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Research Article

Effect of Oral Administration of Honey on Arginase Activity of Rats Exposed to Smoke

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ABSTRACT: In this paper, we report the effect of oral administration of 125 mg/kg honey on the liver and kidney arginase activity of rats exposed to smoke from hydrocarbon-fueled lantern. Eighteen Wistar albino rats (weighing 150-200 g) were randomly assigned into three groups of 6 rats each. Group one served as the control (CTR) that was not exposed to smoke while Group two and three were exposed to smoke alone (SMW) and smoke with honey (SMH) respectively for 12 weeks. Results showed that the inhalation of smoke by the rats for 12 weeks significantly ($p<0.05$) reduced the total weight gain of experimental rats. The integrity of the liver and kidney were compromised in the SMW group as compared to the control and the SMH rats. There was a significant increase in arginase activity of SMW rats as compared to the control rats; Liver (0.71 ± 0.04 µmol/ml/min), Kidney (0.50 ± 1.07 µmol/ml/min). In addition, there was a significant reduction of arginase activity in the SMH rats as compared to the SMW rat; Liver (0.50 ± 0.06 µmol/ml/min), kidney (0.38 ± 0.60 µmol/ml/min). Our findings suggest that honey has a protective effect on liver and kidney in animals exposed to smoke.

KEYWORDS: Smoke, inhalation, honey, arginase, liver, kidney.

INTRODUCTION

Smoke is a mixture of particles and chemicals produced by incomplete combustion of carbon-containing materials (Lee, 2005). All smoke contains carbon monoxide, carbon dioxide and particulate matter (PM or soot) (Butler and Mulholland, 2004). Inhalation of carbon monoxide (CO) can result in poisoning, with symptoms ranging from cough, shortness of breath, hoarseness, headache, acute mental status changes or even death (Ramirez *et al.*, 2014). CO poisoning is often under diagnosed due to exposure to low concentrations goes unnoticed, and threshold values for normal carboxyhemoglobin vary according to different authors (Ramirez *et al.*, 2014).

Several environmental exposures are associated with increased risk of coronary heart disease (CHD), exposure to second-hand smoke may increase the risk by as much as 25% to 30% (Anthony *et al.*, 2014). Exposure to third-hand smoke, residual components of tobacco smoke that remain in the environment after a cigarette is extinguished, also appears to increase risk (Anthony *et al.*, 2014).

Honey is often produced by the honey bees in larger quantity than bee wax, pollen, royal jelly and bee venom which are other beehive product (White, 1979). Honey has three major uses; as food, as medicine, as raw materials. As food, honey is a near perfect food (Cooper *et al.*, 2002; Ladas and Raptis, 1999). As medicinal substance, its anti-bacterial ability and supersaturated sugar solution with high osmotic pressure builds up the immunity level of individual consumers (Dixon, 2003). The curative power of honey can only be ascertained therapeutically as it has positive effects on the diseases. As raw material, honey is hygroscopic and the confectioneries made with honey remain moist most of the time (Delaplane, 2006).

Arginases catalyze the divalent cation-dependent hydrolysis of L-arginine to form the nonprotein amino acid L-ornithine and urea (Berueter *et al.*, 2005). In most mammals the enzyme was found to exist in two forms, has a broad tissue distribution and share ~ 60% sequence identity (Jenkinson *et al.*, 1996). Arginase I functions in the urea cycle, and is located primarily in the cytoplasm of the liver while Arginase II functions primarily in L-arginine homeostasis in non-hepatic tissues (e.g., in regulating L-arginine bioavailability to nitric oxide synthase (NOS) (Jenkinson *et al.*, 1996; Morris *et al.*, 1997; Christianson and Cox, 1999).

The human Arginase I and Arginase II are related by 58% sequence identity (Jenkinson *et al.*, 1996) and are immunologically distinct. The comparative properties of the two arginase isozymes are discussed in a number of recent reviews (Jenkinson *et al.*, 1996; Lyer *et al.*, 1998; Perozich *et al.*, 1998). Arginase activity has been detected in a number of non-hepatic tissues that lack a complete urea cycle; the reaction is thought to provide ornithine, the biosynthetic precursor of proline and the polyamines (Tabor and Tabor, 1984). For example, in lactating mammary gland, arginase activity rises to about 25% that found in liver in order to supply the proline required for milk protein biosynthesis (Yip & Knox, 1972). Myometrial arginase activity increases ≈25-fold during pregnancy to supply the rapidly growing fetus with polyamines to facilitate cell proliferation (Weiner *et al.*, 1996). The requirement of rapidly dividing tissues for enhanced polyamine biosynthesis is apparently met by increased arginase activity as found in gastric cancers (Leu and Wang, 1992; Wu *et al.*, 1994a; Wu *et al.*, 1994b) and in breast cancer (Straus *et al.*, 1992). Arginase activity has been detected in certain human colon cancer and human breast cancer cell lines (Buga *et al.*, 1998; Singh *et al.*, 2000).

This study was aimed at determining the effect of oral administration of honey *on the arginase activity* in rats exposed to smoke. We used hepatic and renal arginase levels as a biochemical parameter for monitoring the smoke-induced pathophysiology in those organs.

MATERIALS AND METHODS

Reagents used for this research work include; sodium hydrogen orthophosphate, Disodium hydrogen orthophosphate salt, Trichloroacetic acid (TCA), Hydrochloric acid which were products of Sigma Chemical company Limited, St. Louise, MO, US; and BDH Chemical Limited, Poole, Dorset, England, UK.

Experimental animal

Eighteen adult Wistar Albino rats weighing between 150-200 g were used for this work; the rats were purchased from the Veterinary Department of the University of Ibadan, Oyo State, Nigeria. They were acclimatized for a period of 7 days, and then treated for twelve weeks. The honey used for this research work is the ILORAA varieties (a local crude and dark coloured honey commonly consumed in Remo land of Ogun State, Nigeria). The weights of the rats were taken from the date of purchase till the day they were sacrificed.

Animal grouping

After acclimatization, the 18 rats were randomly assigned into three groups of 6 rats each. In Group 1 (control group), rats were given saline along with the normal rat chow and water on a daily basis for a period of 12 weeks without exposure to smoke. Group 2 (Smoke without honey treatment (SMW)): Rats in this group were given saline along with the normal rat chow and water on a daily basis for a period of 12 weeks with exposure to smoke from hydrocarbon-fueled lantern. In Group 3 (Smoke with honey treatment (SMH)), rats were given 125 ml/kg of honey along with the normal rat chow and water on a daily basis for a period of 12 weeks with exposure to smoke from hydrocarbon-fueled lantern.

Organ collection and homogenization

At the end of the twelfth week, the rats were anaesthetized with diethylether and sacrificed; the organs used (liver and kidney) were excised, weighed and homogenized separately

in 4 volumes of 0.02 M phosphate buffer. The homogenate was centrifuged at 4000 rpm for 15 minutes and the supernatant was used for the analysis.

Arginase Assay

Arginase activity was determined according to the method of Kaysen and Strecker [25]. The reaction mixture contained 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM $MnCl_2$, 0.1 M arginine and 50 µl of the enzyme preparation was added in a final volume of 1.0 ml. The mixture was incubated for 10 minutes at 37 °C. The reaction was terminated by the addition of 2.5 ml Erhlich reagent (2.0 g of p-dimethylaminobenzaldelyde dissolved in 20 ml of concentrated hydrochloric acid and made up to 100 ml by adding distilled water). The optical density reading was taken after 20 minutes at 450 nm. The urea produced was estimated from the urea curve prepared with varying concentrations of urea (0.1-1.0 mol). The unit of activity of arginase is defined as the amount of enzyme that will produce one mole of urea per min at 37 °C.

Statistical Analysis

All results were expressed as mean ± standard error of mean. All grouped data were statistically evaluated with SPSS version 10.0 software. Hypothesis testing methods include one-way analysis of variance (ANOVA) followed by Duncan multiple Range test. *P* value of less than 0.05 (*P*<0.05) were considered to indicate statistical significance.

Table 1: Total weight gain of the experimental rats exposed to smoke inhalation

Group	Total Weight Gain
CTR (g)	64.75 ±1.74 [c]
SMW (g)	33.10 ± 2.14 [a]
SMH (g)	43.86 ± 2.81 [b]

n= 6, values are expressed as mean ± SEM. Mean values are compared using one–way ANOVA, level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at P < 0.05.

Table 2: Percentage organ to body weight ratio (ROB) in animals exposed to smoke inhalation

Group	Liver	Kidney
CTR (%)	8.30 ± 0.013 [b]	4.81 ± 0.013 [b]
SMW (%)	5.87 ± 0.008 [a]	3.70 ± 0.128 [a]
SMH (%)	7.06 ± 0.026 [b]	4.20 ± 0.09 [b]

n= 6, values are expressed as mean ± SEM. Mean values are compared using one – way ANOVA, level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at P < 0.05.

RESULTS AND DISCUSSION

The results presented in Table 1 showed that there was a reduction in the total weight gain of animals exposed to smoke as compared to those treated with honey (Table 1). Exposure of the rats to smoke affected the liver and the kidney as the percentage ratio of organ to body weight in both organs were reduced on exposure to smoke (Table 2). We determined the level of arginase in selected organs in order to track the extent of tissue damage caused by smoke inhalation. There was an increase in the activity of arginase in the liver and kidney in animals exposed to smoke as compared to those treated with honey (Table 3).

Smoke contain many different chemicals, including aldehydes, acid gases, sulfur dioxide, nitrogen oxides, polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, styrene, metals and dioxins (Butler and Mulholland, 2004; Carlone, 2009; Reuter *et al.*, 2005). The type and amount of particles and chemicals in smoke varies depending on what is burnt, how much oxygen is available, and the burn temperature (Anenberg *et al.*, 2012). This study confirms what is known that smoke has adverse effects on the functions of the experimental animals and significantly impacted overall growth and size of vital organs.

Table 3: Effect of smoke inhalation on levels of arginase in liver and kidney

Group	Liver	Kidney
CTR (µmol/ml/min)	0.42± 0.01 [a]	0.23 ± 1.12 [a]
SMW (µmol/ml/min)	0.71 ± 0.04 [c]	0.50 ± 1.07 [c]
SMH (µmol/ml/min)	0.50 ± 0.06 [b]	0.38 ± 0.60 [b]

n=6, values are expressed as mean ± SEM. Mean values are compared using one – way ANOVA, level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at P < 0.05.

Smoke inhalation reduced the total weight gain in SMW group, administration of honey to the SMH group showed restoration of the body weight gain. Our results also show that exposure of the rats to smoke affected the liver and kidney, the organ to body weight ratio in both organs were reduced on exposure to smoke, this reduction was restored when honey was administered. This observation is in line with the finding of several studies on the effect of smoke (Al-Waili, 2003; Kücük *et al.*, 2007; Mohammed *et al.*, 2011). The

inhalation of a large concentration of soot and toxic gases may lead to lung edema and inflammation, causing death a short time after the fire (Butler and Mulholland, 2004). Harman *et al.* (2005) reported that honey has the potential to stimulate inflammatory cytokine production from monocytes, thus increasing the tissue protection from various scavenging oxidants. This is also in agreement with the findings of Al-Mamarya *et al.* (2002) and Molan (1992) who reported an antioxidant property of honey while Gheldof *et al.* (2002) identified the presence of antioxidants such as ascorbate, selenium and catalase in honey.

Our result shows that arginase activity was significantly increased in the liver and kidney of the rats exposed to smoke (SMW). This could be due to the effects of smoke-induced injury on these organs. An increase in arginase activity has been associated with the pathophysiology of a number of conditions, including an impairment in nonadrenergic and noncholinergic (NANC) nerve-mediated relaxation of the gastrointestinal smooth muscle, vasoregulatory dysfunction in systemic (Demougeot *et al.*, 2007; Johnson *et al.*, 2005; Zhang *et al.*, 2001) and pulmonary hypertension (Morris, 2006; Xu *et al.*, 2004), aging (Santhanam *et al.*, 2007; White *et al.*, 2006), diabetes (Romero *et al.*, 2008), inflammatory stimuli (Morris, 2005; Mori and Gotoh, 2000; Wei *et al.*, 2000), diabetic nephropathy (Morris *et al.*, 2011), erectile dysfunction (Bivalacqua *et al.* 2001) and bronchodilatory dysfunction in asthma (Morris *et al.*, 2004).

Differences in the activities or concentration of certain enzymes between cancer cells and their normal counterparts might be useful as biological markers of malignancy and/or aggressiveness in particular tumors (McIntire, 1984). On the other hand, since the increase in the activities of certain enzymes is an indicator of prominence or abeyance of particular biochemical reactions or metabolic pathways, one might speculate that application of measures correlating to the activities of such enzymes will lead to elucidation of therapeutic approaches when these tissues are damaged (Pamies and Crawford, 1996). The reduction of arginase activity of SMH when compared to the SWH could be attributed to the inhibition of arginase by some components of honey. Proline has been reported as the most prominent amino acid in honey (White and Doner, 1980), this amino acid has also been reported to inhibit arginase activity to certain extent (Carvajal *et al.*, 1987; Carvajal *et al.*, 1994; Fuente *et al.*, 1984).

Conclusion

The findings of this work demonstrate that honey significantly reduced the toxic effects of smoke from the use of hydrocarbon-fueled lanterns on the body weight, liver and kidney integrity in experimental rats. Smoke inhalation also caused a reduction in the activities of arginase in these two organs. We showed that oral administration of honey has a protective effect on the liver and kidney of animals exposed to smoke. We propose further studies to elucidate the exact molecular mechanism of action of honey and the subsequent use of honey as supplement alone or in combination with other drugs in protecting or treating illnesses associated with smoke.

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