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Research Article

Effects of honey supplementation on hydrocarbon-induced kidney and liver damage in wistar albino rats

Fidelis I. Achuba* and Caleb C. Nwokogba.

Department of Biochemistry, Delta State University, PMB 1, Abraka, Delta State, Nigeria

*Correspondence: Fidelis Achuba; achubabch@yahoo.com, +234(0) 8035663720

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ABSTRACT: **This study investigated the chemoprotective and ameliorative effects of natural honey on nephro-and hepatoxocity induced by gasoline and kerosene in wistar albino rats. Ingestion of gasoline and kerosene contaminated diets significantly (P<0.05) increased serum levels of urea, creatinine, potassium ion (K^+), total bihrubin, and the activities of serum hepatic marker enzymes, aspartate aminotransferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP). Conversely, serum levels of sodium ion (Na^+), chloride ion (Cl^-) and bicarbonate ion (HCO_3) were significantly decreased. However, the concentration of these serum metabolites and the activities of the hepatic marker enzymes, AST, ALT and ALP, in rats exposed to gasoline and kerosene and fed simultaneously with natural honey supplemented diet were close to those obtained in control rats. Rats that were exposed to gasoline and kerosene and later given natural honey supplemented diets after four (4) weeks of gasoline and kerosene exposure did not also differ significantly (P<0.05) in serum metabolite concentration and hepatic enzymes activities as compared to the control rats. These observations suggest that the consumption of natural honey supplemented diet has chemoprotective and ameliorative effects against gasoline and kerosene induced kidney and liver tissue damage.**

KEYWORDS: **Honey, contaminated diets, hepatotoxicity, nephrotoxicity**

INTRODUCTION

Petroleum is the most important source of primary energy in the modern world (Ohimain, 2010). However, its exploitation has caused severe deterioration of the environment, causing major changes in several natural habitats. Oil spill is the major source of petroleum in the environment. Spillage occurs during the exploration, production and transportation of crude oil (Medubari and Elijah, 2014). Moreover, exposure of humans to petroleum-derived hydrocarbons could be from gasoline fumes at the filling stations or from spilled oils and chemicals used at home such as pesticides (Al-Helaly and Ahmed, 2014). In addition, some amount may leak from underground storage tanks and enter the ground waters (Al-Helaly and Ahmed, 2014) The ingestion of crude oil or its refined products such as gasoline and kerosene orally or through exposed organisms represents a pathway for delivery of potential toxicants to the human system (Ujowundu et al, 2011).

Petroleum hydrocarbon in its crude, refined or spent form has a negative impact on a variety of living organisms (Gbadebo et al., 2009; Nwaogu and Onyeze, 2010). Several studies have shown crude oil and its refined products including gasoline, kerosene and diesel to cause adverse alterations in both biochemical and histological profile of experimental animals (Achuba, 2005; Achuba and Ogwumu, 2014a; Patrick–Iwuanyanwu et al., 2010; 2011; Ujowundu et al., 2011; 2012; Nwaogu and Onyeze, 2014). Earlier studies have indicated the use of antioxidants in ameliorating the involvement of free radicals in disease process (Farris, 1991; Verma and Nair, 2001; Ognjanovic *et al.,* 2003).

Honey is widely used by humans for nutritional and medicinal purposes (Abubakar et al., 2012; Othman, 2012). Honey is widely accepted to be beneficial to health and the antioxidant activity of honey has been studied (Moussa et al., 2012). It is believed that some of the constituents of honey may act synergistically to produce its antioxidant effect (Gil *et al.*, 1995). Significant correlation was observed between the antioxidant strength of two Malaysian honeys and their total phenolic contents (Aljadi and Kamaruddin, 2004). The administration of honey was associated with amelioration of oxidative stress in kidneys of streptozocin-induced diabetic rats (Omotayo *et al.*, 2010). Some studies have described the utilization of plant products that are rich in antioxidants including *G. latifollium* (Ujowundu et al., 2012), *Ocimum giatissimum* (Ujowundu et al., 2011) and red palm oil (Achuba and Ogwumu, 2014b) in the management and amelioration of petroleum hydrocarbon toxicity. However, studies utilizing animal products especially natural honey are scarce.

The study reported in this paper was designed to investigate the possible chemoprotective and ameliorative effects of natural honey on gasoline and kerosene induced toxicity in wistar albino rats.

MATERIALS AND METHODS

Petroleum products

The petroleum products (gasoline and kerosene) were obtained from Port Harcourt refinery, Alesa, Eleme, Rivers state, Nigeria. All the reagents used for this study were of high quality analytical grades.

Experimental animals

Thirty-six (36) mature male albino wistar rats were obtained from the Animal House, Department of Anatomy, Delta state university, Abraka, Nigeria. The experimental rats were housed in clean wooden cages and left to acclimatize for seven days on grower's mash. The rats were weighted after the acclimatization period and their weights ranged between 120–140 g.

Experimental design and treatment groups

The thirty-six mature male albino wistar rats were equally divided into 6 groups of six 6 rats per group. The control group (Group T) was fed with grower's mash only. Group F were fed with gasoline-contaminated diet plus natural honey while Group G were fed with grower's mash plus natural honey. Group O animals were fed with kerosene contaminated diet plus natural honey, and those in Group W received gasoline and kerosene contaminated diet. Rats in Group Z were fed with diets contaminated with gasoline and kerosene, and later treated with natural honey after 4 weeks of the experiment. The rats in each group were allowed free access to clean drinking water during the experiment. The feeds for the test groups were prepared fresh daily and stable feed remnant were regularly discarded.

Determination of metabolite concentration

At the end of the experiment, the rats were anaesthetized with chloroform soaked in swap of cotton wool in a desiccator. They were then slaughtered and 5 ml sterile syringes with needle were used for collection of blood from the vena cava into properly labeled plain sample bottles.

Serum creatinine was determined by Jaffe-slot alkaline picrate colorimetric method as described by Cheersbrough (2006). The concentration of urea was determined using Randox kits. The bicarbonate ion (HCO_3^-) was determined by titration method as described by Ochei and Kalhatkar (2005). The sodium ion (Na^+) and potassium ion (K^+) were determined using 410 clinical flame photometer (Sherwood Scientific). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the colourimetric method of Reitman and Frankel as described by Ochei and Koihatkar (2005).

Serum alkaline phosphatase (ALP) activity was determined in accordance with the principle of Tietz (1995) as described by Ochei and Kolhatkan (2005). Serum bilirubin was determined by the Jendrassik and Grof bilirubin method as described by Cheersbrough (2006).

Table 1: Effect of natural honey on serum urea, creatinine and electrolytes of rat fed hydrocarbon-contaminated diet.

Rat Grouping	Group	Chloride (mmol)	Sodium (mmol)	Potassium (mmol)	Bicarbonate (mmol)	Urea (mmol)	Creatinine (mmol)
Rat Feed only (control)	T	99.20 ± 2.58^{a}	131.60 ± 3.20^{a}	3.86 ± 0.27^{a}	27.40 ± 2.22^{a}	3.42 ± 0.48^{a}	66.20 ± 3.63^{a}
Mixture of rat Feed, natural Honey and petrol	F	89.60 ± 4.50^{b}	126.60 ± 3.36^{a}	5.22 ± 0.43^{b}	25.80 ± 2.79^{a}	5.59 ± 0.56^{b}	82.40 ± 6.50^{b}
Mixture of rat Feed and natural honey	G	96.40 ± 2.81^{a}	129.20 ± 2.92^{a}	4.04 ± 0.33^{b}	27.80 ± 2.33^{b}	3.14 ± 0.41^{a}	62.00 ± 3.23^{a}
Mixture of rat Feed, natural Honey and Kerosene	O	91.00 ± 3.58^{b}	127.80 ± 3.28^{a}	5.30 ± 0.46^{b}	26.80 ± 2.42^{b}	5.94 ± 1.10^{b}	86.40 ± 6.10^{b}
Mixture of rat Feed with Petrol and Kerosene	W	76.40 ± 4.92^{c}	112.40 ± 4.66^{b}	13.32 ± 0.56^{c}	18.20 ± 3.27^{b}	9.83 ± 0.52^{c}	129.00 ± 7.70^{c}
Mixture of rat Feed with Petrol and Kerosene later treated with natural Honey	Z	93.00 ± 3.0^{a}	132.40 ± 3.96^{a}	4.24 ± 0.29^{a}	28.60 ± 2.89^{a}	3.85 ± 0.52^{a}	73.80 ± 3.98^{b}

Each values is an average of six determinations (Mean ± SEM) Means with different superscript letters in the same column are significantly different at 0.05 (P<0.05) level.

Statistical analysis

All the results were expressed as means ± SEM and all data were analyzed using Analysis of Variance (ANOVA). Significant difference between the control and treatment means were determined at 5% (P < 0.05) confidence level using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The effects of feeding gasoline and kerosene contaminated diets on serum urea, creatinine, and electrolyte concentrations are shown in Table 1. The serum levels of potassium, urea and creatinine were significantly increased while serum chloride, sodium and bicarbonate ions were significantly decreased in wistar albino rats fed gasoline and kerosene contaminated diets as compared to the control. However, supplementation and post-treatment with natural honey brought the values of these parameters close to those of the control rats (Table 1).

Table 2 shows significant (p<0.05) increase in the activities of serum hepatic enzymes, AST, ALT and ALP, and significant increase in total bilirubin in rats fed gasoline and kerosene contaminated diets compared to the control. Supplementation with natural honey and post-treatment with natural honey significantly decreased the activities of AST, ALT and ALP as compared to the group fed gasoline and kerosene contaminated diets.

DISCUSSION

The effects of acute and chronic exposure of animals to toxic chemical compounds including crude oil and its refined products could be studied by evaluating the biochemical changes in various organs especially the kidneys and the liver. The results of this present study showed a significant increase in serum levels of urea, creatinine and potassium ion while sodium, chloride and bicarbonate ions decreased significantly in rats fed gasoline and kerosene contaminated diets relative to the control (Table 1). This agrees with the results of previous studies on the toxicity of crude petroleum and its refined products (Ovuru et al., 2004, Achuba and Ogwumu, 2014b). The increased levels of urea and creatinine observed in this study indicate a deficiency in the kidney's capacity to excrete these waste products. This could be due to a decrease in glomerular filtration rate (Ovuru et al., 2004). Moreover, elevated values of potassium ion and decreased levels of sodium, chloride and bicarbonate ions are indicators of impaired kidney function (Ochei and Kolhatkar, 2005). However, supplementation of the gasoline and kerosene-contaminated diets with natural honey brought the values of these serum metabolites close to the values observed in animals in the control group (Table 1). This indicates that natural honey has a protective effect on hydrocarbon-induced toxicity. Significantly, dietary supplementation with natural honey after the exposure of the animals to hydrocarbon toxicity resulted in a significant restoration of these serum metabolites close to the values of the control rats, suggesting a potential ameliorative effect of honey under these circumstances.

Table 2: Effect of natural honey supplemented diet on serum hepatic enzyme activities and bilirubin in rats fed hydrocarbon contaminated diets

Rat Grouping	Group	AST (µ/l)	ALT (µ/l)	ALP (µ/l)	Total Bilirubin (µmol/l)
Rat Feed only (control)	T	48.40 ± 1.51^{a}	24.60 ± 0.54^{a}	3.04 ± 0.054^{a}	0.39 ± 0.0044^{a}
Mixture of rat Feed, natural Honey and petrol	F	76.40 ± 2.24^{b}	26.80 ± 0.84^{a}	4.42 ± 0.083^{b}	0.42 ± 0.0054^{a}
Mixture of rat Feed and natural honey	G	47.20 ± 1.48^{a}	24.00 ± 0.50^{a}	2.90 ± 0.048^{a}	0.40 ± 0.0046^{a}
Mixture of rat Feed, natural Honey and Kerosene	O	75.20 ± 2.05^{b}	27.20 ± 0.85^{a}	4.44 ± 0.085^{b}	0.41 ± 0.0049^{a}
Mixture of rat Feed with Petrol and Kerosene	W	86.40 ± 3.93^{c}	35.20 ± 1.30^{c}	5.70 ± 0.088^{c}	0.47 ± 0.0060^{b}
Mixture of rat Feed with Petrol and Kerosene later treated with natural Honey	Z	54.00 ± 1.85^{a}	24.60 ± 0.54^{a}	3.16 ± 0.066^{a}	0.40 ± 0.0046^{a}

Each value is an average of six determinations (Mean ± SEM) Means with different superscript letters in the same column are significantly different at 0.05 (P<0.05) level.

Treatment of rats with gasoline and kerosene-contaminated diets resulted in a significant ($p<0.05$) hepatic damaged as evidence by the increased total bilirubin and elevated levels of serum marker enzymes AST, ALT and ALP (Table 2). The elevated serum AST, ALT and ALP may be as result of leakage due to hepatic injury caused by free radicals generated from the metabolism of aliphatic and aromatic hydrocarbons present in the hydrocarbon products (Achuba and Osakwe, 2003; Patrick-Iwuanyanwu et al., 2011).

Interestingly, supplementation of gasoline and kerosene contaminated diets with natural honey as well as post treatment with natural honey significantly ($p<0.05$) decreased the activities of these liver market enzymes and total bilirubin (Table 2). This suggests the protective and ameliorative effect of natural honey on gasoline and kerosene toxicity. Several studies have established antioxidant rich substances such as *Gongronema latifolum, Ocimum gratissimum* and palm oil as hepatic protective by significantly decreasing the activities of serum hepatic enzymes.(Ujowundu et al., 2011, 2012; Achuba and Ogwumu, 2014a). Natural honey is rich in antioxidants and may have played these protective and ameliorative roles by its antioxidant property. The hepatic injuring entities, free radicals, may have been scavenged by the antioxidants present in natural honey (Farombi, 2000; Koleva, 2000; Ujowundu et al., 2011)

Conclusion

This study has shown that gasoline and kerosene intoxication could cause both nephrotic and hepatocellular damage. Our results suggest that consumption of natural honey could protect and ameliorate the integrity of the kidney and the liver in response to hydrocarbon-induced toxicity

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