Research Article

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Study on antioxidant effects of cinnamon and garlic extract in liver, kidney and heart tissue of rat.

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The aim of present study was to investigate the antioxidant enzymes activity and oxidative status of Cinnamon and Garlic - treated rats. 12 male Wistar Albino rats were randomly divided into 3 groups (n=4). Group I: Control rats received distilled water; Group II: Received aqueous extract of Cinnamon (10 mg / kg b.w.) orally for 13 consecutive days; Group III: Received aqueous extract of Garlic (500 mg / kg b.w.) orally for 13 consecutive days. Oxidative status was measured by MDA and 4-HNE and antioxidant enzymes activity was measured by Catalase and GSH levels, meanwhile mean body weight, liver weight, kidney weight and heart weight was also measured. No changed in body weight was observed while decreased liver weight was observed in Garlic treated rats and decreased heart weight was observed in Cinnamon-treated rats. Liver MDA level was increased in Cinnamon -treated rats (P<0.05), while decreased MDA level was observed in kidney (P<0.05) and heart (P<0.05) tissues. Decreased level of MDA was also observed in heart tissue of Garlic-treated rats (P<0.05). 4-HNE level was increased in liver tissue (P<0.01) was observed in Cinnamon - treated rats while decreased 4-HNE was observed in heart tissue (P<0.01). Catalase was increased in kidney tissue of Garlic treated rats (P<0.05). Liver GSH level was increased in Cinnamon-treated rats (P< 0.05), while decreased GSH was observed in kidney tissue (P<0.05) and increased GSH liver tissue (P<0.01) was observed in Garlic - treated rats. Thus the study showed the antioxidant properties of Cinnamon and Garlic. Garlic showed potent antioxidant activity in liver and heart tissue and slightly in heart tissue while Cinnamon markedly showed antioxidant activity in liver tissue.

Key words: Cinnamon, Garlic, antioxidant enzymes, lipid peroxidation

Plants have been essential part of human society since the civilization started. Garlic is widely consumed in many cultures as spice and as condiment in many dishes. Several cultures use garlic for medicinal purposes. A plethora of publications are also available on the pharmacological properties of garlic and their beneficial health effects (Rahman, 2007; Kojuri et al. 2007). Garlic (Allium sativum L.) is used as a spice and medicinal herb. Most recent research on garlic has used garlic in the form of tablets, flesh, raw, boiled, cooked (Gorinstein et and dried al. 2006). Commercially available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (Elkayam et al. 2003). Garlic exhibits a wide range of properties immunomodulatory including hepatoprotective, antimutagenic and anticarcinogenic effects (Uma et al. 2007). Garlic and garlic extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (Rahman and Lowe, 2006; Steiner and Li, 2001) and modulates lipid metabolism (Steiner and Li, 2001). Several studies have also shown that garlic contains active hypocholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide. It has also been reported that garlic supplements in human subjects lead to the increased resistance of low density lipoprotein to

oxidation and may be one of the powerful mechanisms accounting for the antioxidative and anti-atherosclerotic properties of garlic (Borek, 2001, Lau, 2001).

Cinnamon (Cinnamomum zylenicum) is commonly used in the food industry because of its special aroma. Additionally, it has strong antibacterial properties. anticandidial, antiulcer, analgesic, antioxidant hypocholesterolaemic activities and regulates the lipogenesis (Mehmiet et al. 2010; Qin et al. 2010). In this regard we aimed to investigate the effect of garlic and cinnamon extract on oxidative status and antioxidant enzymes in liver, kidney and heart tissues of rat. Moreover, we also assessed the comparative effect of Garlic and Cinnamon on lipid peroxidation and antioxidant enzymes.

MATERIALS AND METHODS

Animals & diet: Wistar albino rats of male sex (200–260g b.w.), purchased from the animal house of ICCBS, (Karachi, Pakistan) were taken for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room ($23\pm 4^{\circ}$ C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

Study Design: Twelve Male Albino Wistar rats were randomly divided into 3 experimental groups (n=4). Each group received the following treatment:

- Group I: Control group remains untreated
- Group II: Received aqueous solution of Cinnamon (10 mg/kg b.w.) orally for 13 consecutive days
- Group III: Received aqueous solution of Garlic orally (500mg / kg b.w.) for 13 consecutive days.

All the rats were weight before treatment.

Sample Collection: After 48 hours of last dose of treated groups, animals were decapitated and blood was sampled from head wound in the lithium heparin coated tubes. Kidneys, Liver and Heart tissues were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were then kept in freezer -70 ° C until analysis.

Kidney Homogenate Preparation: Kidney homogenates were obtained using a tissue homogenator Ultra Taurax T-25 Polytron at 4° C. The homogenates (1:10 w/v) were prepared by using a 100 mmol KCl buffer (7.00 p H) containing EDTA 0.3 mmol, All homogenates were centrifuged at 600 g for 60 minutes at 4° C and the supernatant was used for biochemical assays.

Liver Homogenate Preparation: Liver were perfused with saline and homogenized in chilled KCI (1.17%) using a homogenizer. The homogenates were then centrifuged at 800 g for 5 minutes at 4° C to get post mitochondrial supernatant.

Heart Homogenate Preparation: Homogenate were prepared on ice in the ratio 4 gm tissue for 16 mL of Phosphate buffer (pH = 7.5) containing 1 m M/L Na₂EDTA, 10 MI of 500 m M/L BHT (butylated hydroxytoluene) in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 2000 rpm for 4 minutes at 4 °C and the supernatant was frozen at -70 °C until analysis.

Assessment of tissue Lipid peroxides: 10µl of BHT (0.5 M in acetonitrile) was added to prevent homogenate from oxidation and the homogenate was stored at -70 °C until analysis for MDA and 4-HNE.

Estimation of malonyldialdehyde (MDA): The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al, 1979. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of homogenate. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as

standard absorption.

Estimation of 4-HNE (4-hydroxy-2nonenal): The 4-HNE content was measured by the method of Kinter *et al*, 1996. Briefly, the assay mixture consisted of 2 ml of filtrate with 1 ml of 2, 4 Dinitrophenyl hydrazine and kept for 1 hr at room temperature. Sample was extracted with hexane, and the extract was evaporated at 40 °C. Cool sample was measured at 350 nm against methanol blank.

Assessment of antioxidant status:

Estimation of Catalase: Catalase activity was assayed by the method of Sinha,1972. Briefly, the assay mixture consisted of 1.96 ml phosphate buffer (0.01 M, pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml (10%) homogenate in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 min, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of GSH: GSH activity was determined by the procedure of Carlberg and Mannervik, 1985. The assay solution contained 10 % BSA, 50 m M Phosphate buffer (p H = 7.6), 2 m M NADPH, 20 m M GSSG. Absorbance at 340 nm was recorded at a temperature of 25 °C. The activity was calculated using the molar coefficient for NADPH of 6.22 μ mol⁻¹ x cm⁻¹ and expressed in U/ gm tissue.

Statistical analysis: Results are presented as mean \pm SD. Statistical significance and differences from control and test values were evaluated by Student's t-test. Statistical probability of P <0.01, P<0 .05 were considered to be significant.

RESULTS

Table I showed decreased body weight in Cinnamon and Garlic-treated rats but the results were not significant. While Garlic-treated rats showed decreased liver weight $(4.94\pm0.38, P<0.01)$ and Cinnamon-treated rats showed decreased heart weight $(0.57\pm0.05, P<0.05)$. No significant change was observed in kidney weight in both Garlic and Cinnamon – treated rats.

Cinnamon decrease the MDA level in kidney (27.22±5.49, P< 0.05) and heart tissue (26.74±4.32, P< 0.05)significantly, while increased MDA in liver tissue (37.44±4.60,

P<0.05) as compared to control (table 2). Decreased MDA in heart tissue was observed in Garlic - treated rats (32.57±5.13, P<0.05) while MDA level was similar to control in kidney and liver tissue in Garlic – treated rats. Liver 4-HNE was increased (1131.87±5.84, P< 0.01) and Heart 4-HNE was decreased (568.37±26.89, P< 0.01) as compared to control in Cinnamon-treated rats (table 3). While 4-HNE level was decreased in liver tissue (312.97±163.9, P<0.01) as compared to control in Garlic - treated rats. No significant changes was observed in 4-HNE level in kidney and and heart tissue in Garlic treated rats.

In Cinnamon – treated rats no significant results were observed in catalase level in kidney, liver and heart tissues (table 4). Garlic treatment showed increased Catalase enzyme $(1.01\pm0.15, P<0.05)$ in kidney tissue as compared to control.

Cinnamon and Garlic treated groups showed increased Liver GSH level (524.32±97.98, P< 0.05; 458.67±46.38, P<0.01) respectively as compared to control (table 5). Garlic showed decreased GSH in liver (120.65±44.36, P< 0.05) and heart tissues (87.27±3.8, P<0.05) while no changes were observed in Cinnamon treated kidney and heart tissues.

DISCUSSION

Formation of reactive oxygen species is a normal consequence of a variety of essential biochemical reactions. It is also known that oxygen radicals could be formed in excess in chronic diseases (Elzbieta et al. 2005). Therefore, an adequate range of antioxidative defences within and outside the cells, has also been considered to be very important to offer protection against oxidative damages of cell components including membrane phospholipids (Sun, 1990).

The effects of Garlic and Cinnamon on oxidative status was observed by measuring peroxidation. MDA the lipid (malonyldialdehyde) and 4-HNE (4-hydroxy 2-nonenal) are lipidperoxides involved in lipid peroxidation. It is found that Garlic and Cinnamon both significantly decreased the MDA level in heart tissues, while cinnamon decreased MDA in kidney tissue and increased in liver tissue significantly (Table 2). It is reported previously excessive oxygen production involves radical in lipid peroxidation (Elzbieta et al. 2005), results in cellular membrane degeneration and DNA

rats:			
Parameters	Control	Cinnamon – treated	Garlic – treated
Mean Body weight (g)	232.5±15.02	206±43.5	178.25±18.66
Mean Kidney weight(g)	0.682±0.12	0.68±0.09	0.612±0.05
Mean Liver weight (g)	7.12±0.37	5.95±1.58	4.94±0.38*
Mean Heart weight (g)	0.692±0.04	0.57±0.05**	0.598±0.09

Table 1: Mean Body Weight, Kidney Weight, Liver Weight, Heart Weight in Control, Cinnamon and Garlic - treated

n = 4, Values are Mean \pm SD

Significant differences between Control, Cinnamon and Garlic - treated rats by Student's t-test *P<0.01, **P<0.05

Table 2: Effect on tissue MDA level in Control, Cinnamon and Garlic - treated rats.

Parame	ters		Control	Cinnamon – treated	Garlic - treated
Kidney	MDA	(nM/g	38.26±3.29	27.22±5.49**	28.50±6.26
tissue)					
Liver	MDA	(nM/g	26.24±3.00	37.44±4.60**	42.33±9.58
tissue)					
Heart	MDA	(nM/g	40.33±4.81	26.74±4.32**	32.57±5.13**
tissue)					
= 4: Values	are Mean	+ SD			

n =

Significant differences between Control, Cinnamon and Garlic - treated rats by Student's t-test *P<0.01, **P<0.05

Table 3: Effect on tissue 4-HNE level in Control, Cinnamon and Garlic - treated rats

Parameters		Control	Cinnamon – treated	Garlic - treated
Kidney 4-HNE	(nM/g	734.25±92.23	677.45±104.98	607.58±35.22
tissue)				
Liver 4-HNE	(nM/g	889.94±62.81	1131.87±5.84*	312.97±163.9*
tissue)				
Heart4-HNE	(nM/g	835.24±48.08	568.37±26.89*	698.09±113.8
tissue)				
= 4: Values are Mean	± SD			

n =

Significant differences between Control, Cinnamon and Garlic - treated rats by Student's t-test *P<0.01, **P<0.05

Table 4: Effect on tissue	Catalase level in Control	Cinnamon and Garlic	 treated rats

Parameters	Control	Cinnamon – treated	Garlic - treated
Kidney Catalase (mM/g tissue)	0.623±0.07	0.672±0.10	1.01±0.15**
Liver Catalase (mM/g tissue)	2.04±0.50	2.13±0.40	1.73±0.19
Heart Catalase (mM/g tissue)	1.56±0.16	1.73±0.19	1.737±0.36

n = 4 Values are Mean ± SD Significant differences between Control, Cinnamon and Garlic – treated rats by Student's t-test *P<0.01, **P<0.05

Table 5: Effect on tissue GSH level in Control, Cinnamon and Garlic - treated rats

Parameters	Control	Cinnamon – treated	Garlic - treated
Kidney GSH (U/g tissue)	210.4±31.17	237.6±42.93	120.65±44.36**
Liver GSH (U/g tissue)	250.8±12.38	524.32±97.98**	458.67±46.38*
Heart GSH (U / g tissue)	198.6±49.0	106.0±62.0	87.27±3.8**

n = 4; Values are Mean \pm SD

Significant differences between Control, Cinnamon and Garlic - treated rats by Student's t-test *P<0.01, **P<0.05

damage (Sharma et al. 2001). The increased MDA in liver tissue by Cinnamon - treated rats might have decreased antioxidant enzymes in liver tissue. Similarly, decreased MDA in kidney and heart tissue may be due to reactive oxygen species consumptions (Eander, 1996) by bioactive compounds of cinnamon. Cinnamon decreased the 4-HNE in liver and heart tissues significantly while Garlic showed no significant effects (Table 3). The primary constitutes of cinnamon like cinnamaldehyde, phenols, terpenes (Qin et al. 2010) are responsible for antioxidant activity and thus decreased lipid peroxide. it has been demonstrated that superoxide anion as well alkoxyl peroxyl and radicals could as inactivate one of the antioxidant enzymes catalase and reduce the effectiveness of cells to defend against free radical damage (Mayo et al. 2003). In present study increased level of Catalase was found in kidney tissue of Garlic - treated rats (Table 4). Garlic stimulates the synthesis of antioxidant enzymes as observed in present study. Catalase is used by cells to defend against the toxic effects of hydrogen peroxide, which is generated by various reactions and/or environmental agents or by the action of dismutase, enzymes superoxide while detoxifying superoxide anion (Michiels et al. 1994). Another important finding of this study is the increased level of liver GSH in Cinnamon and Garlic – treated rats (Table 4). The active ingredients of Cinnamon and responsible for increased Garlic are antioxidant enzyme activity showing antioxidant property of these spices.

Selenium is a cofactor of GSH and the major components of Cinnamon (Hoffman and Manning, 2002) and Garlic (Leonge et al. 2010). Garlic contains several organosulfur compounds such as allicin, diallyl sulfide and diallyl disulfide, a valuable precursor for alutathione biosynthesis (Wei and Lau, 1998). Kidney and heart GSH level was decreased in Garlic – treated rats (Table 5). The effectiveness of GSH depend upon the cofactor availability and extent of lipid peroxidation. In present study the Garlic treated rats showed decrease level of GSH in kidney and heart tissue may be due to insufficient availability of selenium to GSH svnthesis.

The Cinnamon – treated rats showed increased MDA and 4-HNE and GSH level. It is found previously that adaptation of the liver to oxidative stress and higher GSH level promote better survival under hyper condition (Kurosawa et al. 2005). It is concluded from the present study that both Cinnamon and Garlic showed antioxidant activity and beneficial effects on liver tissue. Cinnamon showed oxidative stress in liver coupled with increased GSH, could be a compensatory mechanism. Thus constituents of Cinnamon and Garlic could provide better antioxidant activity in kidney, liver and heart tissues of rat against toxic assaults at a given doses under monitored conditions.

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