Vol. 27 (no. 1) 44–49 31 March 2015

Biokemistri

An International Journal of the Nigerian Society for Experimental Biology

Research Article

Effect of honey supplementation on heamatological parameters of wistar albino rats fed hydrocarbon contaminated diets

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

Received 6 March 2015: Revised 23 March 2015; Approved 23 March 2015.

ABSTRACT: The effects of dietary supplementation with natural honey on haematological profile, blood sugar and final body weight of rats fed diets contaminated with hydrocarbon petroleum products (gasoline and kerosene) were evaluated in this study. There was a significant (P<0.05) decrease in haemoglobin (Hb) content, red blood cell (RBC) count, packed cell volume (PCV) and platelet count but a significant increase in white blood cell count (WBCC) in rats fed gasoline and kerosene contaminated diet compared to the control rats. Gasoline and kerosene contaminated diet also resulted in significant (P<0.05) decrease in blood sugar and final body weight of rats as compared to the control. Supplementation of diet and post treatment with natural honey significantly (P<0.05) restored Hb content, RBC, PCV, platelet and WBCC close to control values. Although supplementation of gasoline and kerosene contaminated diet did not substantially improve the final body weight and percentage increase in weight, post treatment with natural honey significantly (P<0.05) improved the blood glucose, final body weight and percentage increase in weight of rats compared to rats fed gasoline and kerosene contaminated diet only. These findings indicate that natural honey has ameliorative effect on gasoline and kerosene induced haematotoxicity, hypoglycaemia and weight loss.

KEYWORDS: Honey, contaminated diets, haematotoxicity, hypoglycemia, weight loss

INTRODUCTION

Petroleum production began in Nigeria in 1958 and the industry has over the years become a vast operation covering both offshore and onshore oilfields (Awobajo, 1981). Crude petroleum is obtained through oil drilling, and refined into a number of consumer products such as gasoline (petrol), kerosene, diesel, asphalt, and chemical reagents used in making plastics and pharmaceuticals (kvenvolden, 2006; Speight, 2007). Kerosene and gasoline contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (Speight, 2007).

A large section of the Nigerian population, especially fuel attendants and auto mechanics, are occupationally exposed to kerosene and gasoline without cognizance for proper protection against possible adverse effects of these petroleum products (Patrick–Iwuanyanwu *et al*., 2011). The volatile components of crude oil have been reported to burn eyes and skin, and can irritate or damage sensitive membranes in the nose, eye and mouth; as well as trigger pulmonary problems when inhaled (Etkin, 1997). Moreover, once inhaled, the volatile hydrocarbons in fuels are transferred rapidly to the blood stream from the lungs and can damage red blood cells, suppress immune systems, strain the liver, spleen, and kidneys and can even interfere with the reproductive system of animals and humans (Etkin, 1997).

A large proportion of crude oil and other petroleum products are lipophilic in nature, a property that promotes their interaction with biological membranes, which could be the target sites where their adverse effects occur (Anozie and Onwurah, 2001). Alterations in the architectural structure of plasma membrane by hydrocarbons result in various altered membrane properties and altered red cell behaviour in circulation (Ita and Udofia, 2011). Crude petroleum and its refined products have been reported to cause oxidative stress (Onwurah, 1999; Achuba and Osakwe, 2003, Achuba and Otuya, 2006), induction of white blood cells and changes in serum metabolites (Kori-Siakpere, 1998; Bartimeous *et al*, 2002; Achuba and Ahwin, 2008, Achuba and Ogwumu, 2014). A previous study indicated that the damaging effect of crude oil is associated with its ability to induce lipid peroxidation in organs and tissue (Achuba and Otuya, 2006).

Several studies have indicated alterations in the hematological profile of rats fed with diets contaminated with crude oil and its refined products (kerosene, gasoline, diesel) (Uboh *et al*., 2009, 2012; Achuba and Awhin, 2008; Patrick–Iwuanyanwu *et al*., 2010, Adegoke *et al*., 2012; Achuba and Ogwumu, 2014). Similarly, the consumption of crude petroleum contaminated diets by experimental animals has been found to induce hypoglycaemia (Achuba *et al*., 2005; Ita *et al*., 2014) and reduced body weight (Patrick-Iwuanyanwu *et al*., 2011; Adegoke *et al*., 2012; Ita *et al*., 2014).

Earlier studies have shown that both plants and animals products of organic and inorganic origin have ameliorative effect on the haematological profile and body weight of rats fed with petroleum contaminated diet. Uboh *et al*. (2009) reported significant improvement in the haematological profile and body weights of rats fed with petroleum-contaminated diet and supplemented with vitamin A and E. Moreover, Achuba and Ogwumu (2014) reported a protective role of palm oil and a replenishing role of beef liver on blood parameters of rats fed with diesel-contaminated diets.

Natural honey is widely used for both nutritional and medicinal purposes (Abubakar *et al.*, 2012; Othman, 2012). Honey has been proven to be rich in both enzymatic and non-enzymatic antioxidants such as catalase, flavonoids and other polyphenols, as well as vitamins such as thiamine, riboflavin, pyridoxine, pantothenic acid, ascorbic acid, and nicotinic acid (Haydak *et al.*, 1942; Schepartz, 1966; Gheldof *et al.*, 2002; Kishore *et al.*, 2011; Abubakar *et al.*, 2012).

In this study, we investigated the protective and ameliorative potential of natural honey on kerosene and gasoline-induced haematotoxicity, hypoglycaemia and weight loss in wistar albino rats.

MATERIALS AND METHODS

Petroleum products

The petroleum products namely 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 weighted 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 6 rats per group. The control group (Group T) was fed with grower's mash only. Group F animals were fed with gasoline-contaminated diet plus natural honey and Group G were fed with grower's mash supplemented with natural honey. Rats in Group O were fed with kerosene-contaminated diet plus natural honey; and those in Group W received gasoline and kerosene-contaminated diet. Those in Group Z were fed gasoline and kerosene-contaminated diet and later treated with natural honey after four (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.

Table 1: Effect of natural honey supplemented diet on haematological profile of rats fed p contaminated diet

Rat Grouping	Group	Hb (g/dl)	PCV (%)	RBC ($x10^6/\mu/l$)	WBC ($x10^3/\mu/l$)	Platelets ($x10^3/\mu/l$)
Rat Feed only (control)	T	16.06 ± 0.25^a	49.80 ± 1.92^a	6.92 ± 0.67^a	14.98 ± 0.19^a	863.00 ± 30.53^a
Mixture of rat Feed, natural Honey and petrol	F	14.44 ± 0.13^b	42.80 ± 1.29^b	6.14 ± 036^a	17.22 ± 0.27^b	469.00 ± 16.79^b
Mixture of rat Feed and natural honey	G	15.50 ± 1.24^a	43.82 ± 1.22^c	6.66 ± 0.66^c	14.44 ± 0.16^a	828.60 ± 28.99^a
Mixture of rat Feed, natural Honey and Kerosene	O	14.48 ± 0.12^b	42.81 ± 1.26^b	5.69 ± 0.22^a	17.24 ± 0.25^c	5.94 ± 1.10^b
Mixture of rat Feed with Petrol and Kerosene	W	11.02 ± 0.19^c	30.20 ± 1.30^c	3.36 ± 0.16^c	20.96 ± 0.35^b	503.20 ± 19.52^c
Mixture of rat Feed with Petrol and Kerosene later treated with natural Honey	Z	14.26 ± 0.24^b	45.20 ± 1.40^c	6.22 ± 0.73^a	16.76 ± 0.15^b	471.2 ± 31.79^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.

Determination of haematological and biochemical parameters

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. The haematological parameters including Haemoglobin (Hb), Packed cell volume (PCV), White blood cell (WBC) count, Red blood cell (RBC) count and platelet count were determined with the aid of an automated haematology analyzer (Mindray Hematology analyzer, BC-2300). Blood glucose was determined using glucose oxidase-peroxidase method as described by Cheersbrough (2006).

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

Table 1 shows the effect of gasoline and kerosene contaminated diets on the haematological paprameters studied, as well as the protective and ameliorative effect of honey on gasoline and kerosene toxicity. The pattern shows a significant decrease in haemoglobin concentration, red blood cell count, packed cell volume, and platelets, and a significant increase in white blood cell of rats fed gasoline and kerosene contaminated diets. Augmentation of gasoline and kerosene contaminated diets with natural honey brought the values of these parameters close to the values of control rats. Treatment with natural honey after four weeks of experiment with gasoline and kerosene contaminated diets restored values of these parameters close to those of the control rats.

Table 2 shows the effect of gasoline and kerosene contaminated diets and the effect of natural honey supplementation on blood glucose and body weight of wistar albino rats. There was a significant ($p<0.05$) decrease in both blood glucose and body weights of rats fed gasoline and

Table 2: effect of natural honey supplemented diet on blood glucose and body weight of rats fed petrol and kerosene diet

Rat Grouping	Group	Glocose (mg/dl)	Initial weight (g)	Final Weight (g)	% weight increase
Rat Feed only (control)	T	65.00 ±.1.00[a]	122 ± 8.5	152 ± 11.5	24.50[a]
Mixture of rat Feed, natural Honey and petrol	F	55.20 ± 0.83[b]	132 ± 11.2	137 ± 10.8	3.78[b]
Mixture of rat Feed and natural honey	G	56 ± 0.91[b]	125 ± 8.5	165 ± 11.7	32.0[a]
Mixture of rat Feed, natural Honey and Kerosene	O	54.00 ± 0.78[d]	125 ± 11.3	132 ± 10.5	5.6[b]
Mixture of rat Feed with Petrol and Kerosene	W	45.00 ± 0.57[c]	135 ± 11.6	139.0 ± 9.9	2.96[b]
Mixture of rat Feed with Petrol and Kerosene later treated with natural Honey	Z	53.60 ± 1.03[b]	135 ± 16.0	152.0 ± 11.5	12.50[c]

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.

kerosene contaminated diets compared to the controls. Supplementation and post treatment with natural honey resulted in significant improvement in both blood sugar and body weights of the wistar albino rats

DISCUSSION

The result of this present study showed a significant ($p<0.05$) decrease in haemoglobin (Hb) content, packed cell volume (PCV), red blood cell (RBC) count and platelet count in rats fed gasoline and kerosene contaminated diets as compared to the control rats (Table 1). This correlates with the results of earlier studies on the haematotoxic effects of gasoline and kerosene (Patrick-Iwuanyanwu *et al*., 2010; Ita and Udofia, 2011). The observed decrease in haemoglobin and RBC may have resulted from the toxic chemicals present in gasoline and kerosene. Petroleum hydrocarbons may induce oxidative stress (Shakirov and Farkhutdinov, 2000) with attendant effect on red cell membrane, making it susceptible to haemolysis (Ita and Udofia, 2011).

The decreased RBC may explain the reduction in PCV and this agrees with the reports of Ita and Udofia (2011). The reduction in the values of haemoglobin, RBC and PCV seen in this study suggests that the treatment induced an anaemic condition (Ita and Udofia, 2011). Supplementation and post treatment with natural honey reversed the anaemic condition as evidenced by significant improvement in the values of haemoglobin, RBC and PCV.

The result of this study also depicted significant ($P<0.05$) increase in white blood cell count (WBCC) (Table 1). White blood cells are primarily concerned with the defense of the body against foreign substances and this is mainly achieved through leucocytosis and antibody production (Marieb, 1995; Cheersbrough, 2006). The observed increase in WBCC may therefore be the result of response to foreign and toxic chemical substance in gasoline and kerosene. The increase in white blood cell counts suggests induction of the immune system defensive mechanism during disease processes (Cheersbrough, 2006). Treatment with natural honey significantly ($P<0.05$) reduced the observed increase in WBCC close to the values obtained in control rats (Table 1).

The result of this present study also showed significant decrease in blood glucose level, final body weight and the percentage weight increase in rats fed gasoline and kerosene diets (Table 2). This result agrees with the findings of Achuba *et al*. (2005), Ita *et al*. (2014), Uboh *et al*. (2009), Patrick–

Iwuanyanwu *et al*. (2011) and Sunmonu and Oloyede (2007). Whereas Achuba *et al*. (2005) and Ita *et al*. (2014) reported hypoglycaemia following the ingestion of petroleum products, Patrick–Iwuanyanwu *et al*. (2011) and Sunmonu and Oloyede (2007) reported marked decrease ($P<0.05$) in final body weight and daily weight gain in rats fed petroleum product contaminated diets. The reduction in blood glucose, final body weight and percentage weight increase may be an indication of the inability of the rats to efficiently convert the feed consumed into useful nutrients (such as glucose) required by the body, resulting in the hypoglycaemia and reduced final body weight (Patrick–Iwuanyanwu *et al*., 2011; Sunmonu and Oloyede, 2007). Further studies would be necessary to establish the precise toxicity mechanisms behind these observations.

Studies on the effect of antioxidant vitamins A and E on petroleum product induced haematotoxicity by Uboh *et al*. (2009) showed that antioxidant vitamins restored the weight loss following exposure of rats to gasoline vapours. Natural honey is rich in antioxidants. Even though supplementation of gasoline and kerosene contaminated diet did not substantially improve the final body weight and percentage increase in weight like the post treatment, both treatments significantly ($P<0.05$) improved the blood glucose of rats compared to rats fed gasoline and kerosene contaminated diet. Supplementation and post treatment of gasoline and kerosene diets with natural honey significantly improved the blood sugar and restored the weight loss.

Conclusion

The result obtained in this study suggests that ingestion of gasoline and kerosene could possibly cause anaemia, hypoglycaemia and weight loss. Moreover, the consumption of natural honey could prevent anaemia and hypoglycaemia, and restore weight loss due to gasoline and kerosene toxicity.

REFERENCES

Abubakar, M. B., Abdullah, W. Z., Sulaiman, S. A. and Suen, A. B. (2012) A review of molecular mechanisms of the anti-Leukemic effects of phenolic compounds in honey. *International Journal of Molecular Science* 13: 15054–15073.

Achuba, F. I. and Ogwumu, M.D. (2014) Possible protective role of palm oil and beef liver on the kidney and liver of wistar albino rats fed diesel-contaminated diet. *Biokemistri* 26: 124-129

Achuba, F. I. and Ogwumu, M.D. (2014) Effect of palm oil and beef liver on diesel-induced haematotoxiaty in wistar albino rats. *Biokemistri* 26: 120–123

Achuba, F. I., Peretiemo-Clarke B.O and Okolie T.C. (2005) Oxidative stress in the brain of rabbits with petroleum-induced hypoglycaemia. *Biological Letters* 42: 33–39

Achuba, F.I. (2005). Effect of Vitamins C and E intake on Blood Lipid Concentration, Liquid Peroxidation, Superoxide Dismutase and Catalse Activities in Rabbit fed Petroleum Contaminated Diet. *Pakistan Journal of Nutrition* 4: 330–335.

Achuba, F.I. and Awhin, P.E. (2008) Protective influence of antioxidant vitamins on haematological indices of rabbits fed crude oil-contaminated diet. *Toxicological and. Environmental Chem*istry 91: 505–510.

Achuba, F.I. and Osakwe, S. A. (2003) Petroleum-induced free radical toxicity in African catfish (*Clarias gariepinus*). *Fish Physiology and Biochemistry* 29: 97–103.

Achuba, F.I., and E.O. Otuya. (2006) Protective influence of vitamins against petroleum induced free radical toxicity in rabbit. *Environmentalists* 26: 295–300.

Adegoke, A.O., Bamigbowu, E.O., Geoge–Opuda, M.I., Awopeju, T.A. and Braid, S.A. (2012) Effect of cassava based diet on some haematological parameters in albino rats fed petroleum contaminated Diet. *International Journal of Applied Research in Natural product* 5: 22–26

Anozie, O .I. and Onwuroh, I N. (2001) Toxic effect of bonny light crude oil in rat after ingestion of contaminated diet. *Nigerian Journal Biochemistry* 16;103–108.

Awobajo, S.A. (1981) An analysis of oil spill incident in Nigeria: 1976-1980: Proceedings of the 1981 international seminar, Nov 9-12, Petroleum Training Institute, pp. 58-63.

Bartimaeus E. S., Igwe C. C. and Igwe F. U. (2002) Effect of exposure to petroleum products on the levels of some haematological and biochemical parameters in motor mechanics in Portharcourt, Nigeria. *International Journal of Science and Technology* 1: 36–39.

Cheesbough, M. (2006) District laboratory practice in tropical countries (Part II). Cambridge University Press Ltd, England.

Duncan, D.B. (1955). Multiple ranges and multiple F. test. *Biometrics* 11: 1–10

Etkin D. S. (1997) The impact of oil spill on marine mammals. OSIR Report.1997. Special Report.

Gheldof, N., Wang, X. H. and Engeseth, N. J. (2002) Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry* 50: 5870–5877.

Haydak, M., Palmer, L., Tanquary, M. and Vivino, A. (1942). Vitamin content of honeys. *Journal of Nutrition* 23: 581.

Ita, S. O. and Udofia,A.U. (2011) Comparative study of some Haematological Parameters in Rat following ingestion of crude oil (Nigeria Bonny Light), gasoline, kerosene and diesel. *Asian Journal of Biological Sciences* 4: 498–505

Ita, S. O., Aluko, E.O, Olubobokun, T.H, Okon, U.A. Anta, A.B and Osim, E.E (2014) the relationship between nigerian bonny light crude oil-induced hypoglycaemia and endogenous serum insulin concentration in male wistar rats: the role of antioxidant vitamins C and E. American *Journal of Molecular Biology,* 4, 81–88.

Kishore, R. K., Halim, A. S., Syazana, M. and Sirajudeen, K. (2011). Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition Research* 31: 322–325

Kori-Siakpere O.(1998) Petroleum induced alterations in the African catish, *Clarias gariepipinus* (Teugels 1984) I – Haematology. *Nigerian Journal of Science and Environment* 1: 91–100.

Kvenvolden, K. A. (2006). Organic geochemistry–A retrospective of its first 70 years. *Organic Geochemistry* 37: 1–3.

Marieb, E.N. (1995) Human anatomy and physiology. 3rd ed. Benjamin and cummings Pub. Co, California, 585–611

Onwurah, I. N. E. (1999) Lipid peroxidation and protein oxidation in Azotobacter vinelandii exposed to mercury, silver, crude oil and Fenton reagent. *Journal of Toxic substances* 18: 167–176.

Orisakwe, O.E., Akumka, D.D., Fonne, O.J. and Gamaniel, K.S. (2000). Investigation into the pharmacological basis for fokloric use of bonny light crude oil in Nigeria. *Indian Journal of Pharmacology* 32: 231–234

Othman, N. H. (2012) Honey and Cancer: Sustainable Inverse Relationship Particularly for Developing Nations: A Review. *Evidence Based Complement Alternative Medicine* http://dx.doi.org/10.1155/2012/410406

Patrick-Iwuanyanwu, K.C. Onyemaenu, C.C., Wegwu, M.O and Ayalogu, E.O. (2011) Hapatotoxic and Nephrotoxic Effect of kerosene and gasoline-contaminated Diet in the Wistar Albino Rats. *Research Journal of Environmental Toxicology* 5: 49–57

Patrick-Iwuanyanwu, K.C., Onyemaenu, C.C., Wegwu, M.O. and Ayalogu, E.O. (2010). Haematotoxic effects of diets contaminated with petroleum products(kerosene and gasoline) in the wister albino rats. *Research Journal of Environmental Toxicology*, 4:134–140

Patrick-Iwuanyanwu, K.C., Onyemaenu, C.C., Wegwu, M.O. and Ayalogu, E.O. (2011). Hapatotoxic and nephrotoxic effect of kerosene and gasoline-contaminated diet in the wistar albino rats. *Research Journal of Environmental Toxicology* 5: 49–57

Patrick–Iwuanyanwu, K.C, Onyemaenu, C.C, Wegwu, M.O and Anyalogu, E.O (2010) Haematotonic effect of diet contaminated with petroleum products (Kerosene and petroleum wistar albino rats. *Research Journal of Environmental Toxicology* 4: 134–140

Schepartz, A. (1966). Honey catalase: occurrence and some kinetic properties. *Journal of Agricultural Research* 5: 167-176.

Shakirov, D.F. and Farkhutdinov, R.R (2000) Identification of high risk groups at the examination of workers engaged in oil-refining industry. *Gigiena Sanitariia* 15: 33–36

Speight, J.G. (2007). The chemistry and technology of petroleum (4th ed). CRC press, Taylor and Francis group, Raton, USA.

Sunmonu, T.O. and Oloyede, O.B. (2007) Biochemical assessment of the effects of crude oil contaminated catfish (*Clarias gariepinus*) on the hepatocytes and performance of rat. *African Journal of Biochemistry Research* 1: 083–089.

Uboh, F.E., Akpanabiatu, M.I., Alozie, Y., Edet, E.E., Ndem, J.I. and Ebong, P.E. (2009). Comparative effect of vitamins A and E on gasoline vapours–industrial haematoxicity and weight loss in male rats. International Journal of Pharmacology 5: 215–221.

Uboh, F.E., Usoh, I.F., Nwankpa, P. Obochi, G.O. (2012) Effect of oral exposure in nitrocellulose thinner on haematological profiles of male albino wistar rats. *American Journal of Biochemistry and Molecular Biology* 2: 227–234.