature (24–26^{o}C), pulverized by a mechanical grinder, sieved through 40 meshes. The powdered material (100 g) was extracted with ratio 4: 2: 2 of acetone, ethanol and methanol in a Soxhlet apparatus. A rotary vacuum evaporator set at 45°C was used to concentrate the extracts. The resultant semisolid mass was preserved in desiccators for further use in phytochemical screening and in-vivo studies [4].

1.2.5 Phytochemical screening

Qualitative phytochemical analyses were performed on the Cajanus cajan acetone, ethanol and methanol extract to identify its various contents, including alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, free amino acids, and triterpenoids.

The pharmacological efficacy of phytochemicals may be due to the synergistic effects [5]. Due to biocompatibility and overlapping mechanisms of action, proper analysis of the active agents is necessary [6].

1.2.6 Animal

In this investigation, Wistar animals of both genders were employed. Rats utilized in the study ranged in age from 2 to 3 months and weighed 140 to 180 g on average. They were purchased from licensed breeders. The animals were housed in polyacrylic cages, with a maximum of than six animals to a cage in a space with regular, regulated environmental conditions. Prior to the trial began, the rats spent 14 days becoming used to the lab environment [7].

Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow.

The experimental animals were randomly divided into five groups, each group contain six animals. The normal control group (I) was orally administered 5ml/kg distilled water, while the

negative control group (II) was given 10ml/kg of a 20%w/v suspension of brewer's yeast in normal saline, the standard group (III) was given 150 mg/kg paracetamol and groups (IV) and (V) were prescribed dose I and II of acetone, ethanol and methanol extract of test drugs, respectively [8].

1.2.7 Grouping

Animals were randomly divided into following groups. Group I: This group was known as a normal control group. 5ml/kg distilled water were administered to this group. Group II: This group was known as a negative control group. This group received with 10 ml/kg brewer's yeast of 20% w/v alone. Group III: This group was known as a standard reference group. This group received with brewer's yeast with 150 mg/kg of paracetamol. Group IV: This group is known as a test group. This group received with brewer's yeast along with 200mg/kg of Cajanus Cajan. Group V: This group was known as a test group. This group received with brewer's yeast along with 400mg/kg of Cajanus Cajan [9].

1.2.7 Induction of pyrexia

After administering 20% w/v Brewer's yeast to rats to generate fever, antipyretic efficacy was examined. Animals received nourishment consistently for 18 hours before receiving medication. Following taking the rats' rectal temperatures with a 1.5 cm thermometer that was inserted into the rectum. Pyrexia was produced by subcutaneously administering a dosage of 20% w/v suspension of yeast that was mined in normal saline equal to 10 ml per kilogram of the animal's weight. Animals with temperature increases of a minimum of 1°C after an 18-hour yeast injection were selected for the study. Following giving the medicine, the operating temperature was taken at 1, 2, 3, 4, 6 and 8 hours [10].

1.2.8 Statistical treatment

The results were subjected to one-way ANOVA followed by Dunnett's test. The data is deemed to be statistically significant if $p<0.05$. Data were represented in mean ± SEM.

2 RESIULT

The antipyretic activity was performed along with water consumption, food consumption and confirmation for the presence of phytochemicals. The water consumption was slightly increased with respect to the time spent while the vice versa in case of food consumption over

the time till 8 hours. The phytoconstituents detected in the leaves include Alkaloids, Flavonoid, Phenols, Tannins, Protein and amino acids, Carbohydrate, Saponins & Terpinoids (Table 1)

Table 1: Phytochemical screening of various leaf extract of Cajanus cajan. (+) represent the presence of different phytochemicals.

Phytochemical Constituents	Status Value
Alkaloids	+
Flavonoid	+
Phenols	+
Tannins	+
Protein& Amino acids	+
Carbohydrate	+
Glycosides	+
Saponins	+
Terpinoids	+

The anti-pyretic activity of ethanol leaves extract of Cajanus Cajan. Against yeast induced pyrexia. The acetone, ethanol and methanol leaves extract of Pigeon pea leaves. At doses of 200 and 400 mg/kg showed significant effect against Brewer's yeast induced pyrexia method. There was a progressive dose dependent reduction in the temperature of rats treated with the extract. The reduction caused by the extract was significant when compared to control.

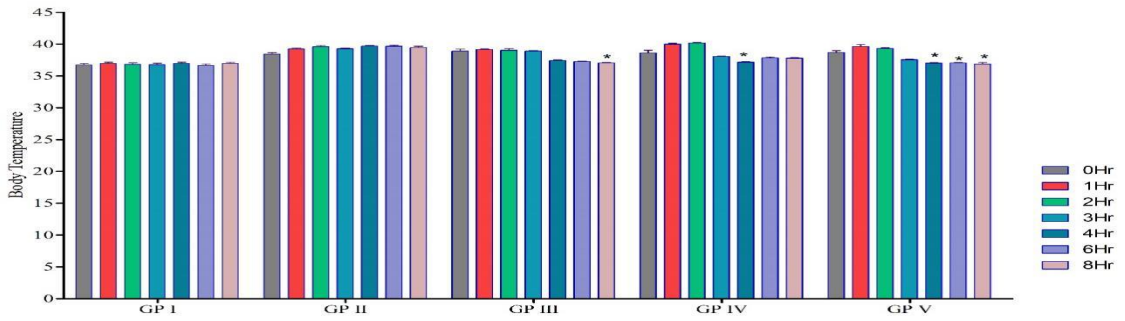

Figure 1: This figure show temperature variation in fever induced in all groups of wistar rats**.** All value expressed as mean ± SEM; Significant (*) was considered at $p<0.05$ when compared with control. Brewer's Yeast Caused Pyrexia in Rats and the Anti-Pyretic Properties of Cajanus Cajan's ethanol, methanol, and acetone Leaves Preparation.

DICUSSION

Although antipyretic activity is usually cited as a quality of drugs or substances that have an inhibitory effect on prostaglandins synthesis, the brewer's yeast-induced hyperpyrexia in rats model was utilized to assess the antipyretic activity of the extracts [11].

Pathogenic fever, also known as yeast-induced pyrexia, is caused by prostaglandins (PGE2) being produced, which raise the thermoregulatory center's temperature. Prostaglandins, which play a role in pyrexia, are known to be targeted by specially flavonoids. Consequently, the flavonoid content of the plant may contribute to its antipyretic action [12].Numerous endogenous pyrogens, including macrophage protein-1, prostaglandins, tumour necrosis factor TNF-, interleukin-1, interleukin-6, and interleukin-8, are known to produce fever. TNF- and phospholipase A2 have the potential to trigger prostaglandin production. TNF- and prostaglandin production are both stimulated by brewer's yeast [13]. By inhibiting Cyclo-oxygenase (COX-2) and so lowering prostaglandin E2 concentration in the brain, particularly in the hypothalamus, the extracts are expected to diminish pyrexia. They may also increase the body's natural synthesis of antipyretic chemicals. So it follows that Cajanus Cajan prevents prostaglandin production. COX-2 is transcribed and induced by two distinct mechanisms, which have been identified. Both routes are stimulated by cytokines including IL-1, IL-6, & TNF, which activates DNA transcription factors notably nuclear factor (NF) kB and the activator and signalling transducer of transcription (STAT-3) to activate central mechanisms [14].

Albino wistar animals were given brewer's yeast to make them ill. Since yeast raises the temperature of the body over the course of around 18 hours, a fever was seen 18 hours following the yeast injections. Brewer's yeast injection under the skin causes pyrexia via boosting prostaglandin production. It is regarded as a helpful testing for determining if synthetic pharmaceuticals or plant-based compounds have an antipyretic activity. Pathogenic fever, also known as yeast-induced pyrexia, has prostaglandin synthesis as one of its possible explanations. Similar to how paracetamol works to reduce fever, prostaglandin production might be inhibited to have an antipyretic effect. Cyclo-oxygenase enzyme production can be blocked to limit prostaglandin formation. The antipyretic action is brought on by the suppression of the many mechanisms that contribute to pyrexia.

When given orally, Cajanus Cajan significantly reduced the temperature of albino rats that had been given a yeast induction. Consequently, it may be hypothesized that Cajanus Cajan included medicinally potent components that prevent prostaglandin release. Throughout three hours of testing, an albino rat that had been given brewer's yeast for inducing pyrexia showed measurable antipyretic efficacy. It was discovered that the resulting substance had antipyretic action that was dosage responsive.

CONCLUSION

The current experimental experiments have verified the antipyretic effect of the acetone, ethanol and methanol extract of Cajanus Cajan leaves. When compared to the temperatures of the rats in the control group, the wistar albino experimental rats treated with Cajanus Cajan extract at two separate dosages of 200 mg/kg and 400 mg/kg had decreased temperature levels. Prostaglandin (PGE2) is inhibited as a result. Based on these findings, it can be said that the Cajanus Cajan leaves extract, at a dosage level of 400mg/kg, significantly reduced the temperature to normal. Consequently, they have antipyretic properties equivalent to those of paracetamol. A plant test extract at a dosage of 200 mg/kg was not as effective in compare to the dose of 400 mg/kg.

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