A COMPREHENSIVE STUDY OF EXTRACTED PECTIN FROM FICUS CARICA FRUIT

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Abstract: This research study aims to evaluate the isolation of Ficus carica pectin extraction, characterization and acute toxic studies. Pharmacognostic studies have been investigated on the ripened Ficus carica fruits using several micro-chemical tests. The fruits were washed, dried and extracted with 95% alcohol. The extract was detected for the identification of phytoconstituent pectin. The evaluation studies were conducted as an important prerequisite step for the determination of the physicochemical properties of the pectin. OECD guidelines are followed for acute toxic studies. The isolated pectin was light brown in colour, rough and odourless with characteristic rough fractures. The pectin was confirmed to have pH as slightly neutral, good flow properties, low moisture content, degree of esterification range was in between 55.6% -65.7 % ; the swelling index was found to be 450. The isolated pectin was characterized using NMR, XRD, FTIR and DSC tests which proved the chemical nature of the pectin is in pure form. No significant changes were observed in haematological parameters, behavioural symptoms, signs of mortality and histopathology studies, during acute toxic studies. The evaluation studies, parameters and results revealed that the extract from the Ficus carica fruit is a novel approach to get high yields of isolated pectin. It can be concluding that Ficus carica fruit pectin can be used as natural disintegrant in oral solid dosage form to prepare ODTs.

Keywords: Ficus carica, Fig, Natural Disintegrant, Pectin Extraction, Acute Toxicity,

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INTRODUCTION

Ficus carica fruit commonly named as “fig fruit” belongs to the family Moraceae. The fresh figs are eaten, dried fruits are in vitro antioxidants for human consumption. The fruits are widely used in ancient medicine through its multiple benefits such as antipyretic tonic purgative, diuretic, astringent, carminative, laxative, stimulant against throat diseases, emmenagogue, emollient, antitussive, and resolvent. It is also used against inflammation, weakness, paralysis, nosebleeds; it also stimulates hair growth [1-3].
Ficus carica polysaccharides are of natural origin and preferred over semi-synthetic and synthetic substances because pectin isolation from Ficus carica is a preferable process as it is cheaper, vastly available, non-irritating, and non-toxic. It is an excellent source of pectin and polysaccharides which act as disintegrants [4-5]. The scientific literature on the Ficus carica proves that the plant is rich in flavonoids, terpenes, alkaloids, polysaccharides, mucilage, and glycosides [6] The most commonly used adjuvant in the different pharmaceutical dosage forms is mucilage and pectin which possess pharmaceutical properties like disintegrating, binding, emulsifying, sustaining, and suspending properties at various concentrations. Pectin has good solubility, a higher swelling index, and it may be used in fast dispersible formulations [7-8]. Not many research studies are found on the use of isolated pectin and polysaccharides from Ficus carica fruit as disintegrants. Hence this research work is designed to characterize toxicity studies, isolate and evaluate the Ficus carica pectin for further studies as a tablet disintegrating agent.

MATERIALS AND METHODS

Ficus carica fruit were obtained from the local fruit market, Hyderabad, Telangana. Chemicals and reagents were procured from Vijaya Chemicals, Hyderabad, Telangana. Analytical grade chemicals and reagents were used in this research work.

Phytochemical Extraction & Pectin Yield Determination

Dried Fig fruit powder about 20 gm was taken in a conical flask, treated distilled water of 250ml distilled water, and heated up to 30 min on a water bath. The extract was filtered with muslin cloth of eight folded. Pectin was precipitated from the filtrate with 95% of alcohol (1:2 v/v) to allow precipitation of pectin which was recovered by refrigerated centrifuge (Lab India) at 1000 rpm for 20 min [9]. The obtained precipitate was kept in a dark room at 25°C of temperature for a day and washed with 70% alcohol. To remove pectin colour added acetone drop-wise and dried in the oven (SISCO instruments) at 65°C - 70°C to get a constant weight. Ficus carica fruit Pectin yield percentage is determined by using below formula - I.

$$\text{The percentage yield of Pectin} = \frac{\text{weight of pectin powder obtained}}{\text{20 gm of fruit powder}} \quad \text{---------- (I)}$$

Determination of pH and Swelling Index

Distilled water about 50 ml is added to one gram of weighed pectin and sonicated for 5 min. Distilled water was topped

upto 100 ml to the mixture and determined the pH by using a digital pH meter (LabIndia).

Swelling index of fig pectin was calculated and weighed on a butter paper size 2X2 cm. The butter paper was kept into a Petri plate having 10 ml of water. 0.1 g of powdered pectin sample was taken and kept on a butter paper placed in a petri dish. After 3 hrs swelling index was calculated by the formula –II [10 -13].

$$\text{Swelling Index} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} X100 \quad \text{----------- (II)}$$

Degree of Esterification

Titrimetric method is used for determination of DE. 0.2 gm of isolated pectin was dried, a drop of alcohol is added and 20 ml of distilled water is used for dissolution. After shaking, the pectin was kept on water bath at 45°C till dissolution was completed. The sample was titrated with 0.1N sodium hydroxide and 0.1 ml of phenolphthalein solution is added. Initial titrant is in pink colour. To neutralize polygalacturonic acid, 10 ml of 0.1 NaOH was added by stirring and kept it for 2 hr at room temperature to de-esterification of pectin [14]. To neutralize the mixture of sodium hydroxide added 10 ml of 01.N HCL and stirred the sample to the endpoint till the disappearance of pink colour. For pectin, 0.1 ml of phenolphthalein is added and titrated with 0.1 NaOH on appearance of pink colour recorded the end point of titration volume. Calculated the DE was in the given formula -III:

$$\text{DE \%} = \frac{\text{Final titration volume ml} * 100\%}{\text{Initial titration volume ml} + \text{Final titration volume ml}} \quad \text{---------- (III)}$$

Evaluation of Moisture Content

About 2 gm is weighed was taken in a porcelain crucibles and kept in a hot oven at a temperature of 105°C temperature for 5 hr and kept in a desiccator for 30 min. Weights of crucibles and pectin were recorded [15].

Pectin Flow Properties Determination

20 gm of powdered pectin was weighed and taken through a funnel and kept in a measuring cylinder, noted the initial volume (V_o). Measuring cylinder is tapped for 100 times on the bench and noted the constant volume (V_f). The Hausner ratio and Carr's index were calculated. Angle of repose is calculated with fixed height method.

Solubility Determination

The pectin powder solubility properties were determined in hot and cold water at 25°C, along with the solvents like petroleum, ether, acetone, methanol, and ethanol. Added 1 gm of sample to above solvents each 50 ml, kept for an overnight. 25 ml of supernatant was added to Petri dishes (pre-weighed) and kept for dryness on a water bath for evaporation. Dried sample are weighed to determine the constant weight of the pectin [17].

Physical Constants Determination

Ethanol -soluble extractive, water -soluble extractive, ash value, water-soluble ash, and acid insoluble ash are determined as per the standard procedure for fig fruit extract to understand the presence of phytoconstituents [18].

Screening of Phytochemical Tests

Phytochemical screening of ethanolic extract was carried out as per the standard procedure. Chemical tests have been conducted for the identification of various phytoconstituents [19].

Molisch's Test

A solution of the fig extract was treated with alcoholic solution of alpha-naphthol and H_2SO_4 acid is added to the side of the test tube.

Fehling's Test

A few drops of the Fehling's reagent is added to a solution of the fig extract.

Benedict's Test

1 ml of Pectin extract is added and heated with 5 ml of Benedict's reagent.

Lead Acetate Test

About 2 ml of each ethanolic extract was added to 10% lead acetate solution for indicative of flavaonoids.

Biuret Test

1 ml of 40% sodium hydroxide solution is added to 2 drops of 1% $CuSO_4$ solution to form blue colour and add 1 ml of the Ficus carica extract for confirmation of proteins.

Specific Chemical Test for Confirmation of Pectin

A 10% (w/v) sample solution on cooling gives a solid gel. A semi gel of the pectin has resulted in interaction with 1 % solution of 5 ml pectin and 2 % solution of 1 ml of KOH, keep the sample mixture at room temperature for 15 min.

Fourier Transform-Infrared Spectroscopy (FTIR)

Infrared spectrum of drug is recorded with potassium bromide dispersion technique using FTIR on the Bruker FTIR instrument. 1 mg pectin in 100 mg potassium bromide is placed on a disc and kept in the sample beam and recorded the spectra from 4000 cm-1 to 400 cm-1 using Bruker FTIR spectrophotometer [20-21].

Differential Scanning Calorimetry (DSC)

It measures the absorbed or released amount of heat and energy when a sample is heated, cooled kept at constant temperature. This is valuable in identification of compounds [22]. The DSC studies of the pure drug were carried out using the DSC instrument Mettler Toledo DSC 822e.

X-ray Diffraction Studies (XRD)

The powder X-ray diffractometer is one of the most powerful and established techniques for material structural analysis. It provides the information about the crystalline material structure at the atomic level [22]. XRD studies are carried out with the Advanced Bruker AXS D8 diffractometer.

NMR Studies

The sample dissolved in a liquid is placed in a magnetic field instrument. The radio frequency waves are forwarded through sample pectin solution by excitation of the nuclei sample solution. The spectrum shows chemical shifts, from these the compound structure can be determined [23].

A simplified description of NMR is that the sample, dissolved in a liquid, is placed into the instrument, which contains a magnetic field. A radio frequency pulse is then sent through the sample solution in order to orient the magnetic moments of the nuclei in the solution. As the magnetic moments relax, they exhibit free induction decay. The free induction decay is Fourier transformed into an NMR spectrum. The NMR spectrum displays chemical shifts for the individual nuclei; and from these chemical shifts, the structure of the compound can be determined.

Zeta Potential

Malvern DLS analyzer is used to measure the Zeta Potential of pectin [23].

SEM Studies

Bruker SEM analyzer is used to analyze the size of the particle; shape and morphology of the isolated pectin Magnified images are created in this technique by using light waves. It produces very high-resolution images of pectin sample surface. SEM analysis was carried out to analyze the crystalline or amorphous nature of the isolated pectin component from Ficus carica fruit [24].

Acute Oral Toxicity Studies

As per the OECD guideline 423 the acute oral toxicity studies were conducted on the extracted Ficus carica pectin. The Wister rats were procured vide IAEC protocol No: I/IAEC/AGI/004/2020WR and were housed in animal house. Defined doses, of (5, 50, 500, 1000, 2000, 5000 mg/kg body weight). Acute toxicity results of substances are classified and ranked as per the Globally Harmonized System (GHS) [25].

Three male Wister rats weighing between 150-180 gm were taken to study. Relatively herbal extracts are non toxic. Dose levels of alcoholic extracts of Ficus carica fruit selected as (5, 50, 500, 1000, 2000, and 5000 mg/ kg/ b.w p.o). The extracts were orally administered to rats. Before administration of the drug the rats were fasted over night with water ad libitum. Rat body weights are noted before and after the treatment. Somatomotor activity, central nervous system, respiratory and circulatory activities, behavioural pattern and any changes are noted pertaining to skin & fur, eyes & mucous membranes. Noted the observations if any, on convulsions, diarrhoea, lethargy, sleep, salivation or any tremor signs. Observations are noted if any, onset of toxicity & toxicity signs.

Repeated Oral Toxicity Studies

Oral toxicity studies are repeated to obtain additional data pertaining to a chemical or drug toxicity profile. These studies are carried for a period of 28 days, where the rats are administered with pectin. The chemical dosage level is lower than acute toxicity to accumulate in the body.

Experimental Procedure

Repeated oral toxic studies on pectin in rats are evaluated as per the following procedure:

Group I: Administered 1% tween 80 (10 ml/ kg/p.o) to control rats for 28 days

Group II: Alcoholic extract of Ficus caricafruit at dosage of 1000 mg/kg/ p.o for 28 days.

Observations, if any, were recorded on toxic manifestation, mortality, body weight, intake of food & water at two intervals. After treatment of 28 days, rats are administered with excess dose of anaesthesia for sacrifice. Haematological tests are carried out on blood collection. Heart, kidneys, liver, lungs, and pancreas were dissected and kept in 10% formalin solution for histopathelogical studies [26].

Haematological Parameters

Red Blood Cell, White Blood Cell count and Haemoglobin percentage and differential counts in the blood are studied.

Pathological Studies

Small pieces of brain, heart, kidney, pancreas, lung, liver were collected and kept in 10% formalin solution for preparation of sections by using microtome. A standard method was used to carry out the histopathological studies [27].

RESULTS AND DISCUSSION

Pectin Yield and Qualitative Identification Tests

The extraction using alcohol was performed by a simple method which resulted in a 16% extractive value. The qualitative screening was conducted using phytochemical tests which could prove the presence of flavonoids, triterpenoids, sterols, tannins, carbohydrates and proteins, as shown in (**Table 1**). The isolated pectin was confirmed by performing the general chemical test for carbohydrates which was found to be positive as shown in (**Figure 1(A)).** The pectin yield was found to be 16% as the weight of the pectin powder obtained was found to be 3.2 g. The isolated pectin is in light brown colour, odourless characteristics with irregular and rough fracture (**Figure 1(B)).**

Figure 1(A) Isolated pectin

Figure 1 (B) Dried pectin of Ficus carica fruit

Table 1. Qualitative Phytochemical analysis of Ficus carica fruit ethanolic extract

Phytochemical tests for detection	Result
Sterol's and Triterpenoids	+ (Positive)
Phenolic Compound	+ (Positive)
Tannin's	- (Negative)
Flavonoid's	+ (Positive)
Proteins & Amino Acid	+ (Positive)
Carbohydrates	+ (Positive)
Free Sugars	+ (Positive)

Molisch's test confirms the presence of carbohydrates on formation of violent color ring. Fehling's test indicates

reducing sugars on formation of red color. Benedict's test confirms an orange or brick red color presence of reducing sugars. Lead acetate test confirms and indicate the presence on formation of violet color. Biuret test confirms the presence of proteins on formation of pale purple color.

Determination of pH and Swelling index

The pH of the sample pectin obtained was found to be 6.0. The swelling index of the pectin was found to be 450 as measured by the formula indicating the physicochemical nature.

Degree of Esterification

DE is found to be in the range of 55.6 - 65.3 % and indicating the free –OH groups

Determination of Moisture Content, Solubility, and Flow Properties

The moisture content of pectin, pH, and flow properties are vital in predicting pectin characteristics. Isolated pectin was completely soluble in hot and cold water along with methanol and ethanol. The pectin was sparingly soluble in petroleum ether. The moisture content was found to be 10%, the Hausner ratio is 1.18, Carr's index is 18.27 and Angle of Repose is 27.5°. The pectin was found to be free-flowing properties and had dispersed particles. All the physico chemical characteristics of Ficus carica pectin fruits are tabled in (T**able 2**).

Table 2. Physico Chemical Characteristics of Pectin Powder of Ficus carica Fruits

Physico Chemical Characteristics	Result
Yield	16%
pH	6.0
Swelling Index	450
Degree of Esterification (DE)	55.6 -65.3 %
Moisture Content	10%
Hausner Ratio	1.18
Carr's Index	18.27
Angle of Repose	27.5°

FTIR Analysis

The isolated pectin spectrum was recorded on the Bruker instrument by the potassium bromide pellet method to identify the pectin shown in (**Figure 2**). There are two peaks in the region of 3412cm-1 and 2924cm-1 respectively, stretching vibrations of molecular groups O-H and C-H (CH, CH2, CH3) in pectin. The peaks are 1749cm-1 and 1624cm-1 were related to absorptions of esterifies carboxyl (-COOR), ionized carboxyl (-COO-), respectively. The 916.88cm-1 curve corresponded to the characteristic band for carbohydrates.

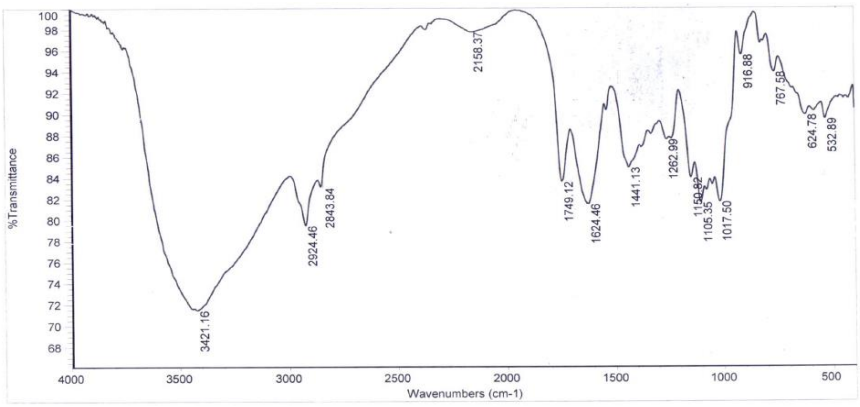

Figure 2. FTIR studies of isolated pectin.

DSC Analysis: At 235.90oC the exothermic peaks (degradation temperature) indicated the stability of pectin and endothermic peaks (melting temperature) are not indicated in (**Figure 3**).

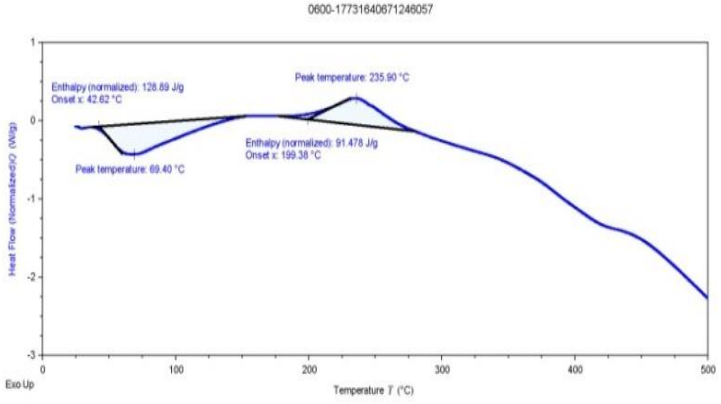

Figure 3. DSC Studies of Isolated Pectin.

XRD Analysis

The XRD analysis was carried out for the isolated pectin to understand the crystallinity. The Braggs peaks were observed at 2θ angles of 19.20, 21.10, 23.60, and 28.30, respectively as shown in **(Figure 4)**. The amorphous nature of the pectin was determined according to the XRD analysis by JCPDS standards (JCPDS 15-03589).

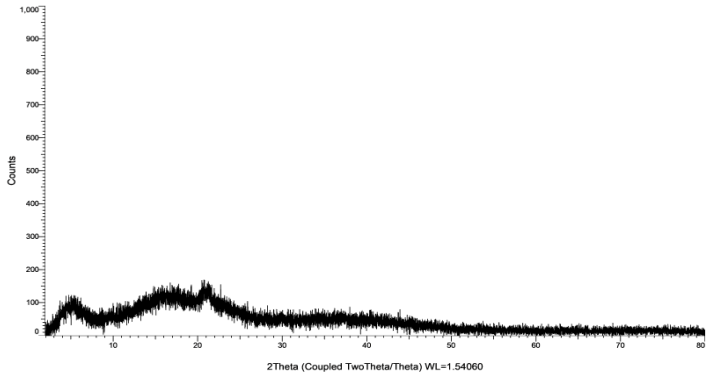

Figure 4. XRD Studies of Isolated Pectin.

NMR Analysis

The NMR analysis was carried out and the peaks in the H1 NMR spectrum reveals the chemical nature of the pectin isolated. The characteristic peaks at certain chemical Acetyl shift values of 5.1 ppm (COOH group), 4.16 ppm (GalaOCH3), 3.99 ppm (H3proton), 2.5 ppm (O-Acetyl), 1.89,1.65 ppm (Rha-CH3) groups indicate the pectin (**Figure 5**).

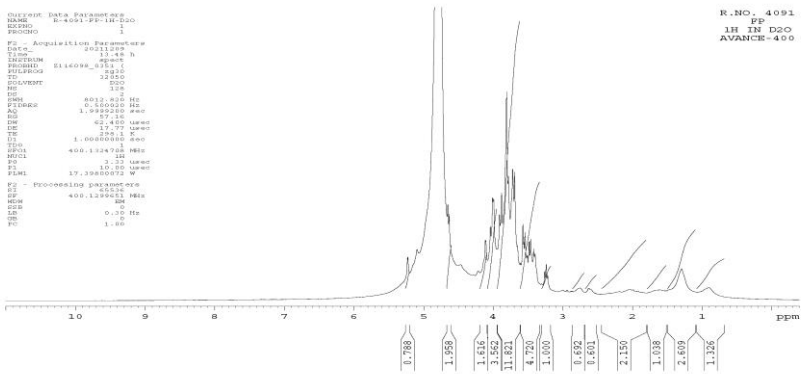

Figure 5. H1 NMR Spectrum of Pectin

Zeta Potential

Pectin Zeta potential was found tobe -23.5mV. According to the literature, the particles are found to be stable within the range of -30mV to +30mV. The zeta potential was found to be within the indicated range the pectin was identified as negatively charged (**Figure 6**).

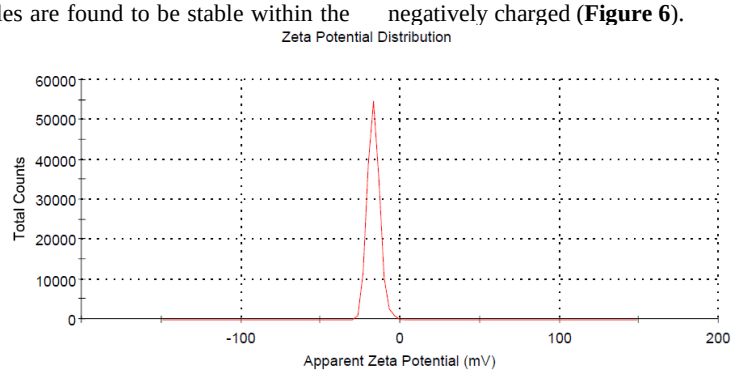

Figure 6. Zeta Potential of Pectin.

SEM Analysis

It has been observed that the pectin particles are in irregular shape with smooth and spherical ends (**Figure 7**).

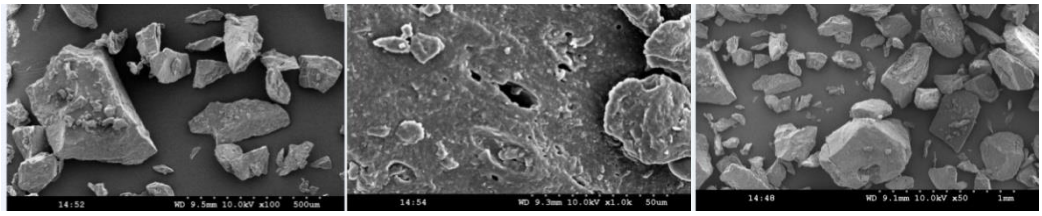

Figure 7. SEM Analysis of Pectin.

Physical Evaluation of the Extract

Physical evaluation of the Ficus carica fruit results are shown in (**Table 3**)

Table 3. Determination of Physical Constants

Physical Constants	Values
Total ash	5.42±0.02
Acid insoluble ash	2.35±0.011
Water-soluble ash	1.35±0.03
Alcohol-soluble extractive	8.95±0.021
Water-soluble extractive	9.53±0.031

Acute Oral Toxicity Studies

Body weights were observed before and after treatment of the experiment. Experiments were repeated again with the same dose levels found to be no signs of toxicity. It was observed for 14 days and found no significant changes. 5000 mg/kg dose is identified as LD50 cut-off. The acute toxicity study result is given in (**Table 4**).

Table 4. Observation of weight and signs of toxicity after treatment of Ficus carica fruit alcoholic extract to rats for 14 days (n=3)

Drug Administered	Dosage	Body weights of rats in gm Treatment		Toxicity Sign	Effect observed	Death
		Before (1st day)	After (14th day)			
Alcoholic Extract of Ficus carica fruit	5 g/kg	171	180	No sign	No effect	Nil
	50 g/kg	158	183	No sign	No effect	Nil
	500 g/kg	168	190	No sign	No effect	Nil
	1000 g/kg	174	180	No sign	No effect	Nil
	2000 g/kg	176	188	No sign	No effect	Nil
	5000 g/kg	179	196	No sign	No effect	Nil

Repeated Oral Toxicity Studies

Alcoholic Ficus carica fruit extract with dosage of 1000 mg/kg/b.w/ p.o. was administered to rats for 28 days. There are no changes in intake of water and food no loss in body weights during the entire experiment. The alcoholic extract of rats treated with Ficus carica fruit has not resulted, any considerable changes/values in haematological parameters (HB, RBC, WBC, neutrophils, monocytes, esinophils and lymphocyte) as shown in (**Table 5**).

Table 5. Observation of Haematological Parameters in rats for 28 days after the treatment of alcoholic extract of Ficus carica fruit

Group	Drug treatment	HB	RBC	WBC	Differential Count			
					Neutrophils	Lymphocytes	Monocytes	Esinophyls
1	Control	13.42 ± 0.1462ns	5.894 ±0.2456ns	8654 ±220.5ns	51.23 ±1.032ns	43.23 ±0.632ns	2.892 ±0.4321ns	2.648 ±0.5234ns
2	Alcoholic extract of Ficus carica fruit (1000 mg / kg)	12.86 ±0.03215ns	6.023 ±0.3268ns	8832 ±224.4ns	52.68±1.246ns	41.83 ±1.236ns	2.489 ±0.5832ns	3.001 ±0.8243ns

* Values are expressed in mean ± SEM (n=3)

Histopathological Examinations

The organs (like heart, liver, brain, kidneys, pancreas and lungs) of rat were studied and found that no considerable damages are caused by alcoholic extract of Ficus carica fruit pectin and at the same time did not show any significant changes in their normal architecture (**Table 6**) and detailed figures of rat internal organs are shown in (**Figure 8**).

Table 6: Histopathological report of the organs of rats treated with alcoholic extract of Ficus carica fruit for 28 days

S.No	Organ	Animals	
		Control	Alcoholic extract of Ficus carica fruit (1000 mg/kg)
1	Brain	Normal brain tissues	Normal brain tissues
2	Heart	Normal cardiac tissues	Normal cardiac tissues
3	Kidneys	kidneys glomeruli & tubules- normal	kidneys glomeruli & tubules - normal
4	Liver	liver tissues are in normal	liver tissues or in normal
5	Lungs	lung tissues with bronchi & alveoli - normal	lung tissues with bronchi & alveoli -normal

6	Pancreas	pancreatic tissues - normal	pancreatic tissues - normal

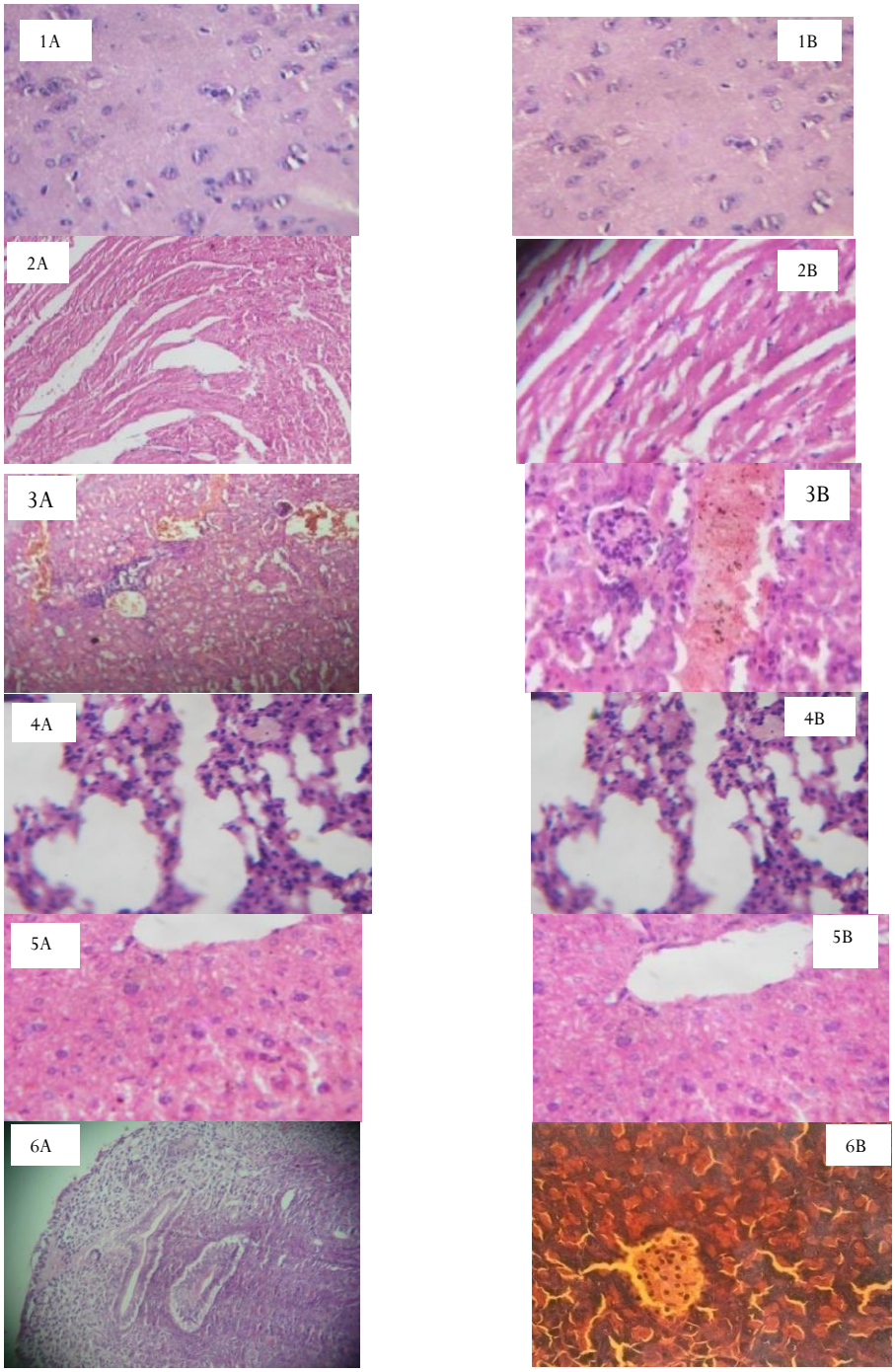

Figure 8: Histopathology of rat internal organs treated with Alcoholic extract of Ficus carica fruit for 28 Days

1A Brain – Control, 1B- Brain Test, 2A- Heart Control, 2B Heart Test, 3A- Kidney control, 3B- Kidney test, 4A-liver Control, 4B- Liver test, 5A- Lungs control, 5B- Lungs test, 6A- Pancreas control, 6B- Pancreas test.

CONCLUSION

This research work reveals that Ficus carica pectin has good solubility, flow properties, low moisture content, accurate neutral pH, and higher swelling index; it may be used in the oro-dispersible formulations. The evaluation studies involving description, solubility, melting point of pectin were found to be within the range. Pectin obtained was light brown in color with a rough texture and was found to be more soluble in warm water than in methanol and ethanol. Further, the chemical tests confirmed the pectin's chemical nature. The pH was found to be slightly neutral and the degree of esterification was found to be between 55.6%-65.7 %. The swelling index was found to be 450. The studies of pectin isolated from Ficus carica with its characterization by FTIR, XRD, and NMR gave confirmation about their purity. DSC measures the exothermic and endothermic responses of the samples, while FTIR in observing the structural changes in physical

and chemical properties of pectin. No significant changes were observed in haematological parameters, behavioural symptoms, signs of mortality and histopathology examination during acute toxic studies. The flow properties, pH, moisture content, and swelling index clearly define the Ficus carica pectin is an ideal super disintegrant. Hence the further studies can be taken up for the preparation of oral disintegration tablets or fast-dissolving tablets.

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Authors Contributions - **All the authors have contributed equally**.

Conflicts of Interest - **The authors report no conflicts of interest in this work**

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