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Analgesic Effect of Ethanol Extracted Leaves of *Psidium guajava* Leaves In Animal Models.

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ABSTRACT

The research was carried out to investigate the analgesic effects of ethanol extract of Psidium guajava leaves. In order to assess the analgesic effects by acetic acid induced writhing response model. Leaf extract were administered (2mg/kg bodyweight) and the obtained effects were compared with commercially available analgesic drug Diclofenac sodium (40mg/kg bodyweight), Distilled water (2ml/kg body weight) was used as control for the study. In analgesic bioassay, oral administration of the ethanol extract of leaves were significantly ($p<0.01$) reduced the writhing response. The efficacy of leaves extract was almost 60% which is found comparable to the effect of standard analgesic drug Diclofenac sodium (75%).we recommended further research on this plant leaves for possible isolation and characterization of the various active chemical substances which has the toxic and medicinal values.

Keywords: Psidium guajava, analgesic, diclofenac sodium.

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INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. Those plants that have healing properties are termed as medicinal plants or herbs. The plant kingdom is divided into several groups, but the botanical classification is beyond the scope of this section. However, medicinal plants can be simply classified as trees, shrubs, woody perennials, annuals and biennials, and climbers (Dr.Everaldo G. Attard, 2005).

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active constituents of plants are alkaloid, tannin, flavonoid and phenolic compounds. In the last few years, a number of studies have been conducted in different medicinal plants indifferent countries to prove the medicinal efficiency many of the indigenous medicinal plants are used for medicinal purposes (Edeogra H.O., 2005).

The advent of science into the search of antibiotics largely depends on some of these medicinal plants as raw materials. For many years medicine had depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use (Anderson *et.al*, 1996).

Some reported adverse events following the use of certain herbal medicines have been associated with a variety of possible explanations, including the inadvertent use of the wrong plant species, adulteration with undeclared other medicines and/or potent substances, contamination with undeclared toxic and/or hazardous substances, over dosage, inappropriate use by health-care providers or consumers, and interaction with other medicines, resulting in an adverse drug interaction. Among those attributable to the poor quality of finished products, some clearly result from the use of raw medicinal plant materials that are not of a sufficiently high quality standard.

Research in recent years has shown the implication of oxidative and free radical-mediated reactions in degenerative processes related to aging and diseases such as atherosclerosis, dementia, and cancer (Pryor, 2000). When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS) in the form of superoxide anion $O_2^{\bullet-}$ hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are generated. Membrane lipids, proteins, and deoxyribonucleic acid (DNA) are the targets of such species and can suffer oxidative damage, causing tissue injury (Wiseman and Halliwell, 1996).

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. In recent years, there has been increasing interest in finding natural antioxidants since the synthetic antioxidants, such as butylated Hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are suspected of being responsible for liver damage and carcinogenesis (Grice, 1988).

The reactive oxygen species, excessive production of reactive nitrogenous species especially nitric oxide (NO) through the activation of the constitutive as well as the expression of the inducible isoform of NO synthase (iNOS) has been shown to play an important role in various models of inflammation (Salvemini *et al*., 1996; Cuzzocrea *et al*., 1998; Marzocco *et al*., 2004).

The formation of peroxynitrite (ONOO−), a highly reactive oxidant radical, results from the combination aforementioned nitrogenous species NO and superoxide anion (Beckman *et al*., 1990). Involvement of ONOO− in carrageenan-induced rat paw edema has been shown by measurement of nitro tyrosine staining (Salvemini *et al*., 1996).

Psidium guajava is an important food crop and medicinal plant in tropical and subtropical countries is widely used like food and in folk medicine around of the world. This aims a comprehensive of the chemical constituents, pharmacological, and clinical uses. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have been carried out. Also have been identified the medicinally important phyto-constituents.

A number of metabolites in good yield and some have been shown to possess useful biological activities belonging mainly to phenolic, flavonoid, carotenoid, terpenoid and triterpene. Extracts and

metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities. A survey of the literature shows *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. It has also been used extensively as a hypoglycemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses.

Review of Literature

Recently, 546 species have been identified as having medicinal properties and therapeutic use (Ghani, 1998). Medicinal plants are the great importance to the health of individual and communities. The medicinal values of those plants possess some chemical active substances that produce a definite physiological action on the human body and animal health. The most important bioactive substances are alkaloid, tannin, flavonoid and phenolic compounds (Edeogra, 2005).Drugs which are used presently for the management of pain and inflammatory conditions are either steroidal like corticosteroids or non-steroidal like aspirin. All of these drugs possess more or less side and toxic effects like renal failure, allergic reactions, hearing loss or they may increase the risk of hemorrhage by affecting platelet function (Thomas, 2000). Moreover, synthetic drugs are very expensive (Ahmad *et al.*1992). On the contrary, many plant origin drugs had been used since long time without any adverse effects. The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif and Banik, 2006). Plants are the cheapest and safer alternative sources of antimicrobials (Doughari *et al.,* 2007).

MATERIALS AND METHODS

Sample collection and leaves extract preparation

The fresh leaves obtained were washed with freshwater immediately after collection. Leaves were then chopped into small pieces, air-dried at room temperature for 10 days. Dried leaves were ground into powder and stored in an airtight container. After that 750gm powder from the leaves were taken and suspended in 1L ethanol for 7 days at room temperature. Ethanol extract was sieved using cotton plug followed by a Whatman no.1 filter paper. The extract was concentrated under reduced pressure below 50ºC through rotatory vaccum evaporator. The concentrated extract were collected in a Petri dish and allowed to air-dry for complete methanol evaporation. finally, 50gm greenish colored, concentrated leaves extract was obtained from the leaves and kept them in fridge (4ºC).

Experimental animals and diets

Wistar Albino rats weighing between 150- 200g are obtained from Vasavi Institute of Pharmaceutical Sciences; laboratory was used for the experiment. The animals were anti acclimatized to room temperature (28±5°C) with a relative humidity (55±5%) in a standard wire meshed plastic cages under a 12 h light/12 h dark cycle for 4-5 days prior to the experiment. The animals were supplied with standard pellet diet by adlibitum water. Laboratory experimentation was performed according to the guidelines of Institutional Animal Ethics Committee (IAEC) of VIPS.

Screening of analgesic activity of leaves extract

Acetic acid induced writhing test model described by Koster *et al.*, was performed to evaluate the analgesic activities of leaves extracts.

Acetic acid induced writhing response model

Fifteen albino rats were randomly divided into three groups and each group consisting of 5 animals. Control group received only distilled water, positive control group received analgesic drug diclofenac sodium at the dose rate of 40mg/kg body weight and treated group received leaves extract at the dose rate of 2gm/kg body weight. Ethanol extract leaves, analgesic drug diclofenac sodium and distilled water were administered orally to particular groups, 30 min prior to acetic acid injection. 1 % acetic acid solution at the dose rate of

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3.3ml/kg body weight was injected intra-peritoneally in mice and the number of writhing and stretching was counted over 20 minutes period. Finally, % analgesic activity was calculated by using following formula:

$$\%Analgesic\ activity = \frac{\text{Mean writhing count (Control group} - \text{Treated group)}}{\text{Mean writhing count of control group}}\ X\ 100$$

RESULTS

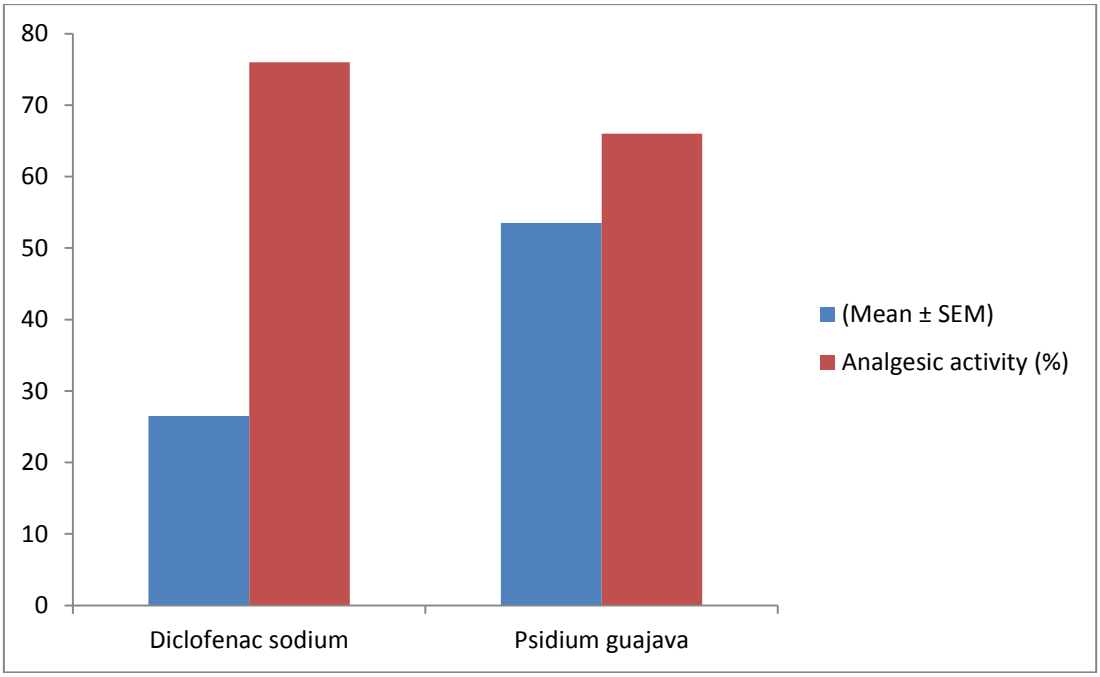
Analgesic activity

Table-1 and Figure-1 shows the pain behavior of writhing response of mice and analgesic activities of Diclofenac sodium and ethanol extract of plant leaves. The control animal showed 66.7 writhing count/20 minutes but, animal treated with Diclofenac sodium caused significant reduction of writhing count, from 66.7 to 26.5 ($p<0.01$). Animals treated with leaves extract of Psidium guajava reduced the writhing count from 66.7 to 53.5. The results suggested ethanol extract leaves and Diclofenac sodium had analgesic action and showed significant ($p<0.01$) reduction of pain in comparison with control group. Psidium guajava had higher analgesic activity (70%).

Table 1. Effect of ethanol leaves extract on acetic acid induced writhing response

Treatment	Dose/Kg body weight	(Mean ± SEM)	Analgesic activity (%)
Distilled water	2 ml	66.7±2.2	-
Diclofenac sodium	40 mg	26.5± 1.7	76
Psidium guajava	2gm	53.5±1.3	66

Figure 1: Graphical presentation of in comparing Psidium guajava leaf extract with Diclofenac sodium



DISCUSSION

The writhing test has long been used as a screening tool for the evaluation of analgesic properties of new substances. Ethanol extracted leaves of Psidium guajava significant inhibition ($p<0.01$) of acetic acid induced writhing response of mice, so it can be suggested that these leaves extract has potential analgesic activities. The analgesic effect of the extract may be either due to its action on visceral receptor sensitive to acetic acid, to the inhibition of the algogenic substances or the inhibition of transmission of painful messages at the central level. The special nerve endings that sense pain are very sensitive to prostaglandin. When

prostaglandin is released, the nerve endings respond to it through prostaglandin E2 (PGE2) receptor by picking up and transmitting the pain and injury messages through the nervous system to the brain and cause visceral writhing stimuli in mice Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably efficient as an anti-nociceptive mechanism in visceral pain Acetic acid induced abdominal constriction is a useful experimental tool in testing of new analgesic drugs The abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid, which results synthesis of prostaglandin via the cyclooxygenase (COX) enzyme The results support the popular use of mentioned plant leaves extract, but phytochemical studies together with pharmacological and toxicological investigations have proven essential for the complete understanding of their medicinal application.

CONCLUSION

The effects of ethanol extracted leaves showed significant reduction of pain in comparison with available commercial analgesic drugs. They had also anti-inflammatory effect. However, further investigation is required for isolation, identification and characterization of different active compounds and their mode of action and therapeutic range.

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