The Effects of Honey Administration on Petrol-Induced Oxidative Stress and Hepatotoxicity in *Sprague-dawley* Male Rats

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Abstract

Background: Exposure to petrol is common among individuals working in oil refineries, oil fields, filling stations and automobile mechanical workshops. Exposure to petrol is associated with a number of health hazards including hepatotoxicity and oxidative stress. Honey is rich in many antioxidants such as catalase, flavonoids, thiamine, riboflavin, pyridoxine, pantothenic acid, ascorbic acid and nicotinic acid and was recently reported to reduce petrol-induced hepatotoxicity and nephrotoxicity.

Materials and Methods: A total of 32 male Sprague-Dawley rats were randomly divided into four groups: control group (exposed to ambient air daily), petrol exposed group (exposed to petrol vapours at 11.13±1.1 cm^3/h, 6 hours daily, six days per week + distilled water 0.5 ml daily for 11 wks) honey treated group (treated with honey at 1.2 g/kg body weight) and petrol + honey group (exposed to petrol vapours at 11.13±1.1 cm^3/h, 6 hours daily + honey at 1.2 g/kg body weight daily for 11 weeks). The total body weight and liver weight of each rat were determined using a digital analytical balance.

Results: The results show that exposure to petrol was associated with significant weight loss and hepatomegaly ($p < 0.001$) and that honey administration did not result in any significant ($p > 0.05$) improvement in these toxicities. No significant alterations were observed in the liver enzymes (AST, ALT and ALP) of petrol exposed group compared with the control group. The activity of glutathione peroxidase was found to be significantly elevated in rats that were exposed to petrol and treated with honey. The activity of glutathione reductase was also found to be significantly reduced ($p = 0.035$) in petrol exposed group.

Conclusion: Exposure to Malaysian petrol may be associated with less adverse effects and honey administration may not improve the petrol-induced hepatotoxicity associated with the petrol exposure.

Keywords: Petrol, oxidative stress, honey, hepatotoxicity, hepatomegaly

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Introduction

Petrol or gasoline is one of the products of crude oil that is commonly used as fuel for automobiles and other internal combustion engines. Many studies have observed that many of the ingredients found in petrol are highly toxic and carcinogenic to humans (1-3). There are many sources of exposure to petrol which include oil refineries, oil fields, filling stations, automobile mechanical workshops, losses from underground tanks, spillage and emissions from automobiles (3,4). A number of studies has documented various health hazards associated with exposure to petrol vapour which include hepatotoxicity (5-7) and oxidative stress (8,9).

Although the hazardous effects of petrol have been previously reported many decades ago (10), recent reports regarding the hazardous effects of petrol exposure and environmental safety resulted in reformulation of petrol constituents which led to the removal of many potentially toxic components from petrol. For instance, there was a switch from the use of leaded petrol to use of unleaded one and there was also introduction of oxygenates to the new petrol formulation (11,12). This approach ensures that little or no harmful heavy metals are present in the petrol mixture and only a limited amount of benzene is traceable in the final reformulated fuel (13). It was recently recommended by the Euro II standard that the research octane number (RON), which is a determinant of fuel performance, should be 97 (14). It also recommended that not more than 500 ppm and 5% of sulphur and benzene respectively should be present in a given RON 97 petrol formulation. The Malaysian Government therefore consequently proposed to join other countries to move to Euro II standard. Despite such important move, recent literature still suggests that exposure to petrol may be associated with some health problems.

It is suggested that administration with appropriate combination of antioxidant vitamins could possibly ameliorate the toxic effects of exposure to petrol among filling station workers. Petrol-induced oxidative stress was found to be significantly improved among petrochemical station workers following administration of tablets rich in vitamins, micro-elements and flavonoids (15). A number of experiments using animal models have demonstrated that treatments of rats exposed to petrol vapour with vitamins A, E and C was associated with significantly reduced toxicities resulting from the exposure (6, 7, 16-18). Since it is established that exposure to petrol is associated with oxidative stress (19-22), it can be hypothesized that administration with natural products rich in antioxidants) like honey may improve the adverse effects associated with the exposure.

The last decade has witnessed a renewed research interest on honey. Honey is a natural yellowish-brown fluid formed from nectar by honeybees. Honey contains many antioxidants including catalase, flavonoids, thiamine, riboflavin, pyridoxine, pantothenic acid, ascorbic acid and nicotinic acid (23-27). Several lines of evidence exist to support the antioxidant effects of honey (28). It has been suggested that the antioxidant effect of honey may be due to the synergistic activity of some of its constituents (29). It was also recently observed that administration of honey was associated with amelioration of liver damage in streptozocin-induced diabetic rats (30); it was also shown to be associated with amelioration of oxidative stress in kidneys of diabetic rats (31). Honey was also recently demonstrated to exert protective effect against petrol-induced hepatotoxicity and nephrotoxicity in rats (32). The aim of the present study was therefore to assess the effect of honey administration on oxidants/antioxidants markers and liver function of petrol rats exposed to petrol vapours.

Materials and Methods

Animals

This study was approved by the Animal Ethics Committee of Universiti Sains Malaysia [USM/Animal Ethics Approval/2011/ (70) (321)]. A total of 32 male Sprague-Dawley rats aged 6-7 weeks (weighing 170-230 g) were obtained from Animal Research and Service Centre of Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia. The animals were randomly divided in to 4 groups (n = 8) and treated as follows:

- **Control group (CG):** Normal control group, administered 0.5 ml distilled water daily for 11 weeks without exposure to petrol.
- **Petrol exposed group (PG):** Exposed to petrol vapours (11.13±1.1 cm^3/h, 6 hours daily, six days per week) and given distilled water 0.5 ml daily for 11 wks
- **Honey treated group (HG):** Normal control group, treated with honey only (1.2 g/kg body weight) for 11 weeks

- **Petrol + honey group (PH):** Exposed to petrol vapours (11.13±1.1 cm^3/h, 6 hours daily, six days per week) and concomitantly treated with honey (1.2 g/kg body weight) daily for 11 weeks

All the experimental procedures were carried out according to the Institutional Guidelines for the Care and Use of Animals for Scientific Research. The animals were housed in typical rat cages with plastic bottoms and metal grid tops (45 x 25 x 30 cm high) and were subsequently allowed to acclimatize to the animal house conditions for a minimum of five days with 12h light/12h dark cycles prior to the experiment.

Source of honey and its preparation

The Tualang honey (Agromas®, Malaysia) used in this study was supplied by Federal Agricultural Marketing Authority (FAMA), Malaysia. This honey was collected in September 2011, filtered to remove solid particles, and then concentrated to 19-20% of water by oven drying at 40°C and was finally packed in to 230 ml bottle container by the supplier.

Treatment of the rats with honey

The animals that were given honey (HG and PH) were treated once daily with 1.2g/kg body weight of honey as previously reported (33) according to the duration specified above.

Rats' exposure to petrol vapours

The animals in the petro exposed groups (PG and PH) were exposed to petrol according to a previous study (34). Briefly, the animals were housed in their cages (two per cage). The cages were then positioned in the exposure chamber (100 cm x 90 cm x 150 cm) (2 cages per chamber). Four calibrated 1000 ml cans containing 500 ml of petrol were then placed in the exposure chamber and the rats were allowed to inhale the vapours evaporating from the can for 6 hours/day. At the end of the experimental period, the animals were sedated and dissected for collection of blood and liver as described below.

Total body weight and liver weight determination

The total body weight and liver weight of each rat were determined using a digital analytical balance before and after the experimental period and documented as initial body weight (IBW) and final body weight (FBW). The liver weight is expressed per total body weight.

Sample collection and processing

Approximately twenty hours after termination of the experiment, the animals were anaesthetized with intraperitoneal pentobarbital (50mg/kg) and samples were collected and analyzed as follows. Blood sample was collected by cardiac puncture into plain tubes and left to clot after which it was centrifuged at 3000 x g for 20 minutes; serum was then collected and stored at - 80˚C until use. The serum was used for the following assay: alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate aminotransferase (AST). The liver was quickly excised, weighed, washed on ice-cold normal saline and immediately stored at –80˚C until use. The frozen liver tissue was thawed, homogenized and made to 10% homogenate (w/v) in ice-cold Tris-HCI (0.1 M, pH 7.4) using glass homogenizing container at 900 rpm. The homogenate was then centrifuged at 1000 X g for 15 minutes and the supernatant was collected and used for the following analyses: total protein, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), total glutathione (GSH) and reduced glutathione (GSSG).

Biochemical analyses

The activities of ALP, ALT and AST were assayed using commercially available kits (BioAssay Systems, Hayward, CA, USA). Laboratory kits from Cayman (USA) were used for the assay of total protein and activities of SOD, GPx, GR, GST and CAT. The total GSH and GSSG concentrations were determined using Laboratory kits from Northwest Life Sciences (USA).

Statistical analyses

Data were analysed using IBM SPSS version 20. The results are expressed as mean (SD). Groups were compared by one-way ANOVA followed by post hoc test to identify differences between two groups. P value < 0.05 was considered statistically significant.

Results

The initial body weight was not significantly different among all groups of rats. However, at the end of the experimental period, it was observed that the petrol exposed groups (PG and PH) had significantly ($p < 0.05$) reduced total body weight compared to the normal control group (CG) and honey treated group without exposure to petrol (HG). No significant ($p > 0.05$) difference was observed between CG versus HG and PG

and PH. The results of effects of honey on liver weight and its enzymes activity are presented in Table 1. It shows that exposure to petrol caused statistically significant ($p < 0.05$) increase in liver weight (hepatomegaly) and that honey treatment did not result in any statistically significant ($p > 0.05$) decrease in liver weight. The results of effects of honey administration on liver enzymes and oxidative markers are presented in Table 2.

Table 1: Effects of honey on petrol-induced hepatomegaly and liver enzymes activity

Group	Treatment	liver weight (per body weight)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
CG	Distilled water	0.0310 (0.0017) *	265 (65)	60.1 (7.9)	72.7 (9.6)
PG	petrol	0.0388 (0.0033) **	325 (63)	61.9 (38.4)	72.0 (25.8)
HG	honey	0.0292 (0.0049) $^{-}$	314 (125)	63.3 (16.5)	64.2 (12.7)
PH	Petrol + honey	0.0372 (0.0037) $^{-}$	346 (57)	72.5 (9.3)	56.2 (10.9)

CG: normal control group, PG: petrol exposed group, HG: honey treated group, PH: petrol exposed, and honey treated group. Data was analyzed using one -way ANOVA followed by post hoc test. Values are expressed as mean (SD), n = 8, * $p = 0.001$ compared to PG, ** $p < 0.001$ compared with HG, $^{-}p < 0.001$, $^{-}p = 0.007$.

Table 2: Effects of honey on liver enzymes and antioxidants levels

Group	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	GR (U/mg protein)	GST (U/mg protein)	GSH/GSSG (U/mg protein)
CG	7.50 (1.91)	0.11 (0.04)	0.50 (0.38) *	0.50 (0.23)	24.46 (5.75)	3.43 (2.02)
PG	7.00 (1.85)	0.09 (0.01)	0.57 (0.31) **	0.33 (0.16) $^{-}$	32.44 (3.73)	4.71 (1.35)
HG	5.58 (1.00)	0.10 (0.02)	0.80 (0.27)	0.63 (0.14)	28.84 (4.91)	3.86 (1.58)
PH	7.20 (0.62)	0.10 (0.02)	1.27 (0.67)	0.30 (0.20) $^{-}$	32.08 (10.45)	5.27 (1.70)

CG: normal control group, PG: petrol exposed group, HG: honey treated group, PH: petrol exposed, and honey treated group. Data was analyzed using one -way ANOVA followed by post hoc test. Values are expressed as mean (SD), n = 8, * $p = 0.009$ compared to PH, ** $p = 0.019$ compared to PH, $^{-}p = 0.035$ compared to HG, $^{-}p = 0.016$ compared with HG.

Discussion

The present study demonstrates that exposure to petrol vapours is associated with poor weight gain, hepatomegaly and an imbalance in the oxidants/antioxidants status. The reduced weight seen in petrol exposed group of rats is in agreement with previous reports (34, 35). Our study also demonstrates that honey administration may not ameliorate the poor weight again associated with the petrol exposure, which is also in agreement with a previous study (33). It was previously reported that liver is highly susceptible to petrol vapour toxicity (5). The petrol-induced hepatomegaly observed in our study is corroborated by previous studies (7, 36) and may suggest liver tissue injury, increased metabolic workload or chronic inflammation due to toxic effects of constituents of petrol such as the hydrocarbons and other additives which may be metabolized in the liver. In this study, honey administration did not result in any significant improvement in the petrol-induced hepatomegaly.

Studies on changes associated with liver enzymes (AST, ALT and ALP) due to exposure to petrol have been inconsistent (6, 7, 37-39). In the present study, no significant alteration in liver enzymes activity was observed. Our findings on AST and ALT are in agreement with a previous study of petrol station workers (37) but in disagreement with previous studies in animal models (6, 7) and human subjects (38, 39). The absence of

significant effect on AST, ALT and ALP in the present study may be partly attributed to the improved quality of Malaysian petrol (14, 40) which perhaps differ from other developing countries.

The effects of exposure to petrol may be tissue specific and exposure to petrol can induce oxidative stress in many ways. For instance, benzene which is a constituent of petrol can cause oxidative stress by undergoing metabolic activation resulting in generation of reactive oxygen species (15). Another important constituent of petrol, methyl tertiary butyl ether (MTBE) is predominantly metabolized in the liver and its toxicity may be ascribed to induction of oxidative changes (20, 41, 42). A recent study (34) could not establish significant oxidative changes in the erythrocytes of rats exposed to petrol vapours, however, another study (8) demonstrated that exposure to petrol vapours was associated with oxidative stress in liver cells of male and female rats. In the present study, the activity of GPx was found to be significantly higher in PH group that were exposed to petrol and treated with honey compared to the control group; it was also found to be insignificantly higher in the HG group that received honey without exposure to petrol compared with the remaining groups. This suggests that honey administration was associated with elevated GPx activity. The present study demonstrated a reduced (statistically insignificant) GR activity among PG rats exposed to petrol compared to the normal control group. The GR activity was also significantly lower in PG rats compared to HG rats that received honey without exposure to petrol suggesting that the exposure affected the GR activity.

The present study and another previously reported similar study on Malaysian petrol (34) suggest that petrol-induced toxicities may be milder compared to studies from other countries (6, 7, 9, 32, 43-45) in which more severe toxicities were reported. Available findings suggest that the antioxidant effects of honey may depend on several factors which include the nature of the tissue and disease model investigated as well as the origin of the honey itself; hence, this may clarify why honey administration causes increased activity of certain antioxidant enzymes in certain disease model and/or tissue and in other situations causing reduced activity of the same enzymes in another disease model and/or tissue (31-33, 46, 47). In the present study, honey did not appear to show any significant improvement in petrol-induced toxicities.

Conclusions

Our study suggests that exposure to Malaysian petrol may be associated with less toxicity as evident by lack of alteration in antioxidant levels and Malaysian Tualang honey administration may not ameliorate liver damage associated with the petrol inhalation. Additional research especially in human subjects is warranted to ascertain the toxic effects that may be associated with the currently used Malaysian petrol.

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Conflicts of interest

The authors declare no conflicts of interest.

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