



Comparative Effect of Cinnamon and Garlic Aqueous Extract on Liver Enzymes and Electrolytes Homeostasis in Experimental Rats

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ABSTRACT

Introduction: Cinnamon and garlic have been used in foods since centuries. Recent studies have reported antioxidant effects of garlic and variable effects of both cinnamon and garlic on electrolytes. **Aims & Objectives:** The objective of this study is to evaluate the comparative effect of cinnamon and garlic extract on liver enzymes and electrolytes homeostasis in experimental rats. **Place and duration of study:** The study was conducted in Clinical Biophysics Research Unit, Department of Biochemistry University of Karachi, from March 2017 to January 2018. **Material & Methods:** 18 Albino Wistar rats of male sex (200 -250 g body weight) were randomly selected and divide into three groups (n= 6). Group I was labeled control and received normal rat diet; while Groups II and III received 10 mg/kg b.w. cinnamon extract, and 500 mg garlic extract/kg b.w for 13 consecutive days respectively. Kit method was used to measure liver function tests. While ion selective method was used to measure plasma calcium. Plasma and erythrocytes Na⁺ and K⁺ levels were measured by flame photometer. **Results:** A significant increase in total and direct bilirubin and alkaline phosphatase and decreased serum calcium level was noted in both cinnamon and garlic treated groups when compared with control. Whereas decreased Na⁺ K⁺ ATPase and increased erythrocytes Na⁺ level was observed in cinnamon treated group only. **Conclusion:** Cinnamon and garlic extract raised the liver enzymes and electrolytes homeostasis in almost similar manner except for decreased Na⁺ K⁺ ATPase and elevated erythrocyte sodium level in the case of cinnamon. This alteration was dose and duration dependent.

Key words: electrolytes, liver enzymes, garlic extract, cinnamon extract.

INTRODUCTION

A plethora of studies have been made on medicinal plants for treatment and maintenance of health¹. Garlic is one of the documented example of plant employed for the treatment of diseases and maintenance for health. It's distinctive constituent Sulfur and sulfur containing compounds (γ -glutamyl-S- alkyl S- cysteines and S – alkyl –L- cysteine sulfoxides) provides characteristic beneficial effects on breast, colon, skin, uterine, oesophagus and lung cancers². Garlic protects arteries from inflammation by inhibiting prostaglandin synthesis. It also reduces the risk factor of heart attack by reducing the level of LDL, cholesterol, triglycerides, blood pressure^{3,4}. It has

antifungal⁵, antibacterial⁶, and anti allergic effect⁷ as well. It gives vigorous effect on improving stress, folate deficiency, sickle cell anemia⁸, UV induced immunosuppression⁹.

Likewise, Cinnamon is a culinary and medicinal plant having promising effect on treatment of dyspepsia, bronchitis, coughs, loss of appetite. It has antibacterial, antifungal effects Cinnamaldehyde, eugenol, cinnamic acid, weitherhin, diterpenes, proanthocyanidins are its active constituents. They are used in potentiating effect of insulin¹⁰. Its essential oil is used as aromatherapy to promote blood circulation¹¹.

Cinnamon and Garlic both have antioxidant and antimicrobial effects and are both used as food preservatives. The study is designed to evaluate the comparative effect of aqueous extract of cinnamon

and fresh garlic on liver enzymes and electrolytes homeostasis in experimental rat model.

MATERIAL AND METHODS

Animals and diet:

18 male Albino Wistar rats (200-260 g b.w.), purchased from ICCBS (International center for Chemical and Biological Center Karachi, Pakistan) for the study. Animals were acclitized to the laboratory environment for at least 7-10 days before the start of experiment and caged in a quite temperature controlled room ($23 \pm 4^{\circ}\text{C}$). Drinking water and standard rat's diet are easily available for rats. The experiments received approval from University Review Board and followed in accordance with Ethical Guidelines of internationally accepted principles for Laboratory Use and Care in Animal Research (Health Research Extension Act of 1985).

Dose Preparation:

Aqueous cinnamon extract preparation: 200 mg of cinnamon powder was weighed and dissolved in 5 ml of deionized water, mixed thoroughly and stored at 4°C .

Aqueous garlic extract preparation; 1000 mg of garlic powder was weighed and dissolved in 5 ml of deionized water, mixed thoroughly and stored at 4°C till use.

Study Design:

18 rats were randomly divided into 3 groups, six rats in each group and received the following treatment:

Group I: Control group remains untreated

Group II: Cinnamon –treated: received 10 mg/ kg b.w. cinnamon extract orally for 13 consecutive days

Group III: Garlic – treated: received 500 mg/ kg b.w. garlic extract orally for 13 consecutive days.

The rats were sacrificed after 48 hrs of last dose. The blood was collected in a lithium heparinized coated tubes, mixed gently, plasma was separated by centrifuge at speed of 2000 rpm for 20 minutes. Plasma was stored at -70°C in disposable Eppendorff tubes.

The RBC's was washed and processed for analysis of electrolytes and ATPase.

Liver function tests:

Commercially available kit method is used to measure the total (TB) & direct bilirubin (DB), alanine transaminase (ALT) and alkaline phosphatase (AP).

Assessment of Plasma electrolytes homeostasis:

Flame photometry (Corning 410) was used to detect the plasma sodium and potassium, Ion Selective Electrode Method Jenway (Ion Meter 3345) was used to detect the plasma calcium ion.

Estimation of intra-erythrocyte sodium and potassium¹²

RBC's were washed three times at room temperature with MgCl_2 solution (112 M), centrifuged at 12000 rpm at 4°C for 5 minutes. Packed cell volume was estimated by microhaematocrit capillary tubes. Lysis of the cell suspension was done with 0.01 mL of saponin solution (20 % in MgCl_2 112 mM) then intra-erythrocytes sodium and potassium was determined by taking 0.3 mL of lysate to 10 mL of lithium nitrate diluent (15mM). Intensities of erythrocyte sodium and potassium was calculated as mM by using flamephotometry.

Erythrocyte membrane preparation

The washed RBC's then treated with 25 volumes of 0.011M Tris-HCl buffer at $\text{pH} = 7.4$ at 4°C , centrifuged at 12000 rpm for 30minutes 3 X. The membrane yield was ~ 4 mg protein/mL of Tris buffer. Biuret method was used to detect the concentration of protein¹³ and stored at -80°C (Bhuiyan AI 2015)(Takasu J 2006)(Savory J 1968) until the analysis.

Erythrocyte Na^+ - K^+ -ATPase activity measurement¹⁴

Briefly a mixture consists of 92 mM Tris-HCl ($\text{pH} = 7.4$), 100 mM NaCl, 20 mM KCl, 5 mM $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ and 1mM EDTA were preincubated with membrane pellet (400 μg) for 10 minutes at 37°C . Na-K-ATPase activity was calculated by measuring the differences of the release of inorganic phosphate with and without incubation for 10 minutes with 1Mm Ouabain (Na-K-ATPase inhibitor). Inorganic phosphate concentration released and calculated as nm/mg protein/hour. Activity was corrected to a nanomolar concentration.

Estimation of plasma magnesium¹⁵

Briefly, 0.2 mL of plasma was mixed with 1.8 mL of TCA (5% w/v) to make protein free filtrate. To 1mL of filtrate add 1.5mL of titan yellow (0.05%) and 0.5mL of NaOH (4 N). Deionized water is used as bland & treated similarly. The color intensity was measured against blank at 540 nm after 15 minutes of incubation at room temperature on Shimadzu-spectrophotometer UV-120-01.

Statistical analysis:

Results are presented as mean ± SD. One way ANOVA with post hoc test is used to analyze the level of significance between control and experimental groups. P<0.05 is considered significant.

RESULTS

Effect of Cinnamon and Garlic on liver function test:

Significant increased level of total bilirubin was observed in both cinnamon and garlic extract while decreased level of direct bilirubin was observed in both treated groups when compared with control (Table-1). An increased level of ALT was observed in garlic extract treated rats while no changes were observed in ALP level when compared with control.

Effect on intra –erythrocytes electrolytes in group I, II, III:

No significant changes were observed on intra-erythrocytes level in both cinnamon and garlic extract treated rats while significant decreased level of Na⁺ K⁺ ATPase level was observed in cinnamon extract treated rats when compared with control (Table-2).

Effect on serum electrolytes:

Treatment of cinnamon extract showed significant increased serum Na⁺ level when compared with control. While decreased serum Calcium level was observed in both cinnamon and garlic extract treated rats (Table-3).

Parameters	Group I	Group II	Group III
TB (U/L)	0.33±0.007	0.43±0.007 ^a	0.35±0.007 ^c
DB (U/L)	0.97±0.13	0.35±0.06 ^a	0.48±0.89 ^b
AP (U/L)	124.45±25.9	142.17±24.75	188.21±98.80
ALT (U/L)	20.80±1.00	49.30±6.86	59.49±13.85 ^b

Table-1: Effect of Cinnamon and Garlic on liver function test

Parameters	Group I	Group II	Group III
Na ⁺ (mmol/L)	7.71±0.78	7.46±0.52	8.32±0.51
K ⁺ (mmol/L)	72.82±4.08	70.11±1.4	81.12±10.36
Na ⁺ K ⁺ atpase (nm/mg protein)	24.68±3.33	15.17±0.40 ^a	23.12±2.40 ^c

Table-2: Effect on intra –erythrocytes electrolytes in group I, II, III

Parameters	Group I	Group II	Group III
Na ⁺ (mmol/L)	135.75±0.95	138.75±0.95 ^a	137.5±1.73
K ⁺ (mmol/L)	4.8±0.08	4.95±0.191	4.9±0.17
Ca ⁺⁺ (mmol/L)	4.66±0.76	2.73±0.66 ^a	2.27±0.52 ^b
Mg ⁺⁺ (mmol/L)	1.31±0.22	1.73±0.16	1.68±0.31
Nitrate (mmol/L)	1.81±0.64	1.9±0.83	1.83±0.3

Table-3: Effect on serum electrolytes

Results are presented as mean ±SD. Data is calculated by ANOVA with Tukey's post hoc test. a: group II compared with group I; b: group III compared with group I; c : group II compared with group III.

DISCUSSION

We found that fresh garlic extract significantly decreased the level of direct bilirubin and increased level of liver enzyme ALT as shown in Table-1. ALT is suggested as one of the liver specific enzymes. High doses of garlic altered its activity by disrupting the plasma membrane of liver resulting its level increased. Various studies have¹⁶ found that low doses of garlic either orally or intraperitoneally had little effect on lungs and liver, while high doses disrupt the hepatocytes. Another study conducted by Banerjee SK 2001 explained that lower doses of garlic potentiate the antioxidant effects while high doses reverse this effect and exhibits the morphological changes on liver enzymes.¹⁷

The total bilirubin was significantly increased in cinnamon and garlic treated rats (Table-1). Bilirubin is a major breakdown product of hemoglobin. Increased bilirubin production results from impaired hepatic excretion or regulation of conjugated and unconjugated bilirubin from the hepatocytes. Our results indicated that garlic dose 500 mg/kg b.w. increased bilirubin level, although this dose is considered as low. Bunyamin Akgul et al, found that garlic accelerates the RBC's turnover by inducing hemeoxygenase 1, which would then produce Fe²⁺, biliverdin and CO.¹⁸

Electrolytes and water secretions are the means of defense mechanism in the intestine by decreasing the bacterial adhesion to the tissue. ChlorideCl⁻ and bicarbonate ions HCO₃²⁻ secretion by intestinal mucosa is described as a means of defense for the animal to remove the harmful bacterias and foreign bodies like antigens¹⁹. Cinnamon aldehyde

decreases the bacterial adhesion to the epithelial tissues by regulating the anion secretion in small intestine which activate the epithelial nicotinic receptors.

Cinnamon and garlic both significantly decreased the level of calcium when compared. Magnesium has a very delicate role on balancing the calcium regulating hormones calcitonin and parathyroid hormone (PTH). Magnesium suppresses the PTH and stimulates the calcitonin, helps to put calcium into bones and remove it from soft tissues. Increased level of calcium without adequate magnesium stops this chemical process and leads to osteoporosis and arthritis²⁰. Srivastava studied the epinephrine – induced aggregation by garlic extract and suggested that it may be inhibiting uptake of calcium into platelets thereby lowering cytosolic calcium concentrations²¹. Although no significant changes were observed in magnesium, potassium and nitrite level in present study. Ajene found increased potassium and decreased sodium level when treated with garlic extract²². Adenosine triphosphate (ATP) plays an important role in intracellular functions and critical for cellular viability as they control many functions and considered to be a sensitive indicator of toxicity. We found an increased level of Na⁺ in Cinnamon treated rats while no changes were observed in garlic treated rats when compared. Na⁺-K⁺-ATPase is the major transporter of sodium ions in renal basolateral epithelia throughout the nephron²³.

Na⁺ K⁺ ATPase is the major determinant of cytoplasmic Na⁺. It has an important role in regulating cell volume, cytoplasmic PH and Ca⁺⁺ levels through the Na⁺ / H⁺ and Na⁺/ Ca⁺⁺ exchangers, respectively and driving a variety of secondary transport process such as Na⁺ dependant glucose and amino acids²⁴. It provides the driving force for nutrients, electrolytes and water reabsorption²⁵. It is membrane bound enzyme and any change in the lipid component of membrane directly effects Atpase activity. Usta et al, investigated that water extract of cinnamon and clove inhibited the activity of liver ATPases and stimulated F^oF¹ ATPase ; similarly Kreydiyyeh et al. investigated that extract of clove and cinnamon had the most potent inhibitory effect on intestinal Atpase as compared to extracts of other spices.

CONCLUSION

It is concluded that garlic at a dose of 500 mg/kg and cinnamon extract 10 mg/kg b.w. significantly altered the electrolytes homeostasis and liver enzymes in almost similar manner. The important compounds in garlic and cinnamon are responsible for electrolytes and liver enzymes alteration. Likewise dose and duration of garlic and cinnamon extract are complementary/ dependent factors.

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