Screening of Analgesic Activity of Flacourtia jangomes Flesh on

Swiss Albino Mice.

By

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A project submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

> School of Pharmacy Brac University January, 2023

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Declaration

It is hereby declared that-

1. The thesis submitted is my own original work while completing degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material, which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Screening of analgesic activity of *Flacourtia jangomes* flesh on Swiss Albino mice" submitted by Mehenaj Akter (ID-18346001), of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethical Statement

Ethical permission has been achieved from Department of Pharmacy, Jahangirnagar University.

Abstract

Flucortia jangomes is a wild fruit species founded in hill tracks of Bangladesh. This is a less explored plant but carries many phytochemical constituents and has a variety of pharmacological activities. This study investigated central and peripheral analgesic activity of methanolic extracts of *F. jangomes* flesh on Swiss Albino mice. After extraction two main methods were used here – tail immersion method and acetic acid induced writhing method. Three different doses 100 mg/kg, 200mg/kg and 300mg/kg were administered to mice. A dose dependent activity was observed during the experiments and noticeable analgesic effects were seen. Statistical analysis of values indicates ** p < 0.01 and * p < 0.05 vs. Control and 60.01% inhibition was observed at 300 mg/kg dose. However, standard solution shows higher (72.78%) percent inhibition that the extract solution. The result of experiments show potential analgesic activity of *F. jangomes* fruit flesh and further studies are needed to identify the active phytochemical constituents which is responsible for this anti-nociceptive activity.

Keywords: Flucortia jangomes, analgesic activity, tail immersion, methanol, acetic acid.

Dedication

This project is dedicated to my family, faculty members and friends.

Acknowledgement

All praises to Almighty Allah and I would also like to convey my sincere gratitude to my supervisor Dr. Farhana Alam Ripa, Assosiate Professor, School of Pharmacy for providing continuous guidance and support to complete my project.

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List of Acronyms

BNH	Bangladesh National Herbarium
WHO	World Health Organisation
BARI	Bangladesh Agricultural Research Institute
SEM	Standard Error Mean
NSAID	Non Steroidal Anti Inflammatory Drug
ICDDRB	International Centre for Diarrhoea Disease Research, Bangladesh
MFJF	Methanolic Flucortia jangomes Flesh Extract
OGTT	Oral Glucose Tolerance Test

Chapter 1

Introduction

Mankind has always depended on the curative powers of medicinal plants, long before the advent of modern medicines. There are others who place a high value on these plants because of the long-held conviction that they were designed specifically to meet man's needs for sustenance, medicine, and other benefits. WHO estimates that of the world's 5.2 billion inhabitants, 80% reside in less developed nations, and that the majority of these individuals use only traditional medicine for their primary healthcare. More than 3.3 billion people in developing nations regularly use medicinal plants because they represent the "backbone" of traditional medicine. Approximately all of the world's nearly 2,000 recognized ethnic groups have their own unique body of traditional medical knowledge and set of medical experiences (*Screening of Ten Medicinal Plants of Bangladesh for Analgesic Activity on Swiss-Albino Mice*, 2013).

1.1 Using Medicinal plants in Bangladesh

Traditional medicine in Bangladesh makes use of the country's rich flora and fauna to treat a wide range of health problems. Plants utilized by indigenous people have been recognized as the original source of several significant medications currently in use. It has been estimated that 64 percent of the world's population still relies on alternative treatments including traditional medicine and herbal remedies. Traditional medicine has gained popularity among both scientists and the general public in recent years for a number of reasons, including the high price of allopathic drugs that puts them out of reach for the poorer segments of society in almost every country, the lack of access to medical clinics and hospitals by the rural population of developing countries, the side-effects and toxicities of modern synthetic drugs,

and the realization that phytochemicals present in plants can be effective in treating a variety of diseases. Bangladesh is a subtropical nation; hence it has an abundance of plants that may be used to treat various ailments. Even back in the 1980s, local woods provided 80 percent of the raw materials for the country's Ayurvedic and Unani herbal medicine manufacturers, with the remaining 20 percent coming from overseas. The situation has changed for the better since then. Currently, imported goods account for 80% of the local demand, while locally grown therapeutic plants account for only 20%. Research conducted by the Bangladesh Agricultural Research Institute (BARI) has revealed that the country is home to 722 (Rahmatullah et al., 2009).

Table 1: Few Medicinal Plants used in Bangladesh (Rahmatullah et al., 2009).

Scientific Name	Local Name	Parts used	Therapeutic Activity
Justicia adhatoda L.	Basok	Leaf	Common cold, cough
Andrographis paniculata	Kalomegh	Leaf, stem	Fever, Pain
Rauwolfia serpentina Benth.	Sarpogondha	Stem, leaf	Dysentery, Hypertension
Phyllanthus emblica L.	Amloki	Fruit	Hair damage, indigestion
Ocimum tenuiflorum L.	Tulsi	Leaves	Anti bacterial, cough
Cinnamomum zeylanicum	Daruchini	Bark	Spice, dysentery
Cymbopogon citratus	Lemon ghas	Leaf	Stop bleeding
Nerium indicum Mill.	Korobi	Leaf	Skin disorder
Azadirachta indic	Neem	Leaf	Skin infections
Allium sativum	Rosun	Stem	Antidiabetic

1.2 Importance of Medicinal plants as analgesic drugs

Analgesics (painkillers) are any drugs that reduce pain by either a central or peripheral mechanism. Humans have been concerned about pain ever since the ancient age, and this worry led them to seek for and eventually find medicines derived from plants. There are several possible phytochemicals in medicinal plants that might have analgesic activity, and these compounds have less negative side effects than manufactured medications. As a result,

the study of traditional medicinal herbs with analgesic properties has gained prominence and received permission all around the world. Numerous medicinal plants and the phytochemicals extracted from them have been studied for their potential to alleviate pain (Khan, 2012).

Table 2: Plants having analgesic activity (Sengupta et al., 2015).

Botanical name	Parts used	Chemical constituents
Allium stracheyi	Leaves	Saponin, steroid
Murraya paniculata	Bark	Cumarines
Scoparia dulcis L	Whole herb	Tannin, glycoside, alkaloid
Polyalthia longifolia	Leaves	Alkaloid
Hibiscus rosa sinensis	Leaves	Flavons, alkaloids, vitamins

1.3 Description of sample *Flucortia jangomes*

The coffee plum, Indian plum, Indian cherry, Paniala, and Puneala all refer to the same kind of wild fruit, *Flacourtia jangomas* (Lour) Raeusch. Originally from Bangladesh, India, and Myanmar, this tiny (6-10 m tall) tropical deciduous tree is now widely grown over most of Southeast Asia, Eastern Malaya, the Philippines, and even, to a lesser extent, in Surinam, Trinidad, Puerto Rico, and Southern Florida. This tree is found in the wild in the Bangladeshi regions of Chittagong, Chittagong Hill Tracts, Sylhet, and Cox Bazar. *F. jangomas* produce rounded fruits that are approximately 1 cm in diameter, succulent when fresh, dark crimson or purple to practically black in color, smooth, and contain 6-10 tiny, flattened seeds. The flavor ranges from sour to sweet, and the fruit can be consumed in its raw form or processed into a drink or preserve. The fruits have a high protein content (3.9%), vitamin C content (2.2 mg g-1), sugar content (21% sucrose), and mineral content (significant amounts of Ca, K, P, Fe, and Mg), all related to their dry weight (Hossain et al., 2011).



Figure 1: (A) Fully grown tree of F. jangomes (Hossain et al., 2011).

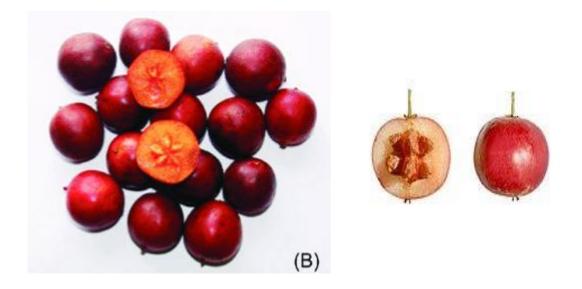


Figure 2: Ripe fruit of F. jangomes (Hossain et al., 2011).



Figure 3: Immature F. jangomes fruit (Hossain et al., 2011).

1.3.1 Botanical description

This little deciduous tree usually reaches heights of 6-10 m but has been known to grow as high as 14 m on rare occasions. Both the trunk and the branches of mature trees are devoid of the woody thorns that are found on trees when they are still young. Bark ranges in color from pale brown to copper red to pinkish buff and peels off in thin, lenticelled lamels. The male flowers are filamentous, glabrous, and found either alone or in small groups, whereas the female flowers always bloom alone. Trees bearing male and female flowers are kept apart. Many oblong to suborbicular anthers make up the androecium. From December through April, not only do flowers but also new leaves emerge in a stunningly vibrant green. The fruits are ellipsoid, subglobose berries that grow 1.5–2.5 cm in diameter and ripen between March and July. They are dull-brownish red or purple and eventually black, and their flesh is greenish–yellow. Single, short style columns ending in minute stigma points top the seeds. Seeds are used to grow new trees. Since seed germination is so sluggish, inarching or budding onto self-seedlings is the preferred method of propagation. Birds consume the ripe fruits and spread them far and wide, allowing the species' range to expand dramatically (Sasi et al., 2018).

1.3.2 Taxonomic classification of F. jangomes

Kingdom – Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Violales

Family - Flacourtiaceae

Genus - Flacourtia

Species - Flacourtia jangomas (Lour.) (Parvin & Hosain, 2014).

1.3.3 Traditional uses of F. Jangomes

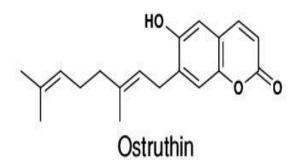
The luscious fruits of the F. jangomas tree are often consumed due to their deliciously tangy flavor. Dark crimson or purple when mature, the fruits can be eaten fresh or used in jams and preserves. The meat has a firm texture, has a muddy green color, and is rather juicy. It's used in sweet stews, as well as in drinks, syrups, jams, marmalades, pickles, and sauces. In order to lessen their astringency, some fruits are rolled in the palms of the hands before eating. When picked when still immature, it is perfect for use in jellies. Fruits, particularly those native to the Indian state of Kerala, are revered for both their culinary and medicinal value. In Indonesia, the young, acidic shoots are a popular food. The wood is a deep red or crimson color, has a tight grain, is extremely strong and brittle, lasts a long time, and takes a high shine. You may use it to make tools for the farm or a block. The southern Indian states of Tamil Nadu, Kerala, and Karnataka occasionally harvest the wood for timber. It's commonly substituted with more costly materials like Teak. In fact, the Queensland fruit fly, Bactrocera tryoni, relies on this plant more than almost any other for sustenance (George et al., 2017).

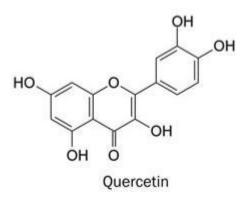
1.3.4 Phytochemical constituents of F. Jangomes

It is fair to say that F. jangomas is one of those plants that has not been studied well in the lab. Studies on the plant's phytochemical components are few. In reality, the identification of a series of cytotoxic diterpenes in Casearia sylvestris only in the last several years brought attention to the plants of the family Flacourtiace. However, only a small number of species have had their phytochemicals reported, and the family's chemistry is currently poorly known. To far, research has demonstrated that the Flacourtiaceae family elaborates a wide variety of compounds, including terpenoids, alkaloids, flavonoids and tannins, lignans and flavanolignans, glucosides, coumarins, and isocoumarins (Ahmad et al., 2020).

Table 3: Chemical compounds of different parts of F. jangomes (Mishra & Rai, 2020).

Parts	Chemical Compounds
Plant	Tannin and fixed oil
Bark	Tannins, flacourtin
Leaves and young shoots	Xanthones, quinones, tannins limonoids and phenazines
Stem and bark	Two limonoids, namely limolin and Jangomolide, ostruthin
Ripe Fruit	Protein, fat, sugar, amino acid, vitamin c, potassium, calcium, phosphorus
Unripe fruit	flavonoids, alkaloids, tannins and total phenols





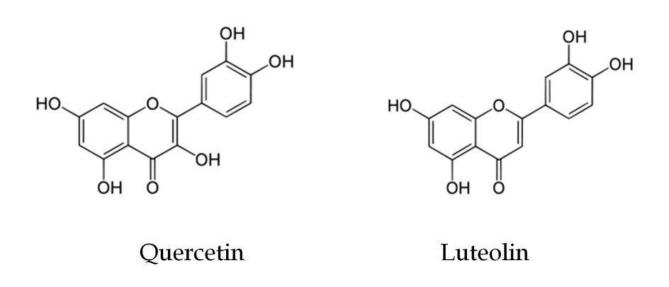


Figure 4: Few chemical constituents of F. jangomes (Sarma & Mahanta, 2020).

1.3.5 Pharmacological activities of F. jangomes

This is a very remarkable fruit tree, rich in both nutritional value and health benefits. Ripe fruits are packed with nutrients, including fiber, protein, low fat, and monounsaturated fatty acids in greater quantity than polyunsaturated fatty acids. Beta-carotene is the most abundant carotenoid in it, followed by lutein, zeaxanthin, retinol, and phylloquinone (vitamin K), all of which have a role in the control of hemoglobin and fibrinogen in the human body. Anti-inflammatory, antibacterial, anti-diarrheal, antiviral, antioxidant, and anti-amylase activity are only some of the pharmacological effects that have been studied for this plant and some of its active chemical ingredients (Kumar, 2015).

Table 4: Pharmacological activity of different part extracts of F.	Jangomes (Barbhuiya et al., 2020).

No	Plant extracts	Pharmacological activity
1	Chloroform- Root	Antibacterial
2	Methanolic- flower	Antioxidant
3	Methanolic- stem	Antidiabetic
4	Ethanolic- leaves	Antidiarrheal
5	Methanolic- bark	Antifungal
6	Methanolic- fruits	Analgesic
7	Ethanolic- fruits	Anti-amylase
8	Methanolic- flower	Cytotoxic

1.4 Rational of the study

Discovering new, potent therapeutic chemicals from natural sources like plants has become crucial for scientists and researchers in order to reduce the side effects of traditional medications and drug resistance. This study assesses the analgesic activity of fruit pulp extract from *F. jangomes* in Swiss albino mice, which can be used as a foundation for further research into this plant to create a more secure and efficient natural analgesic drug compound.

1.5 Aim of the study

This study aims to estimate analgesic activity of *Flacourtia jangomes* using different concentrate doses on Swiss Albino Mice against standard solution Diclofenac Sodium.

1.6 Objective of project

Main objective of this study is to evaluate therapeutic value mainly analgesic activity of Flacourtia jangomes flesh, validation of the claim of F. jangomes having analgesic activity and determining analgesic effect using different doses of flesh extracts on mice.

1.7 Literature review

Previously published studies on *F. jangomes* highlighted various therapeutic effectivity of this plant regarding traditional medicine. Those studies showed multiple therapeutic and pharmacological effects of *F. jangomes* such as analgesic, antioxidants, antibacterial, antiamylase and so on. For example, in vitro cytotoxic study and antibacterial screening indicates its potential for further research on anticancer drug investigation. Additionally, methanolic extract of fruit and leaf of *F. jangomes* showed promising hepatic-protective activity against paracetamol induced hepatotoxicity (*Hepatoprotective Activity of Flacourtia Jangomas* (*Lour.*) Raeusch Leaves and Fruit Methanolic Extract on Paracetamol-Induced Hepatotoxicity in HepG2 Cells, 2021). Methanolic extracts of *F. jangomes* fruit remarkably reduced blood glucose levels in OGTT (Rahmatullah, 2018).

Chapter 2

Methodology

Three main processes were involved in the extraction of *F. jangomes* fruit flesh- collecting the fruits, preparing sample and finally extraction.

2.1 Collection of F. jangomes fruit

After the selection of F. jangomes for pharmacological investigation, ripe fruits were collected from Barisal, Bangladesh in January 2022. Following this, *F. jangomes* was identified and authenticated by a taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession number: 87043).

2.2 Preparation of sample

Firstly, fruits were washed properly using tap water after collecting from vendor. Then fruits were rinsed using distilled water. After that, seeds of fruits were separated from the flesh using hand gloves. Only the flesh parts were used in our project. Following that flesh parts were poured inside a big glass jar with tightly sealed closure. Methanol was then added in the jar and soaked the flesh for 15 days. The jar with the sample was kept in a cool place to avoid heat and sunlight and after one hour the closure was opened and stirred occasionally to remove the gas babble which was generated inside the jar.

2.3 Extraction process

After soaking the flesh in methanol for 15 days, filtration is done to separate the methanolic extract of F. *jangomes* flesh using filter paper. After that the obtained filtrates were evaporated by rotary evaporator. This evaporation is conducted to get gummy concentrate

which will be needed to conduct the further experiments. Finally this crude methanol extract was transferred to a airtight container.

2.4 Drugs

"Diclofenac Sodium" was used in this experiment which was manufactured by Square Pharmaceuticals Ltd. Bangladesh.

2.5 Experimental animals

Swiss albino mice were procured from the animal facilities of Jahangirnagar University and the ICDDRB in Bangladesh and used for therapeutic research. The mice's body weight was calculated to be between 29 and 34 g. Food and drinks were provided in sufficient quantities to them to feed them. Additionally, a favourable environmental condition was maintained to the mice. They were maintained at a relative humidity of 55 to 65%, a 12-hour light/dark cycle, and temperature: 24 °C.

2.6 Ethical approval

Ethical approval was achieved from Jahangirnagar University, Department of Pharmacy and it was kept within the institutional animal ethics committee's guidelines.

2.7 Therapeutic investigation of flesh extracts

To determine the pharmacological activity of flesh extracts of *F. jangomes*, bellow mentioned investigation was done –

• Analgesic activity or anti-nociceptive activity

2.8 Analgesic activity of F. jangomes flesh extract

Two methods were used to estimate analgesic activity of Flacourtia jangomes flesh which is

- Acetic acid induced writhing method
- Tail immersion method

2.9 Analgesic experiment structure

At the beginning, healthy 30 mice were taken and their weight were measured carefully and recorded. Then they were divided into 5 groups each contain 6 mice. After that, all the mice were marked and were given their specific treatment. Using the body weight of mice, dose calculation was done for both control and test sample.

Group	Treatment	
Control (I)	Tween 80 solution	
Standard (II)	Diclofenac sodium 100 mg/kg	
Group III	MFJF 100 mg/kg	
Group IV	MFJF 200 mg/kg	
Group V	MFJF 300 mg/kg	

Table 5: Groups name and treatments.

2.10 List of equipment, reagents and other chemicals

Table	6: Name of equipment,	reagents and chemicals	s used in this experiment.
			· ····································

Equipment, reagents and chemicals	Sources	
Acetic Acid	Germany	
Diclofenac Sodium	Square pharmaceuticals Ltd	
Digital Palanaa	USA	
Digital Balance	USA	
Tween 80	Sigma Aldrich	
Saline solution	Opso saline	
Syringe (Sterile)	India	
Ball shaped end Tuberculin syringe	Germany	
Hot Water Bath	India	

2.11 Standard solution and other chemical solution preparation

Standard solution: To prepare the standard solution for experiment, required amount of Diclofenac sodium at a dose of 100mg/kg was taken and dissolved in 0.9% saline water. After that, solution was administered to the mice.

Extract solution: For preparing this solution at 100, 200 and 300 mg/ kg according to body weight of mice and mixed with tween 80 and saline water.

2.12 Process of acetic acid induced writhing test using *F. jangomes* flesh extract

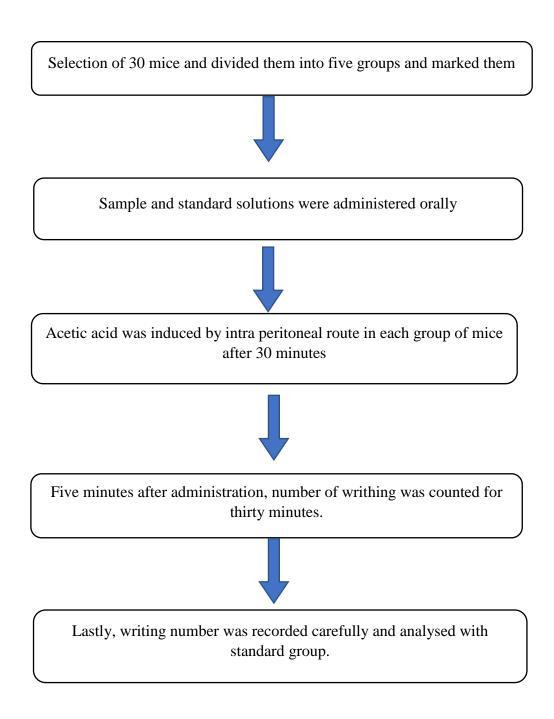


Figure 5: Acetic Acid induced writhing test procedure (Kumar, 2015).

2.13 Process of Tail immersion test using F. jangomes flesh extract

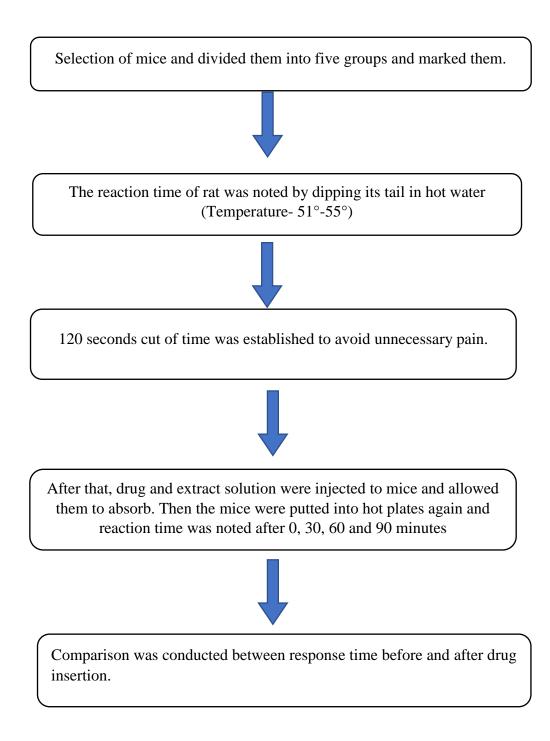


Figure 6: Process of Tail immersion method of mice (Kumar, 2015).

2.13.1 Recording average time of tail immersion of mice

During the experiment, average time of tail immersion of mice was determined using a stop watch and each time was recorded carefully.

2.14 Statistical analysis

All data values that collected from experiments are demonstrated as mean \pm standard error of the mean (SEM). One-way Analysis of Variance (ANOVA) followed by Dunnet's test were used to evaluate the statistically obtained data.

Chapter 3

Result

3.1 Analgesic activity of F. jangomes flesh extract on mice

Two selected methods were used to demonstrate analgesic activity of *F. jangomas* flesh extract – acetic acid induced writhing test and tail immersion method.

3.1.2 Acetic Acid induced writhing test

To determine analgesic activity of *F. Jangomes* flesh, 100 mg/kg, 200 mg/kg and 300 mg/kg doses were used in mice. Based on the experiment result, it was observed that among three doses 300mg/ kg dose of methanolic *F. jangomes* flesh extract shows some noticeable analgesic activity. In compare to standard Diclofenac Sodium solution, flesh extracts show less % of inhibition.

Group	Treatment	Number of writhing	% of Inhibition
Control (I)	Tween 80 solution	20.83±1.47	
Standard (II)	Diclofenac sodium 100 mg/kg	5.67±1.51**	72.78
III	MFJF 100 mg/kg	15.00±0.89*	27.99
IV	MFJF 200 mg/kg	12.67±0.82*	39.17
V	MFJF 300 mg/kg	8.33±0.52*	60.01

The values are demonstrated as mean \pm SEM (n = 6); One-way Analysis of Variance (ANOVA) followed by Dunnet's test. ** p < 0.01 and * p < 0.05 compared to control.

3.1.3 Tail immersion method

Based on the experiment results, methanolic extract of F. jangomes flesh 100mg/kg, 200 mg/kg and 300 mg/kg show some noticeable outcomes. Among these doses, 300 mg/kg shows bit more effective result. However, extracts solutions shows lower results than standard Diclofenac Sodium.

Table 8: Central analgesic effect of F. Jangomas flesh extract on mice by tail immersion method.

Group	Treatment	Average Time of Tail Immersion of Mice				
		Time (in Sec) after Loading the Plant extracts/Drug				
		Standard				
		0 min	30 min	60 min	90 min	
Control	Tween 80 solution	2.63±0.22	2.55±0.26	2.98±0.54	3.39±0.24	
(I)						
Standar	Diclofenac sodium	3.65±0.04*	7.41±0.23**	11.73±0.15**	19.25±0.33**	
d (II)	100 mg/kg					
III	MFJF 100 mg/kg	5.28±0.12*	4.44±0.10	6.20±0.08*	8.26±0.13*	
IV	MFJF 200 mg/kg	3.08±0.05*	5.30±0.17*	7.33±0.11*	9.28±0.12*	
- '		2.00_0.05	0.00_0.17	,	2.20_0.12	
V	MFJF 300 mg/kg	2.96±0.07*	5.83±0.32*	8.38±0.43*	11.19±0.74*	

The values are demonstrated as mean \pm SEM (n = 6); One-way Analysis of Variance

(ANOVA) followed by Dunnet's test. ** p < 0.01 and * p < 0.05 compared to control.

Chapter 4

Discussion

First of all, for this study Flucortia jangomes was chosen due to its renowned used as traditional medicine. To evaluate the anti-nociception activity of *F. jangomes* flesh, two different methods were used in Swiss Albino Mice. Methanol extract of *F. jangomes* was prepared at first stage of this experiment and three different doses 100mg/kg, 200mg/kg and 300 mg/kg were administered. In this experiment, Diclofenac sodium (NSAID) was used as standard drug. The experiment result proves pharmacological effectiveness of *F. jangomes* fruit flesh and provide some noticeable outcomes. Acetic acid induced writhing test and tail immersion test was conducted to investigate the analgesic activity.

Acetic acid induced Writhing test: This test was conducted to determine peripheral analgesic activity of *F. jangomes*. According to the test results (Table 8), methanolic extracts shows reduction of writhing in a dose dependent manner. Percent of inhibition at 100, 200 and 300 mg/kg dose was found 27.99%, 39.17% and 60.01% respectively. At a higher dose percent of inhibition was more effective. For standard drug Diclofenac Sodium percent of inhibition was found 72.78% which is higher than the extract solution. That indicates a lower activity of extract solution than standard solution.

Tail immersion method: This test was conducted to determine central analgesic activity of *F. jangomes* flesh extract. According to the test results (Table 9), basal reaction between several interval 0, 30, 60 and 90 minutes. There was an observable increase in reaction time with increased dose. Extract solution 100, 200 and 300 mg/kg and Diclofenac sodium 100 mg/kg was used in this method and after administering to mice reaction time in different intervals were observed. However, the reaction time of standard solution was higher than the extract solution.

Chapter 5

Conclusion

Central and peripheral analgesic effects of F. jangomes meat extracts on mice were confirmed in this investigation. Much interest has recently been focused on the plant's bioactive chemical constituents, which have been isolated and characterized so far. These constituents engage in a wide range of pharmacological activities, including antibacterial, antifungal, analgesic, antidiarrheal, anti-oxidant, and cytotoxic activities (Bio et al., 2019).

In conclusion, better, safer, and more cost-effective medications cannot be produced without substantial study into the isolation and characterisation of the active principles responsible for the activity, as well as an understanding of the specific mechanism of the therapeutic action.

Future perspective: The use of traditional analgesics for extended periods of time can lead to undesirable adverse effects such as gastrointestinal irritation, renal damage, asthma, cardiac irregularities, etc. Therefore, efficient medicines with fewer side effects are still needed despite the progress in pain management therapy. According to the results of the pharmacological studies, the plant extract and some of its bio-functional elements can be synthesized into products that will be beneficial to society as it explores new areas of complementary and alternative medicine (Mishra & Rai, 2020).

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