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RESEARCH ARTICLE
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ALTERATIONS IN SERUM ELECTROLYTES FOLLOWING ACUTE AND CHRONIC DOSING OF SOME PARTS OF PAPAYA TREE UNLASHED THEIR POTENTIAL TOXIC EFFECTS. A THOUGHT PROVOKING FINDING FROM NUTRITIONAL AND HEALTH PERSPECTIVE.

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Abstract
Consumption and utilization of plant based products as medicine for treating unhealthy conditions is increasing all over the world. There is a general belief that these plant based products are safe to be consumed, are economical, easy available and have no adverse effects. Awareness about the toxic and adverse effect potential of these plant based products and reporting of these adverse effects is very uncommon. Papaya plant is very famous all over the world due to its unique nutritional and therapeutic composition of each part. A large portion of population consume different parts of papaya for different purposes for e.g. Ripe fruit is consumed to relieve constipation, seeds are used as spice in cooking, unripe fruit is used as meat tenderizer, leaves are used in fever conditions etc. This study aimed to investigate the effects of different papaya parts i.e. ripe fruit, seeds, unripe fruit and leaves on serum electrolytes. The findings of this study revealed that each part has the tendency to raise serum potassium, serum calcium and serum magnesium both after acute and chronic dosing which suggests that daily consumption of any of these part can lead to certain consequences due to fluctuations in serum electrolyte concentrations.

Keywords: - Hyperkalemia, Hypercalcemia, Hypermagnesemia, Hypernatremia, Carica Papaya, Serum Electrolytes

INTRODUCTION

History of consuming plant and plant derived preparations in treating various pathological conditions is as old as the history of mankind[1]. These herbal preparations are considered pure, safe and healthy since they are of natural origin[2]. According to an estimate, 80% of world's population consumes plant or plant based products for treating their disease states[3]. Almost 50% of medicines used globally are

of natural origin[4]. In nature, majority of the plants possess substantial pharmacological potential[5]. Despite of tremendous scientific and medical advancement, still a gigantic investigation on plants is going on to explore new therapeutic options[6].

Carica papaya Linn. is a tropical plant belongs to the family of Caricaceae. Due to its unique health promoting benefits and nutraceutical potential, this plant has been a centre of attraction for many researchers and scientists. Various parts of this herbaceous plant such as fruits, leaves, roots, peel, seeds have different distinctive therapeutic significance. Fruit of papaya has been used traditionally in making of jams, jellies, pickles etc. Seeds of papaya has anti-tumor activity whereas leaves of papaya facilitates bowel movements[7].

The increasing popularity and consumption of herbal remedies all over the world has also brought many clinical challenges. The OTC nature of these herbal remedies and their ease of availability largely facilitate the population upon its utilization in different disease states along with conventional drugs and supplements which not only raise chances of adverse effects but herb-drug and herb-supplement interactions[8]. Due to wide consumption of different parts of papaya plant for different therapeutic purposes, this study is planned to observe and investigate the effect of short-term and long-term dosing of different edible (ripe and unripe fruit) and non-edible (seeds and leaves) parts of papaya on serum electrolytes in rabbits.

MATERIALS AND METHODS

Research Animals

Albino rabbits of male gender were selected for this study. 40 rabbits weighing between 2.4 to 2.7 kg were separated out from the breeding area of animal house of Pharmacology Department of University of Karachi. The selected animals were given free access of food and water and were kept under 12 hours light and 12 hours dark cycle with controlled temperature (23 ± 3ºC) and humidity (61%). The guidelines mentioned by NRC (National Research Council) were strictly adopted for animal care and handling[9]. Proper animal ethical approval was taken before initiating the study from ASRB (Advanced Study and Research Board) (ETHICAL APPROVAL: [BASR/No./ 02145/Pharm]).

Identification of plant

All the study parts of papaya i.e. fruit (ripe & unripe), leaves and seeds were identified by Herbalist/Pharmacognocist Prof. Dr. Iqbal Azhar (Professor & Chairman Department of Pharmacognosy & Ex-Dean, Faculty of Pharmacy & Pharmaceutical Sciences)

Extract Preparation

Aqueous extract of study parts were prepared according to the method mentioned in Table I.

TABLE I

S.NO	STUDY PART	PROCEDURE
1.	Ripe Fruit (AEPF)	Ripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. All seeds were removed. AEPF was prepared in 100mg/ml concentration for which 6 grams of ripe fruit was homogenized and blended with 60 millilitres of purified water using local mixer & grinder. The extract prepared was then stored in a glass bottle and denoted by "AEPF"[10].
2.	Papaya Seeds (AEPS	Ripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. Seeds were separated and washed with purified water and were dried at room temperature. The seeds were then manually crushed using domestic mortar & pestle. This mixture (6 gram)

		was then added to the grinder along with the purified water (60ml) for the purpose of blending and grinding to produce 100mg/ml concentration of AEPS. The extract prepared was stored into a glass bottle and denoted by "AEPS"[10].
3.	Unripe Fruit (AEPUF)	Unripe papaya fruit was obtained from the Karachi local market. The fruit was washed with tap water and peel was removed. All seeds were removed. AEPUF was prepared in 100mg/ml concentration for which 6 grams of unripe fruit was homogenized and blended with 60 millilitres of purified water using local mixer & grinder. The extract prepared was then stored in a glass bottle and denoted by "AEPUF"[10].
4.	Papaya Leaves (AEPL)	Fresh mature leaves of papaya of size (8 to 9 inch) were plucked from papaya tree. Leaves were washed with tap water thoroughly and air dried. These air-dried leaves (60gm) were added to the local mixer/grinder along with purified water (60ml) where they were properly grinded and blended. AEPL prepared was of 1000mg/ml concentration, which was stored in a glass bottle and denoted by "AEPL"[11].

Grouping of study animals and their dosing protocol

5 groups of 10 animals were set for biochemical testing. Group I was control whereas the rest of the groups i.e. group II, III, IV and V were the treatment groups. Animal groups and their dosing is represented in Table "II"

The dosing was continued for 2 months (60 days) and all dosing was by oral route. The blood was withdrawn on day 61st for estimation of serum electrolytes[12,13,14].

TABLE II

GROUP	DOSING MATERIAL	DOSE
Group I	Distilled water	2ml daily
Group II	AEPF	250mg per kg daily
Group III	AEPS	200mg per kg daily
Group IV	AEPUF	250mg per kg daily
Group V	AEPL	800mg per kg daily

Estimation of Serum Electrolytes

For estimation of serum electrolytes, blood was withdrawn from the marginal ear vein into electrolyte tubes. After blood withdrawal, these tubes were centrifuged for 600 to 900 seconds at 3000 RPM to get the pure plasma which was then analyzed using Automatic Electrolyte Analyzer (Human-Germany) for the estimation of serum sodium, potassium, magnesium and calcium. For estimation of these tests, standard kits were used which were purchased from the Human company.

Statistical Analysis

The data collected was expressed as Mean ± Std.Dev and analyzed using SPSS version-20. ANOVA (one-way) followed by post-hoc Tukey's test is used for evaluation of statistical significance. All P-values of less than 0.05 were considered significant. However P-values **p<0.05** *#!$^, **p<0.01** **##!!$$^^, **p<0.001** ***###!!!$$$^^^ represent level of significance i.e. significant, very significant and highly significant difference in comparison to control, AEPF, AEPS, AEUPF and day 11th of dosing respectively.

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RESULTS

Table III, IV, V and VI demonstrates the acute and chronic effects of different parts of papaya tree on serum sodium, potassium, calcium and magnesium respectively.

As represented in Table III, acute dosing of all parts of papaya i.e. AEPF, AEPS, AEUPF and AEPL does not affect serum sodium levels in comparison to control nor any significant difference among the treatment group was noted. However after chronic dosing AEPF and AEPS not only lowered serum sodium in comparison to control but also in comparison to their respective acute dosing sodium levels. On the other side chronic dosing of AEUPF and AEPL not only raised serum sodium levels in comparison to control but also in comparison to their respective acute dosing sodium levels. Among all the treatment groups AEUPF showed highest tendency to raise serum sodium levels.

As represented in Table IV, acute as well as chronic dosing of all parts i.e. AEPF, AEPS, AEUPF and AEPL markedly raised serum potassium levels in comparison to control group. In acute dosing AEPS showed highest tendency to raise serum potassium whereas in chronic dosing AEPL showed highest potential of raising serum potassium.

As represented in Table V, acute as well as chronic dosing of all parts i.e. AEPF, AEPS, AEUPF and AEPL markedly raised serum calcium levels in comparison to control group. In acute dosing AEPL showed highest tendency to raise serum calcium whereas in chronic dosing AEPS showed highest potential of raising serum calcium levels.

As represented in Table VI, acute as well as chronic dosing of all parts i.e. AEPF, AEPS, AEUPF and AEPL markedly raised serum magnesium levels in comparison to control group. In acute dosing almost all parts showed similar effects on serum magnesium levels, However after chronic dosing AEPS showed highest potential of raising serum magnesium levels. Both AEPF and AEPS markedly raised serum magnesium when compared with their respective acute dosing levels.

Table Iii Acute and chronic effects of different parts of Papaya plant on serum sodium

GROUPS	Sodium (mEq/L)	
	Acute dosing (MEAN±S.D)	Chronic dosing (MEAN±S.D)
Control	141.6±2.45	142.1±2.46
AEPF	141.1±2.76	135.5±2.17$^{***,\wedge\wedge}$
AEPS	140±2.94	137.2±2.39***
AEUPF	139.1±2.84	154.5±1.43$^{***, ###, !!!,\wedge\wedge\wedge}$
AEPL	138.6±3.06	146.3±2.87$^{**, ###, !!!, \$\$\$,\wedge\wedge\wedge}$

Table Iv Acute and chronic effects of different parts of Papaya plant on serum potassium

GROUPS	Potassium (mEq/L)	
	Acute dosing (MEAN±S.D)	Chronic dosing (MEAN±S.D)
Control	3.93±0.23	3.89±0.24
AEPF	6.21±0.31***	5.52±0.24$^{***,\wedge\wedge\wedge}$
AEPS	6.22±0.29***	5.51±0.25$^{***, \wedge\wedge\wedge}$
AEUPF	5.17±0.21$^{***, ###, !!!}$	5.71±0.19***
AEPL	5.83±0.17$^{***, \#, !, \$\$\$}$	6.28±0.34$^{***, ###, !!!, \$\$\$,\wedge\wedge}$

Table V Acute and chronic effects of different parts of Papaya plant on serum calcium

GROUPS	Calcium (mg/dL)	
	Acute dosing (MEAN±S.D)	Chronic dosing (MEAN±S.D)
Control	12.59±0.76	12.87±0.86
AEPF	13.68±0.68***	16.63±0.31$^{***,\wedge\wedge\wedge}$
AEPS	14.50±0.36$^{***, \#\#}$	18.83±0.97$^{***, ###,\wedge\wedge\wedge}$
AEUPF	14.78±0.31$^{***, ###}$	15.16±0.38$^{***, ###, !!!, \wedge}$
AEPL	15.34±0.32$^{***, ###, !!}$	15.35±0.33$^{***, ###, !!!}$

Table Vi Acute and chronic effects of different parts of Papaya plant on serum magnesium

GROUPS	Magnesium (mg/dL)	
	Acute dosing (MEAN±S.D)	Chronic dosing (MEAN±S.D)
Control	2.44±0.41	2.97±0.41
AEPF	3.20±0.42***	5.14±0.34***,^^^
AEPS	3.32±0.12***	5.38±0.27***,^^^
AEUPF	3.53±0.17***	3.61±0.35**, ###, !!!
AEPL	3.41±0.18***	3.72±0.21***, ###, !!!

DISCUSSION

Globally numerous plants and plant based preparations are used therapeutically in treating different unhealthy conditions. Based on the fact that these herbal remedies also possess potential of abuse, there is an urgent need to evaluate these products for their safety, toxicity and adverse effect potential[15].

Different parts of papaya are used for different purposes like papaya fruit is commonly consumed in Asian region for the relief of constipation, unripe fruit is commonly used as meat tenderizer, seeds are used as spice/condiment, leaves in different infectious fever states. Due to its frequent utilization this study is designed to investigate the short term and long term effects of papaya fruit, seeds, unripe fruit and leaves on serum electrolytes.

In human beings, normal serum sodium concentration is 137 to 142 mEq per Litre[16]. In clinical medicine, disorders of serum sodium are the most and the most poorly understood disorders today. A low level of serum sodium below the normal range is called hyponatremia. Usually at serum sodium levels between 125 to 132 mEq/L the patients are asymptomatic, However, the symptoms specifically gastrointestinal and neuro-psychiatric symptoms begin to show once the levels fall below 125 mEq/L. Untreated severe symptomatic hyponatremia may leads to fatalities. A high serum sodium concentration above 142 mEq/L is called hypernatremia. Unlike hyponatremic state, hypernatremia symptoms are not that much harsh. Most common symptoms of hypernatremia are muscle twitching, seizures, restlessness, altered mental status, irritability, lethargy, intense thirst etc.[17]. Coming towards the finding of our study, acute dosing of all 4 study parts had no effect on serum sodium levels but after chronic dosing two parts i.e. AEPF(fruit) and AEPS (seeds) decreased serum sodium levels but not significant enough to cause hyponatremia. On the other side AEPUF (unripe fruit) and AEPL (leaves) increased serum sodium levels after chronic dosing. This property of ripe fruit and seeds of decreasing serum sodium can be beneficial to be used concurrently with drugs and diseases causing hypernatremia whereas the potential of unripe fruit and mature leaves of raising serum sodium can be beneficial in drug induced hyponatremic states such as with diuretics. However these are only suggestions and future clinical studies are mandatory to evaluate their efficacy in serum sodium disorders. The finding of this study also raised a warning that these papaya parts have the tendency to disturb serum sodium levels and daily chronic consumption of these parts may cause fluctuations in serum sodium concentration and can lead to serious consequences which means their daily use should be avoided despite of the fact that they are natural products.

In human beings, normal serum potassium concentration is 3.5 to 5.5 mEq per Litre. A low level of serum potassium below the normal range is called hypokalemia. One of the main causes of hypokalemia in clinical practice is alkalemia. A high level of serum potassium above the normal range (5.5 mEq/L) is called hyperkalemia. Serum potassium levels above 6.5 mEq/L may lead to very serious consequences and should be treated as emergency [18]. The findings of our study revealed that all 4 parts of papaya have the tendency to significantly raise serum potassium both after acute and chronic use. This ability can be utilized in treating various acute hypokalemic states such as diarrhoea, vomiting etc. and chronic hypokalemic states such as with loop diuretics but for this clinical studies must be conducted to evaluate the efficacy of each part. On the other hand due to this hyperkalemia

causing potential, each part should be very cautiously used both for acute and chronic purpose since high serum potassium concentration not only affects major organs like kidneys and heart but also may cause serious comorbidities.

Calcium is considered as the most abundant mineral of human body. Normal serum calcium concentration in human beings is between 4.3 to 5.3 mEq/L (8.5 to 10.5 mg/dL). Hypocalcemia is low serum calcium concentration which has both neuro-psychiatric and cardiac consequences such as neural excitation, convulsions and cardiac arrhythmias respectively. Hypercalcemia is high serum calcium concentration which affects kidney and heart functioning[19]. The findings of our study revealed that all 4 parts of papaya have the tendency to significantly raise serum calcium both after acute and chronic use. This ability can be utilized in treating various acute and chronic hypocalcemic states but for this clinical studies must be conducted to evaluate the efficacy of each part.

Magnesium is considered as the 2nd most abundant mineral of human body. Normal serum magnesium levels in human beings is between 1.5 to 2.0 mEq/L. Low serum magnesium levels below 1.4 mEq/L is hypomagnesemia whereas high serum magnesium levels above 2.0 mEq/L is hypermagnesemia. Both hypomagnesemia and hypermagnesemia states have serious consequences on heart [20]. The findings of our study revealed that all 4 parts of papaya have the tendency to significantly raise serum magnesium both after acute and chronic use. This ability can be utilized in treating various acute and chronic hypomagnesemic states but for this clinical studies must be conducted to evaluate the efficacy of each part.

CONCLUSION

In the light of above discussed findings and evidences, it is concluded that each part of papaya i.e. ripe fruit, seeds, unripe fruit and leaves have strong tendency to disturb serum electrolyte concentrations. This ability might be helpful therapeutically in various electrolyte imbalance states but for this clinical studies are mandatory. On the other hand it is strongly recommended to not consume these parts acutely and chronically for any purpose without the instructions and supervision of concerned health care provider due to their ability to make changes in serum electrolytes which may lead to serious consequences.

CONFLICT OF INTEREST

There is no conflict of interest

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