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EXTRACTION AND APPLICATION OF PAPAIN ENZYME ON DEGRADATION OF DRUG

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ABSTRACT

Extraction of papain enzyme from unripe papaya gives around 80–90% activity. Lumps Formations and Absorptiometry methods were studied to know the action of papain enzyme. The enzyme was extracted and shows degradation capability on Levetiracetam and Granisetron HCl drug compounds which has harmful effects on cellular systems. The efficacies of the enzymatic activities were checked when drugs were analyzed by TLC, spectrophotometer before and after the treated with papain enzyme.

KEYWORDS

Back solvent extraction, papain, degradation.

1. INTRODUCTION:

The papaya, papaw, or pawpaw is the fruit of the plant *Carica papaya* (Caricaceae). It is native to the America [1]. Papain and chymopapain are the two main enzymes of papaya. Papain's enzymatic use was first investigated by G.C. Roy at 1873.Papain name was first given by Wurtz and Bouchut in the late 19th century who accomplishes to partially purify the product from the sap of papaya. Papain is cysteine protease, also known as papaya proteinase I, from the peptidase C1 family and may be extracted from the plant's latex, fruit, leaves and roots. Papain is simple enzyme which contains 212 amino acid residue chains. Papain can be fold in two different size of domains, each having hydrophobic core [2]. In hydrophobic core, substrate binding pocket situated between two different sizes of domain. Active sites of papain are Cys-25, His-159 and Arg-175.The present study is about the papain activity towards the degradation of Levetiracetam [3-6] and Granisetron HCl [7-9] drugs which are showing toxic effect on human cellular system. We used surfactant (TOMAC) method to extract papain from unripe papaya. This study founds on the degradation property of papain towards the drug molecule. So that we could say that Papain is able to degraded drug molecule.

2. MATERIALS AND METHODS:

2.1 Material

Sodium bis (2 ethylhexyl) sulfosuccinate (AOT) and tri-n-octylmethyl-ammonium chloride (TOMAC) are purchase from Sigma-Aldrich. Spectrophotometer - JASCO V 5.0.

2.2 Extraction Methods

Remove the peel and weight 300 g of papaya and grind it then transfer into in a clean glass 250 ml beaker and add 120 ml of distilled water. Mix well on a stirring hot plate, raise the temperature to 75°C, and then cool liquid to 30 °C. Add in 1.2 grams of sodium bis (2-ethylhexyl) sulfosuccinate (AOT). Stir well again for 15 minutes. Check pH and adjust with if acidic with dilute ammonia and if basic with 10% acetic acid and maintain to 6.3 pH. Now, for back extraction. Add 1.09 grams of counter-ionic surfactant tri-n-octylmethyl-ammonium

chloride (TOMAC). Stir again 15 minutes then "Back extract" the final liquid with 10% ethanol and fine filter paper or use vacuum filter. [10] Activity of papain was assayed by Krishnaiah method [11]

2.3 General Procedures for Degradation

We took 0.015 g of drug in stopped test tube & Then Added 5 ml extracted solution of papain enzyme & put in ice bath to maintain temperature to 25-30 ^{o}c shaked well properly and note observation.

2.4 Analysis of drug molecule

The proteolytic activity of papain towards drug molecule was carried out by two methods

2.4.1Thin layer chromatography (TLC)

In TLC method, we measured Rf value of drug by using solvent system which is mention in **Table: A**. Then we added 5 ml extracted solution of papain enzyme. Then by TLC it is conformed the degradation of drug molecule takes place.

Table A: RF value of drug before and after treated with enzyme

Drugs	Rf value of drug on TLC (before treatment)	Rf value of mixture on TLC (after treatment)	M.W.	Solvent system Methanol : $CHCl_3$
Levetiracetam	0.70	0.89	170.2	5:5
Granisetron HCl	0.83	0.76	312.4	5:10

2.4.2 Spectrophotometer: We measured the wavelength of drug molecule with mention concentration in below table: B. Then the drug treated with 5 ml of extracted solution of papain enzyme, degradation of drug take place with the same reaction time which is mention as above. We again measured wavelength of mixture. Wavelength between drug and mixture (drug + enzyme) is change which is shows that degradation of drug molecule is done by papain enzyme. Result is mention in **Table: B**

Table B: wavelength of drug before and after treated with enzyme

Name of drug	wavelength of drugs (nm)	Wavelength of enzyme (nm)	Wavelength of mixture (nm)	Concentration of drug
Levetiracetam	228	299	304	0.0242 g/ 5 ml
Granisetron HCl	300	299	303	0.0150 g/ 5 ml

3.0 RESULT AND DISCUSSION

We have observed variation in Rf value of drug and mixture and also observed the change in λ max of the same. Result is mention in table: A and B.This shows the degradation of drug molecules has been take place. According to mechanism of papain enzyme, the mechanism by which it breaks peptide bonds involves deprotonation of Cys-25 by His-159.The sulfhydryl group on Cys-25 frequently forms covalent bonds with substrates. His-159 supports Cys-25, and while Arg-175 keeps histidine-159 in its stabilized imidazole form. Both histidine-159 and cysteine-25 take part in the actual catalytic mechanism. Cys-25 acts as a nucleophil and it attack on the carbonyl carbon of a peptide backbone. This frees the amino terminal of the peptide, and forms a covalent

acyl-enzyme intermediate. The enzyme is then deacylated by a water molecule, and releases the carboxy terminal portion of the peptide [12] it may be combined in papain enzyme that's why toxic effect of drug molecule may be reduced on living organ system.

CONCLUSION

From the present study, we have found that the drugs can be degraded by papain enzyme. So, we concluded the toxicity or adverse effect of drugs can be reduced with the help of enzyme.

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