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Effects of Petrol Exposure on Glucose, Liver and Muscle glycogen levels in the Common African toad *Bufo regularis*

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Summary: This study investigated the effects of exposure to petrol on blood glucose, liver and muscle glycogen levels in the common African toad *Bufo regularis*. A total of 126 adult toads of either sex weighing between 70-100g were used for this study. The experiment was divided into three phases. The phase 1 experiment the acute toxicity test consisted of animals divided into six groups of 10 toads per group and were exposed to water (H_2O), H_2O + Tween 80, 2ml/l, 3ml/l, 5ml/l, and 10ml/l of petrol respectively for 96 hours using the static renewal bioassay system. In the Phase 2 experiment, the animals were exposed to H_2O, H_2O + Tween 80, 0.14ml/l, 0.3ml/l, 0.6ml/l, and 1.13ml/l of petrol respectively for 3 days; while in phase 3 experiment they were exposed to petrol solutions for 14 days. After the various exposures, the blood glucose, liver and muscle glycogen contents were determined using standard methods. The results of the study showed that the median lethal concentration of petrol (96 hours LC_{50}) was 4.5ml/l and sub-lethal concentration of petrol caused mortality of animals. Exposure to petrol solutions for 3 days had no significant effect on blood glucose level of the animals but caused significant decrease in the liver and muscle glycogen levels at high concentrations. In the animals exposed to petrol solutions for 14 days, there was a significant increase in glucose levels and significant reduction in liver and muscle glycogen levels at high concentrations when compared with the control. The results show that sub-lethal concentrations of petrol can cause mortality of animals, hyperglycemia and reduction in liver and muscle glycogen levels. The effects of petrol exposure on carbohydrate metabolism depend on the concentration and duration of exposure.

Keywords*:* Petrol, Blood glucose, Liver glycogen, Muscle glycogen

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INTRODUCTION

Several factors have been reported to affect the carbohydrate metabolism of amphibians. The factors include season, sex, temperature, dietary status, and method of handling and geographical location (Farrar and Frye, 1979a, Isehunwa and Alada, 2016).

Recently, environmental pollution has been reported to disrupt the normal blood glucose integrity.For instance, air pollution was reported to cause metabolic disorders such as diabetes mellitus (Brooks et al, 2008; Pearson et al, 2010; Kramer et al, 2010). Andersen et al (2012) reported that long-term exposure to traffic-related air pollution contributes to development of diabetes in healthy and active individuals. Previous studies have shown that exposure to benzene, mercury, lead, chromium, cadmium and nickel affected blood glucose and caused reduction in liver and muscle glycogen stores (Ozdikicioglu and Dere, 2004; Vinodhini and Narayanan; 2009; George et al, 2012). Environmental pollution has been reported to cause decline in the amphibian population (Blaustein and Wake, 1995; Alford and Richards, 1999; Blaustein et al, 2003).

Crude oil and petroleum products contribute to environmental pollution. Some of the petroleum fractions contaminate plants grown in the soil and can accumulate in the body of aquatic organisms e.g. fishes, frogs, crabs, crayfishes thereby posing great threat to the food webs (Ujowundu et al, 2011).There is also indiscriminate abuse of petrol which include people sucking it with mouth, washing hands and legs with it, the use of petrol as solvent, as insect repellent and sniffing for pleasure (Anigbogu et al, 2011).Petrol is a major source of power for generating electricity in our homes and offices and has increased the rate of exposure. Previous studies showed that petrol affected different systems of the body including the respiratory, cardiovascular, nervous system and kidney (Litovitz,1988; Schneider *et al.*, 1991, Cairney *et al.*, 2005). However, there is dearth of information on the effect of petrol on carbohydrate metabolism in amphibians. Ezike and Ufodike (2008) reported that sublethal concentration of water soluble fraction (WSF) of petrol increased plasma glucose level and decreased liver glycogen of catfish while blood glucose and cortisol levels increased in Nile tilapia, *Oreochromis niloticus* exposed to kerosene, diesel or gasoline (Abdel-Tawwab, 2012). There is little or no

information on the effect of petrol on carbohydrate metabolism in the Common African toad, *Bufo regularis*. The common African toad, *bufo regularis* is commonly found in Nigeria especially during the rainy season. This study was therefore designed to investigate the effect of exposure to petrol on blood glucose and glycogen levels of the common African toad, *bufo regularis*.

MATERIALS AND METHODS

Procurement of Animals and Petrol

A total of 126 adult toads of either sex weighing between 70-100g were used for this study. The toads were obtained from the banks of slow-moving streams, around ponds and wet bushes in Ibadan metropolis. The collection process was that of a randomly picking the toads as one finds them during the night search. Hence, selection of the animals was unbiased. Each animal was fasted overnight before start of the experiment.

The petrol (gasoline) was obtained from NNPC (Nigerian National Petroleum Corporation) fuel station along Iwo road, Ibadan. The experiment was divided into three phases. Phase 1 was acute toxicity test while Phases 2 and 3 consisted of animals exposed to petrol solutions for 3 and 14 days respectively.

Experimental Design

PHASE ONE: Preliminary Study (Acute Toxicity Test)

A total of 60 toads were used in this phase. The animals were divided into six groups of 10 animals per group. Groups 1 and 2 (controls) were put inside ordinary water (H_20) and water plus tween 80 respectively while groups 3, 4, 5, and 6 were exposed to 2ml/l, 3ml/l, 5ml/l, 10ml/l of petrol solutions respectively for 96 hours.

Experiments were carried out using daily static renewal system where we maintained renewal of test solutions every 24 hours.

The petrol was dissolved with tween 80 at a ratio of 10:1 (i.e. 10ml of petrol to 1 ml of tween 80).

Determination of LC_{50}(The median lethal dose)

LC_{50} was determined according Spraque (1975) and calculated using a graphical method.

PHASE TWO (3 Days Exposure)

Thirty toads were collected and randomly divided into six groups of 5 animals per group. Groups 1 and 2 (control groups) were exposed to H_20, and tween 80 plus H_20 respectively. Groups 3, 4, 5, and 6 toads were exposed to 0.14ml/l, 0.3ml/l, 0.6ml/l, and 1.13ml/l of petrol solutions respectively. The animals were exposed to petrol solutions for 3 days and allowed free access to insects. A daily static renewal system was used in which the test solutions was renewed every 24 hours. At the end of 3 days of exposure, each animal was anesthetized with sodium pentobarbitone 3mg/kg given intra-peritoneally. The animal was secured on its back on a dissecting board. The thorax was opened and the truncus arteriosus was dissected free from surrounding connective tissue and used for blood sample collection to determine blood glucose. Blood glucose was determined immediately using modified glucose oxidase (Trinder, 1969). The whole liver and gastrocnemius muscle of each animal were removed rapidly under anaesthesia, cleared of adherent tissues, and blood was blotted away using blotting paper. The glycogen content was determined using anthrone reagents method (Seifter et al, 1950; Jermyn, 1975).

PHASE THREE (14 Days Exposure)

For this phase 36 toads were collectedand divided into six groups of 6 animals per group. Groups 1 and 2(controls) were exposed to water, and water plus tween 80 respectively. Animals in groups3, 4, 5, and 6 were exposed to 0.14ml/l, 0.3ml/l, 0.6ml/l, and 1.13ml/l of petrol solutions respectively. The exposure period was 14 days and the animals were allowed free access to insects. A daily static renewal bioassay system was used for this experiment where we maintained renewal of test solutions every 24 hours.

At the end of 14 days exposure, blood was collected from the truncus arteriosus for glucose determination while liver and muscle were removed to determine the glycogen levels. Blood glucose was determined by modified glucose oxidase method (Trinder, 1969). The glycogen content was determined using anthrone reagents method.

Statistical Analysis

The mean, standard deviation, and standard error of mean (S.E.M) of all the values gotten from each group were calculated. The data obtained were statistically evaluated using one-way analysis of variance (ANOVA) followed by Dunnett post hoc test using software Prism, version 5 (Graph-Pad Software Inc. San Diego, CA. USA). Statistical significance was considered at $p<0.05$ level of significance.

RESULTS

PHASE ONE (Acute Toxicity Study)

After 96 hours of exposure to different concentrations of petrol solutions, no mortality was observed in the control groups (water, tween 80+water respectively) while 0%, 20%, 60%, and 100% mortalities were recorded in toads exposed to 2ml/l, 3ml/l, 5ml/l and 10ml/l of petrol solutions respectively (Table 1).

96 HOURS LC_{50} DETERMINATION

Medial lethal concentration (LC_{50}) was determined from this study using graphical method, and it was found to be 4.5ml/l.

Table 1. Toxicity test after 96 hours exposure to petrol solutions

	Treatments					
	Water	Water + Tween 80	Petrol Concentrations (ml/l)			
			2	3	5	10
No. Exposed	10	10	10	10	10	10
No. Surviving	10	10	10	8	4	0
% Alive	100	100	100	80	40	0
% Dead	0	0	0	20	60	100

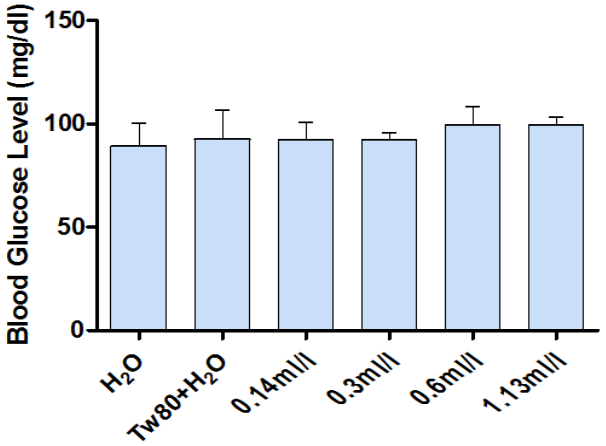

Figure1: Effect of 3-day exposure to petrol solutions on blood glucose levels**.**

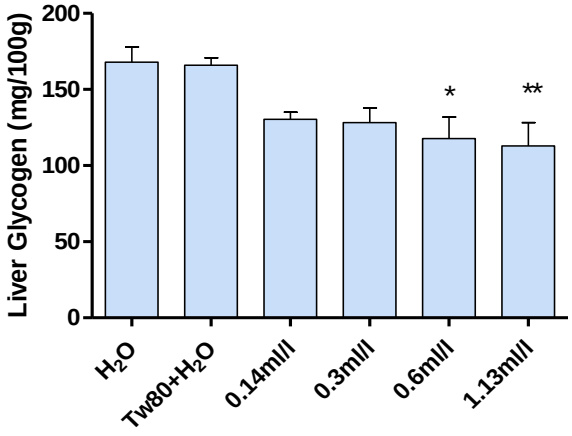

Figure 2: Effect of 3-day exposure to petrol solutions on liver glycogen in *bufo regularis.* *p <0.05, **p <0.01.

Effect of 3 days exposure to petrol solutions on blood glucose level in *Bufo regularis*

There were no significant differences in blood glucose of the animals exposed to 0.14 ml/l petrol (92.20 ± 8.61mg/dl), 0.3 ml/l petrol (92.20 ± 3.58 mg/dl), 0.6 ml/l petrol (99.60 ± 8.65 mg/dl), and 1.13 ml/l petrol (99.40 ± 3.96 mg/dl) when compared with the control groups H_20 (89.40 ± 10.96 mg/dl) and Tween 80+H_20 (92.60 ± 13.91 mg/dl) (figure 1).

Effect of 3 days exposure to Petrol solutions on liver glycogen in *Bufo regularis*

There were no significant differences in liver glycogen of the animals exposed to 0.14 ml/l petrol (130.50±4.58 mg/100g), and 0.3 ml/l petrol (128.20 ± 9.72 mg/100g) when compared with the control

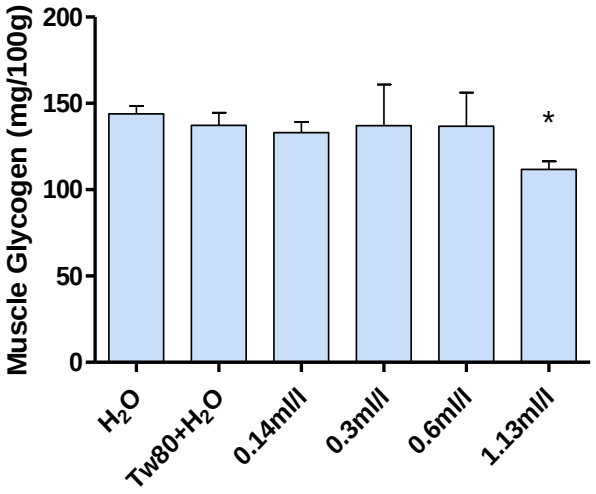

Figure 3: Effect of 3-day exposure to petrol solutions on muscle glycogen. *p <0.05

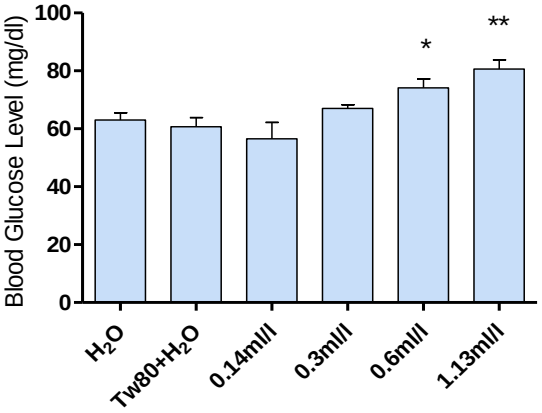

Figure 4: Effect of 14-day exposure to petrol solutions on blood glucose levels. *p <0.05, **p <0.01.

groups H_20 (167.00 ± 9.89 mg/100g) and Tween 80 + H_20 (165.96 ± 4.76 mg/100g). However, exposure to 0.6 ml/l petrol (117.70±14.21 mg/100g) and 1.13 ml/l petrol (113.00 ± 15.34 mg/100g) caused significant decrease in liver glycogen ($p<0.05$) when compared with the control groups (figure 2).

Effect of 3 days Exposure to Petrol on Muscle Glycogen in *Bufo regularis*

There were no significant differences in muscle glycogen of the animals exposed to 0.14 ml/l petrol (133.04 ± 6.22 mg/100g), 0.3 ml/l petrol (137.02 ± 23.92 mg/100g) and 0.6 ml/l petrol (136.75 ± 19.56 mg/100g) when compared with the control groups H_20 (143.98 ± 4.43 mg/100g) and Tween 80 + H_2O (137.24 ± 7.40 mg/100g). But exposure to 1.13 ml/l petrol caused significant ($p<0.05$) decrease in muscle glycogen (111.81 ± 4.63 mg/100g) when compared with the control groups (figure 3).

Effect of 14 days Petrol Exposure on Blood Glucose Level in *Bufo regularis*

There was no significant difference in blood glucose of the animals exposed to 0.14 ml/l petrol (56.51±5.71 mg/dl) and 0.3 ml/l petrol (67.00±1.32 mg/dl) when

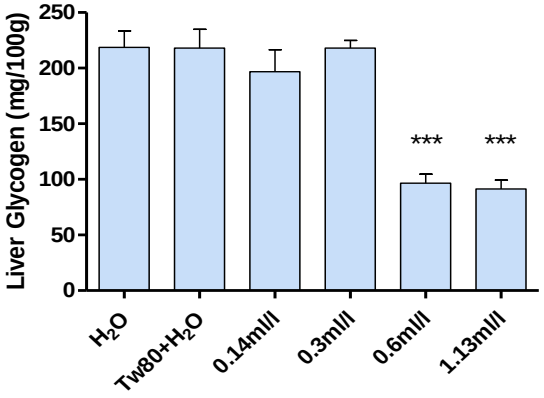

Figure 5: Effect of 14- day exposure to petrol solutions on liver glycogen. ***p<0.001.

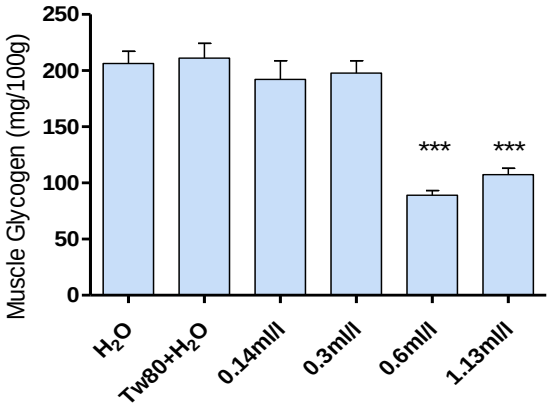

Figure 6: Effect of 14-day exposure to petrol solutions on muscle glycogen. ***p<0.001.

compared with the controls. However, animals exposed to 0.6 ml/l petrol (74.17±3.04 mg/dl) and 1.13 ml/l petrol (80.67±3.08 mg/dl) had significant increases in glucose level when compared with the control groupsH_20 (63.00±2.54 mg/dl) and Tween 80+H_20 (60.67 ± 3.24) (figure 4).

Effect of 14 days Petrol Exposure on Liver Glycogen in *Bufo regularis*

Exposure to 0.14 ml/l petrol (196.80 ±19.67 mg/100g), and 0.3 ml/l petrol (218.10 ±6.78 mg/100g) did not produce any significant effect on liver glycogen of the animals when compared with the controls. However, there was significant decrease ($p < 0.05$) in the liver glycogen of animals exposed to 0.6 ml/l petrol (96.58±8.13 mg/100g) and 1.13 ml/l petrol (91.45±7.88 mg/100g) when compared with the control groups H_20 (218.00 ±14.76 mg/100g), Tween 80 + H_2O (218.10 ±16.75 mg/100g) (figure5).

Effect of 14 days Petrol Exposure on Muscle Glycogen in *Bufo regularis*

There was no significant difference in the muscle glycogen of the animals exposed to Tween 80 + H_2O (211.20 ±12.95 mg/100g), 0.14 ml/l petrol (192.30 ±16.53 mg/100g), and 0.3 ml/l petrol (197.80 ±10.99 mg/100g) when compared with the control (206.40 ±10.90 mg/100g). However, there were significant reductions ($p < 0.05$) in the muscle glycogen of animals exposed to 0.6 ml/l petrol (89.12±4.21 mg/100g) and 1.13 ml/l petrol (107.50 ±5.58 mg/100g) when compared with the control (206.40 ±10.90 mg/100g) (Figure 6).

DISCUSSION

Acute toxicity of petrol exposure on common African toad (*Bufo regularis*)

The results of the present study showed that exposure to petrol solution was toxic to the toads and caused their mortality even at a concentration as low as 0.3ml/l. The mortality rate increased as the concentration of petrol increased. Based on the international classification of toxicity of substances using median lethal concentration (LC_{50}), petrol is toxic to the toads. The results of this study revealed the medial lethal concentration (96 LC_{50}) to be 4.5ml/l. The results also suggest that sub-lethal concentration of petrol can cause mortality in animals. This is consistent with the studies in fishes (Moles and Norcoss, 1998; Oladimeji and Onmumere, 1988; Dede and Kaglo, 2001; Fafioye, 2006, Renner et al, 2008).

Effect of Exposure to petrol solution on Blood Glucose levels

The results of the present study showed the effects of 3 days and 14 days exposure to petrol solutions on the carbohydrate metabolism of the Common African toad *bufo regularis*. The observation of the present study in which 3-day exposure to petrol solutions failed to produce significant effect on the blood glucose while the 14- day exposure to petrol solutions caused significant increase in blood glucose levels at high concentration seems to suggest that the effect of petrol exposure on glucose level depends on the concentration and duration of exposure to petrol solution. This is consistent with studies of (Wedemeyer et al, 1984; Omoregie and Ufodike, 1999; Ezike and Ufodike, 2008; Simonato et al; 2013) in fishes. They observed increases in plasma glucose levels of fishes exposed to water soluble fraction (WSF) of petrol. The hyperglycemia developed was dose dependent. Golet et al, (2002) also reported higher glucose levels in pigeon guillemots (*Cephus Columbia*) exposed to oil spillage caused by increased corticosterone levels. Almeida *et al.* (2001) attributed the increase in blood glucose in Nile tilapia *Oreochromis niloticus* to intensive glycogenolysis. Glucose level is an important indicator of stress (Barton, 2002). The observation of the present study in which exposure to petrol caused increase in glucose level probably indicates that petrol induced stress in the toads by causing hyperglycemia. This agrees with the studies of (Wedemeyer et al, 1984; Omoregie and

Ufoke, 1999) in fishes. The increase in glucose level is caused by stress hormone catecholamines which caused mobilization of glucose from glycogen stores to enable the animal cope with increased respiration rate (Nakano and Stimpso, 1967). Pollutants such as cadmium, chromium, nickel and lead, which are petrol metallic components have been reported to cause hyperglycaemia in fishes and common carp (*Cyprinus carpio L*) respectively through glycogenolysis (Zikic *et al.* 1997; Vinodhini and Narayanan,2009). However, the observation of the present study contrasts the findings of (Newman et al, 2000; Alonso-Alvarez et al,2007) who reported decrease in circulating glucose levels of America coots *(Fulica Americana)* and seabirds after short and long-term exposure to crude oil from oil spillage.

Effect of Petrol Exposure on Liver and Muscle Glycogen

The results of this study showed that 3-day and 14-day exposures to petrol solutions at high concentrations caused significant decreases in liver and muscle glycogen levels of the toads. This probably suggests that the hepatic and muscle glycogens contributed to the observed increase in blood glucose in the present study. The results also show that the effect of petrol on glycogen stores depends on the concentration and duration of exposure to petrol solutions. This is consistent with the studies of (Oladimeji and Ologunmeta, 1987; Omoregie et al 1995; Ezike and Ufodike, 2008) who reported significant reduction in liver and muscle glycogen of fishes exposed to WSF crude oil and petrol. The observation of the present study also suggests that petrol probably induced stress in the animals and caused release of stress hormones such as catecholamines and cortisol. Catecholamines and adrenocorticosteroids have been reported to be secreted in increased amounts as a result of stress stimuli and caused marked changes in carbohydrate reserves of fishes (Nakano and Stimpson, 1967). Petrol constitutes an environmental stressor, and the body responds to such stress in an attempt to maintain the homeostatic state. The blood glucose increase, liver and muscle glycogen decrease are part of the metabolic responses that occur in such state. However, Adebrium (1976) attributed the reduction in liver glycogen to enzymatic activities of the liver such as glycogen phosphorylase. Also, Levasque *et al*(2002) reported that the heavy metals in petrol could cause decrease in glycogen reserve of fishes and invertebrates by affecting the activities of enzymes that function in carbohydrate metabolism. For instance, glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase, and succinate dehydrogenase may have been stimulated by lead (Sobha *et al.*, 2007). Benzene in petrol has been shown to deplete glycogen storage (Moszczyski and Lisiewicz, 1978) and affect enzymes in the glycogen pathway (Samon and Philip,1974). The study of Ozdikicioglu and Dere (2004) showed that liver and muscle glycogen of benzene-treated groups was lower than that of the control group. And that benzene or its metabolites may have affected glycogen metabolism. The results of the present study suggest that exposure to sub-lethal concentrations of petrol induced stress in the animals and caused hyperglycemia probably through reduction in glycogen levels. The effects of petrol on the carbohydrate metabolism also depend on the concentration and period of exposure to petrol solutions.

It can be concluded that petrol is toxic to the toads and the median lethal concentration of petrol (96Lc50) is 4.5ml/l. Exposure to sub-lethal concentrations of petrol could cause mortality of animals and hyperglycemia through glycogenolysis. The effects of petrol on carbohydrate metabolism depend on the concentration and duration of exposure.

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