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RESEARCH ARTICLE

EFFECTS OF PETROL CONTAMINATED DIET AND WATER ONGLUCOSE, LIVER GLYCOGEN, PLASMA LACTATE AND LIPID PROFILE IN MALE WISTAR RATS

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Abstract

Exposure to petrol have been shown to cause detrimental health effects. Literature is sparse on whether exposure to petrol causes metabolic disorders. This study investigated the effects of petrol contaminated diet andwater on blood glucose, liver glycogen, plasma lactate, triglycerides and lipid profile in male Wistar rats. The study was divided into two phases. Phase one study consisted of 15 male Wistar rats divided into three groups (n = 5/group). Groups 1-3 were given distilled water, petrol-contaminated diet (20 mL petrol/200 g rodent chow; PCD), and petrol-contaminated water (0.5 mL/kg; PCW) orally and respectively for .two weeks. In the phase two study, 15 male Wistar rats were divided into three groups (n = 5/group), animals in groups 1-3 received distilled water, petrol contaminated diet, and petrol-contaminated water orally and respectively for four weeks.At the end of the experiment, fasting blood glucose, liver glycogen, plasma lactate, triglycerides and lipid profie were measured.The results of this study showed that the administration of petrol contaminated diet and water caused significant increase in plasma lactate levels, triglycerides (TG),total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL)and a significant reduction in liver glycogen and high density lipoprotein(HDL) in male Wistar rats. In conclusion, the study revealed that, exposure of rats to petrol contaminated diet and water caused changes in lipid profile, altered glucose and lipid metabolism in male Wistar rats. Exposure to petrol should therefore be restricted or avoided.

Introduction:-

Petrol or gasoline,also known as premium motor spirit (PMS), is obtainedfrom fractional distillation of petroleum, and used as a fuel in internal combustion engines. Petrol is used as fuel for motor vehicles, generators, and power plants (Momoh and Oshin, 2015).Exposure to petrol occurs during refueling at gas stations, illicit disposal, spillage, and underground water contamination (drinking water wells) as associated with areas with frequent tanker falling and fuel exploitation. Exposure also occurs through inhalation, skin contact, contaminated food, and water (Ubani et al, 2009).

Petrol consists of hydrocarbons, some additives like tetraethyl lead, ethanol and some other constituents including benzene and toluene (Adegoke et al, 2020).Petrolhas been reported to cause changes in blood chemistry and induced

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anemia through bone marrow hypoplasia (Okoro et al, 2006).Petroleum hydrocarbons or carbon-containing compounds are converted into free radicals or activated metabolites during their oxidation in thecells. These activated metabolites react with some cellular components such as membrane lipids andproduce lipid peroxidation products which may lead to membrane damage (Odo et al., 2012). The consumption of petroleum hydrocarbon (PHC)-contaminated diets has been reported to cause liver enlargement, growth suppression and histological changes (Onwurah and Eze, 2000). Liver plays central role in maintaining lipid homeostasis. Ingestion and exposure to crude oil fractions also has been reported to affect lipid metabolism (Uboh et al, 2005). Lipids are organic compounds containing hydrocarbons and important for the structure and functions of living cells. Lipids are also used as metabolic fuels. Environmental exposure to benzene and toluene has been reported to disrupt lipid metabolism in humans (Shin et al, 2022).

Previous studies have reported that exposure to water and diet contaminated with various fractions of petroleum causedoxidative stress on different tissues. However, there is limited information on the effects of petrol-contaminated diet and water on glucose metabolism, and plasma lactate level. Therefore, this study investigated the possible effects of petrol contaminated diet and water on glucose metabolism, plasma lactate and lipid profile inmale Wistar rats.

Materials and Methods:-

The study was carried out following the National Institutes of Health Guidelines for the Care and Use of Laboratory animals. Thirty male Wistar rats weighing(180±20g)were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were kept in well-aerated plastic cages covered with wire mesh, under an ambient temperature of 25±2^{0}C humidity and light/dark cycle of 12/12 h and were fed ad libitum with standard rat chow and had access to tap water. The study was carried out in two phases. After two weeks of acclimatization, the animals were randomly assigned to two groups (n=15/phase) for the two phases.

Phase one study consisted of fifteen rats randomly divided into three groups (n=5/group). Group I (Control) rats were fed standard chow plus10mg/kg of distilled water; group II was given petrol contaminated diet(PCD) containing (10% of petrol incorporated into 200 g of rat chow(Ogara et al. 2016) and group III received petrol contaminated water (PCW)0.5ml/kg of petrol contaminated water given orally (Azeez et al. 2013; Momoh and Oshin 2015). The animals were fed for two weeks.

Phase two study, fifteen rats randomly divided into three groups (n=5). Groups 1-111 rats were administered 10mg/kg distilled water (control), petrol contaminated diet (PCD) and petrol contaminated water (PCW) respectively for four weeks.

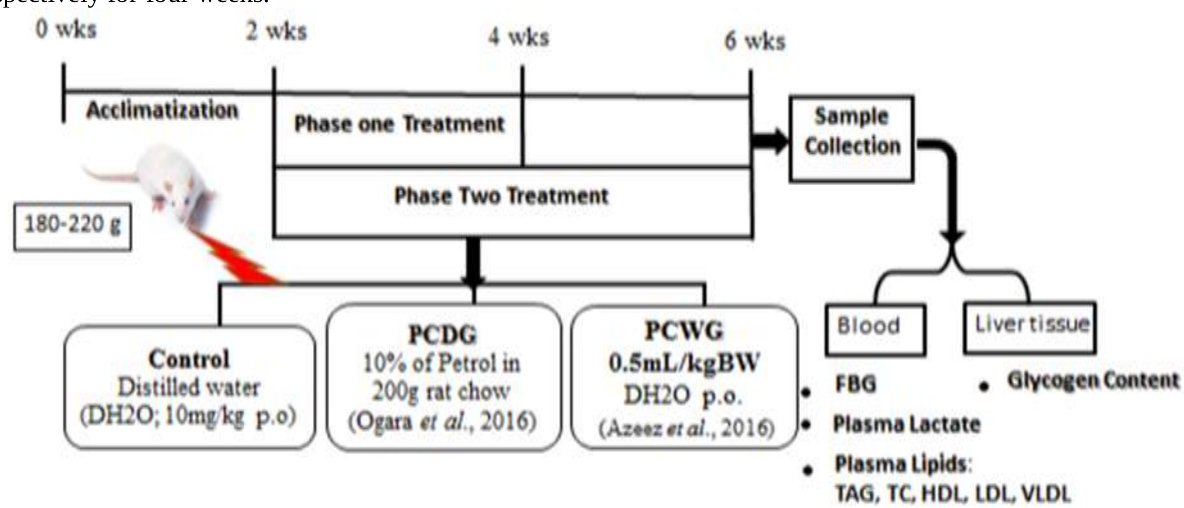

Figure 1:- showsexperimental Protocol.

Purchase of Petrol

The petrol also known as premium motor spirit (PMS) was obtained from Nigerian National Petroleum Corporation (NNPC) fuel station along Iwo Road, Ibadan, Nigeria.

Animal Sacrifice, Tissue Collection, and Processing

At the end of the experiment, blood was collected from animals via retro-orbital puncture using heparinized capillary tubes into an EDTA container and placed over an ice pack. The blood samples collected were centrifuged at 3000rpm for 10 mins to obtain the plasma samples and stored in a refrigerator before biochemical assay.Then, the liver tissue was excised immediately following the cervical dislocation of the animals and glycogen content determined immediately.

Measurement of Blood and Glucose Glycogen levels

The fasting blood glucose (FBG) was determined immediately at the end of each phase of the study. Blood glucose was estimated by modified glucose oxidase method (Trinder, 1969).

Glycogen concentration of liver tissue was determined using modified Anthrone reagent method(Seifter **et al**, 1950, Jermyn, 1975).

Estimation of Plasma Lactate and Lipid Profile

The blood plasma was used for measurement of plasma lactate and lipid profile. Plasma lactate, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels were determinedby standardized enzymatic colorimetric methods using an assay kit obtained from Fortress Diagnostic®, (United Kingdom). The values of very low-density Lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated mathematically (Sampson, **et al**., 2020).

VLDL = Triglyceride/5 and LDL = Total Cholesterol - (Triglyceride/5 + HDL)

Statistical Analysis

Data were presented as Mean ± SEM of the variables measured. Differences in mean values were compared using student’s t test and one-way of variance (ANOVA) followed by Tukey post-hoc test using software Prism, version 9.0.0 (Graph-Pad Software Inc. San Diego, CA. USA). Statistical significance was considered at $p<0.05$ level.

Results:-

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on fasting blood level in male Wistar rats

Treatment of rats with petrol contaminated diet (PCD) and petrol contaminated water(PCW) for two weeks and four weeks respectively caused insignificant increase in fasting blood glucose compared with the control groups (figure 2).

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on liver glycogen

Rats treated with PCD and PCW for two weeks and four weeks respectively had significant ($p<0.05$) reduction in liver glycogen levels compared with control groups (figure 3)

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on plasma lactate levels in male Wistar rats

There was significant increase ($p<0.05$) in plasma lactate levels in animals fed with petrol contaminated diet (PCD) for two weeks and four weeks compared with control groups (figure 4). However, there was insignificant increase in plasma lactate levels of animals given PCW for 2 weeks and 4 weeks respectively compared with control groups(figure 4).

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on lipid profile in male Wistar rats.

Treatment of animals with PCD and PCW for two weeks and four weeks respectively caused significant reduction ($p<0.05$) in plasma HDL compared with control groups (figure 5).

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on plasma total cholesterol level in male Wistar rats

Animals treated with PCD and PCW for 2 weeks had significant increase ($p<0.05$) in plasma total cholesterol compared with control group (figure 6).

Treatment of animals with PCD and PCW for 4 weeks also caused significant increase in plasma total cholesterol compared with control group (figure 6).

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on plasma triglycerides (mg/dl) in male Wistar rats

Treatment of animals with PCD and PCW for two weeks and four caused significant increase ($p<0.05$) in plasma triglyceride compared with control groups (figure 7).

Effects of petrol contaminated diet (PCD) and petrol contaminated water (PCW) on LDL in male Wistar rats

There was significant increase ($p<0.05$) in plasma LDL in animals treated with PCD and PCW for two weeks and 4 weeks respectively compared with control groups(figure 8).

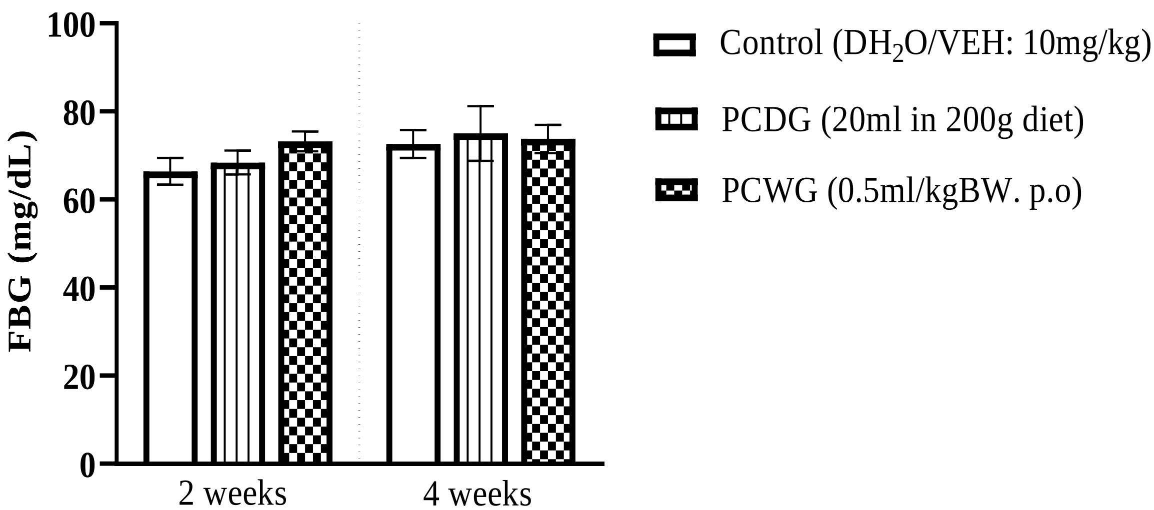

Figure 2:- Effects of Petrol Contaminated Diet (PCD) and Petrol Contaminated Water (PCW) on blood glucose level in male Wistar rats.

Exposure toPCD and PCW for 2 and 4 weeks caused insignificant increase in blood glucose. Bar represents the mean ± S.E.M (n=5).

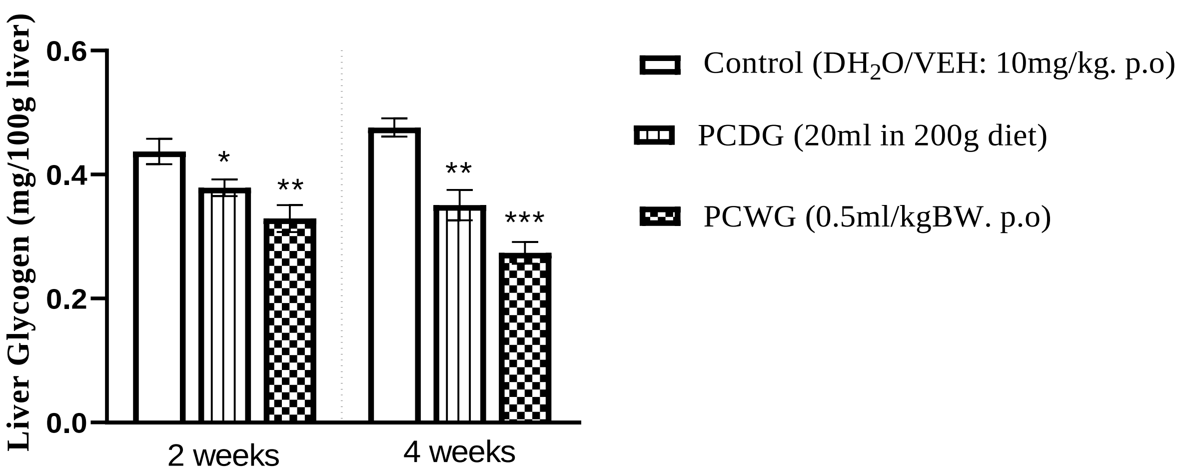

Figure 3:- Effects of Petrol Contaminated Diet and Petrol Contaminated Water on Liver Glycogen in Male WistarRats.Exposure to PCD and PCW for 2 and 4 weeks caused significant decrease in liver glycogen. Bar represents the mean ± S.E.M (n=5).

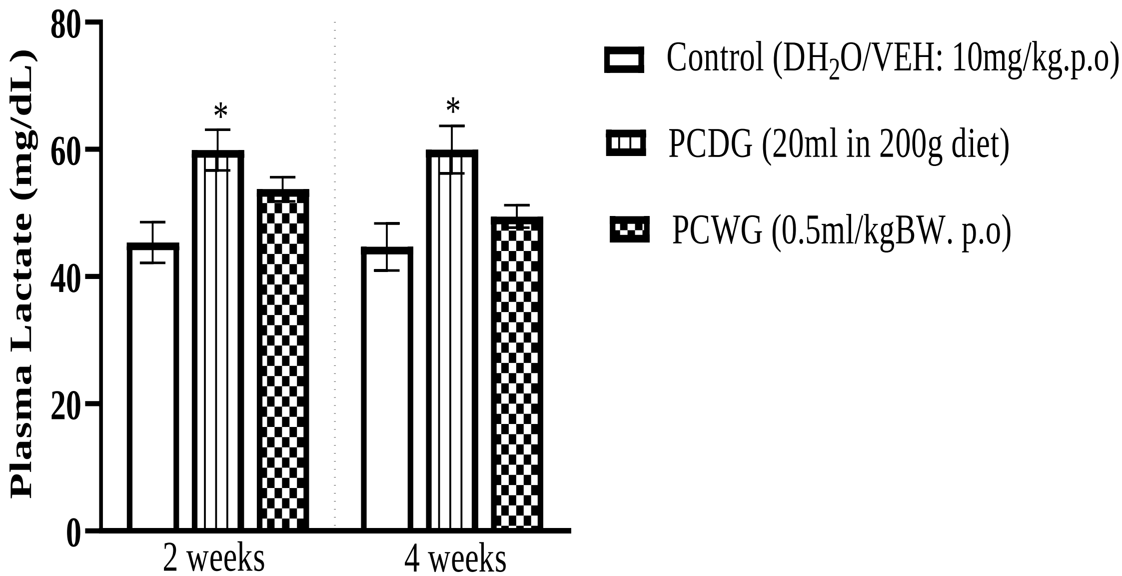

Figure 4:- Effectsof Petrol Contaminated Diet and Petrol Contaminated Water on plasma lactate (mg/dl) in male Wistar rats.Exposure to PCD for 2- and 4-weeks caused significant increase in plasma lactate levelsand insignificant increase in rats exposured to PCW. Bar represents the mean ± S.E.M (n=5).

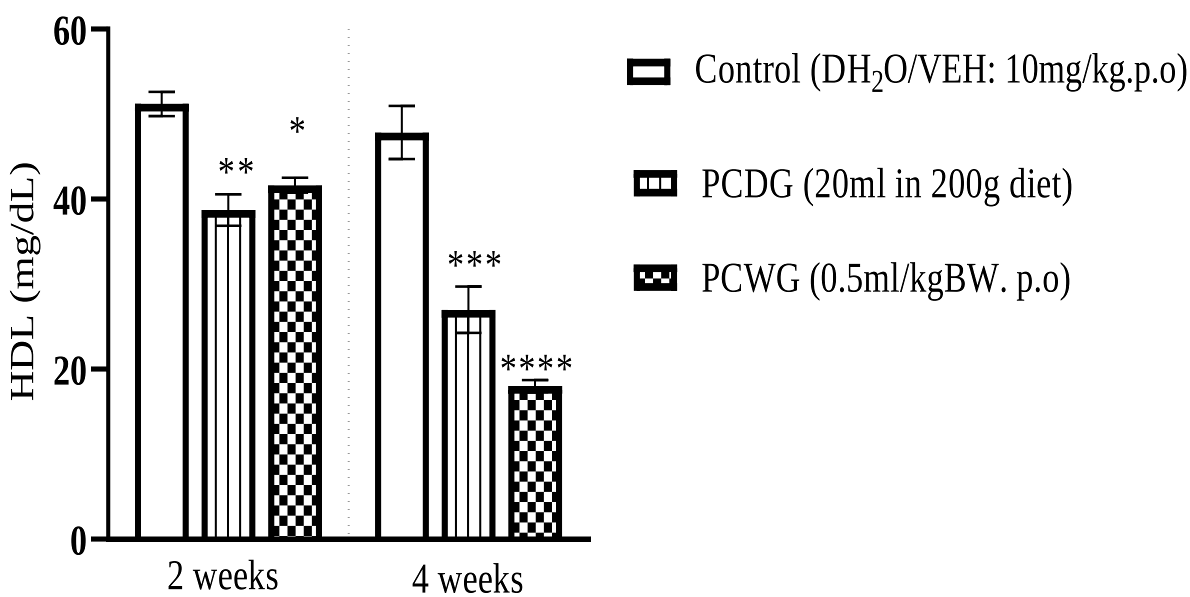

Figure 5:-Effectsof Petrol Contaminated Diet and Petrol Contaminated Water on Plasma high density lipoprotein (HDL) Level in Male Wistar Rats. Exposure of animals to PCD and PCW for 2- and 4-weeks caused significant reduction in HDL level.

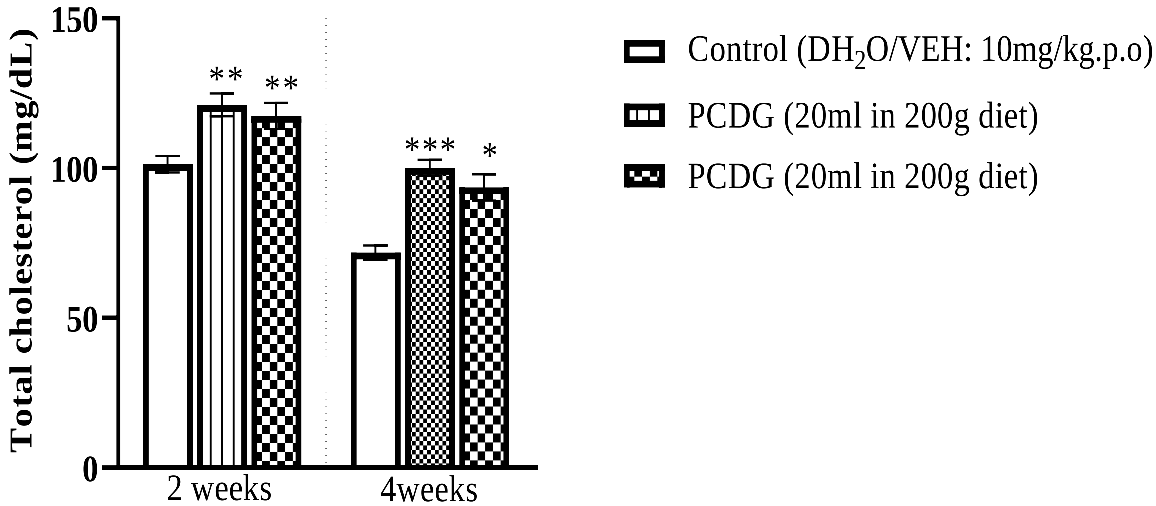

Figure 6:- Effectsof Petrol Contaminated Diet (PCD) and Petrol Contaminated Water (PCW) on total cholesterol level (TC). There was significant increase in total cholesterol levels in animals exposed to PCD and PCW for 2 and 4 weeks respectively. Bar represents the mean ± S.E.M (n=5).

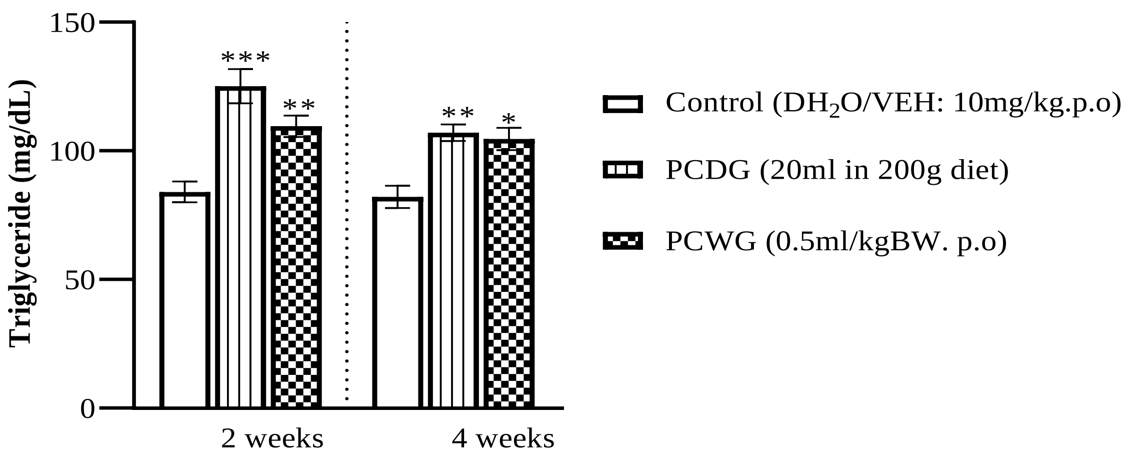

Figure 7:-Effectsof Petrol Contaminated Diet and Petrol Contaminated Wateron plasma Triglyceride (TG) (mg/dL) in male Wistar rats.Exposure to PCD and PCW for 2 and 4 weeks caused significant increase in plasma TG Levels of the animals. Bar represents the mean ± S.E.M (n=5).

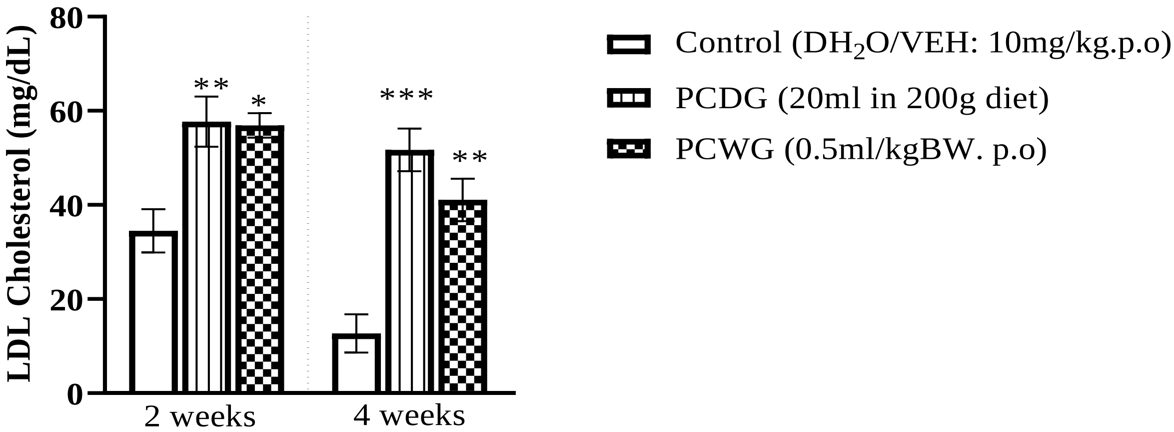

Figure 8:- Effectsof Petrol Contaminated Diet and Petrol Contaminated Wateron plasma Low Density Lipoprotein (LDL) (mg/dL) in male Wistar rats.Exposure to PCD and PCW for 2 and 4 weeks caused significant increase in plasma LDL Level. Bar represents the mean ± S.E.M (n=5).

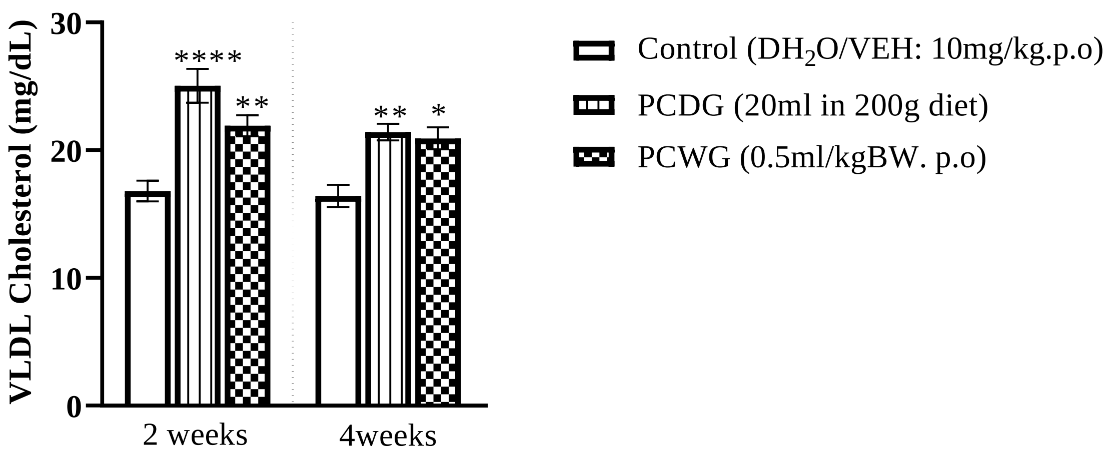

Figure 9:-Effectof Petrol Contaminated Diet and Petrol Contaminated Wateron plasma Very Low-Density Lipoprotein (LDL) cholesterol (mg/dL) in male Wistar rats. Exposure to PCD and PCW for 2 and 4 weeks caused significant increases in plasma VLDL Level. Bar represents the mean ± S.E.M (n=5).

Discussion:-

Despite several health challenges associated with petrol usage such as headache, eye irritation, dizziness, cough, nausea, vomiting, confusion, and breathing difficulties the use of petrol remained inevitable (Abubakar et al., 2015). This study investigated the effects of petrol on blood glucose, liver glycogen, plasma lactate, triglycerides and lipid profile in male Wistar rats.

The observed increase in fasting blood glucose in this study is consistent with previous studies(Alkindi et al, 1996, Simonato et al, 2013, Isehunwa et al, 2016, Owumi et al. 2021). The increase in blood glucose may be due to increase in energy demand and release of stress hormone. In an earlier study,ingestion of soluble fractions of Omani crude oil was reported to cause increase in plasma cortisol and glucose levels (Alkindi et al, 1996). There was also the report of increased glucose levels in pigeon guillemots (Cephus Columbia) exposed to oil spillage caused by increased corticosterone level (Golet et al, 2002). However, this observation contrasts the studiesof (Achuba, 2005, Alonso-Alvarez et al, 2007, Ita et al, 2014) which reported reduction inblood glucose of rats exposed to gasoline-contaminated diet and crude oil. The mechanism for the reported hypoglycemic effect is not clear (Ita et al, 2014).

The reduction in liver glycogen and increased blood glucose observed in the present studyagrees with thestudies of (Ezike and Ufodike, 2008; Isehunwa **et al**. 2016). They reported hyperglycemia and significant reduction in liver and muscle glycogen of fishes fed with WSF crude oil and in the common African toad **Bufo regularis** exposed to petrol solution respectively.Thereduction in liver glycogen and increased blood glucose may be due to stress caused by ingestion of petrol contaminated diet and water and activities ofstresshormonessuch as corticosterone and catecholamines. It has been reported that rats exposed to gasoline fumes had increased level of corticosterone (Owagboriaye et al, 2016). This may explain partly the reduction in glycogen level and increased blood glucose observed in the present study. Rapid secretion of corticosterone is part of physiological response to stress (Owagboriaye et al, 2016). The heavy metals in petrol have been reported to cause a decrease in glycogen reserves of fishes through the activities of enzymes that function in carbohydrate metabolism (Levesque et al, 2002). Benzene also has been reported to cause decrease in glycogen content of rats (Ozdikicioglu and Dere, 2004).

Lactate is produced by most tissues especially the muscle and is rapidly cleared by the liver and small amounts by the kidneys (Lowis et al, 2021). Lactate can be a source of energy for the cell in which it was produced or nearby cells or whole body (Cori cycle) (Garcia-Alvarez et al, 2014).The findings of the present study in which ingestion of petrol contaminated diet caused increased plasma lactate may be due partly to stress response with increased metabolic rate and accelerated glycolysis. The increased plasma lactate in rats may also be attributed to petrol-induced damage to the liver. This may have compromised the liver of its effectiveness in clearing the plasma lactate. Studies have reported toxic effects of gasoline on liver and the kidney (Bokolo and Ligha, 2013, Oyebisi et al, 2013).

Effects of petrol contaminated diet and water on lipid profile in male Wistar rats.

Lipid plays important roles in the body to store energy and as the components of biological membranes, steroid hormones, bile acids, and vitamins. They are supplied from diets or the de novo synthesis in the liver. Liver plays central role in lipid homeostasis (Roger et al, 2003). The findings of this study in which exposure to PCDand PCW caused significant changes in lipid profile is consistent with previous studies (Ubani et al, 2009, Anigbogu and Ojo, 2009). They reported that rats fed with contaminated gasoline diet showed significant increase in total cholesterol, LDL-cholesterol,and triglycerides. Earlier study has also reported increase in total triglyceride and total cholesterol in rats exposed to petrol and kerosene fumes (Uboh et al, 2005). The hypercholesteremic effect observed in this study may be due to liver damage caused by petrol leading to alterations in serum lipids. Lipid metabolism is affected when there is liver damage (Ubani et al, 2009). Ogara et al (2016) reported significant increase in cholesterol concentration and lipid peroxidation in Wistar rats fed with crude oil contaminated diet. There was also the report of reciprocal relationship between HDL-Cholesterol and LDL-Cholesterol in rabbits fed with crude petroleum contaminated diet (Achuba, 2005). In this study, the observedsignificant increase in total cholesterol, LDL-Cholesterol and significant decrease in HDL-Cholesterolagrees with the findings of Adegoke et al (2020). The alterations in lipid profile might have resulted from petrol-induced liver damage leading to functional abnormalities in hepatocytes. There was also the report of disruption of lipid metabolism and increased triglycerides in humans exposed to benzene and toluene (Shin et al, 2022). The observation of the present study is consistent with recent study which reported significant increase in plasma levels of total cholesterol, triglyceride and low density lipoprotein in benzene workers at Khartoum State (Dahab et al, 2024).However, the study of (Ojo et al, 2015) reported no significant alterations in lipid profile of rats exposed to crude oil.

When petroleum hydrocarbons are oxidized in cells, they produce free radicals or activated metabolites which may react with some cellular components such as membrane lipids leading to lipid peroxidation products and membrane damage (Ogara et al, 2016).Consumption of petroleumcontaminated diets has been reported to cause liverenlargement, growth suppression and histologicalchanges (Onwurah and Eze, 2000). Toxicity of petroleum fractions is related to its hydrophobicity. Lipid solubility determines the passage of petroleum components through plasma membrane of cells and the degree of membrane disruption (Freedman, 2000).However, the findingsof the present study in which exposure to PCD and PCW caused significant increase in triglyceride levels is in agreement with the studies of (Ubani et al, 2009, Shin et al, 2022, Dahab et al, 2024) but contrasts the studies of (Aberare et al, 2011, Adegoke et al, 2020) which reported significant decrease in the levels oftriglycerides of rats exposed to fumes of premium motor spirit.It has been reported that gasoline caused cellular damage and functional abnormalities in hepatocytes leading to redistribution of cholesterol among the lipoprotein fractions (Ubani et al, 2009).

The results of this study showed that ingestion of petrol contaminated diet and water altered glucose and lipid metabolism in the rats probably as a result of damage to the liver. It has been reported that gasoline caused cellular damage and functional abnormalities in hepatocytes leading to redistribution of cholesterol among the lipoprotein fractions (Ubani et al, 2009).

Conclusion:-

In conclusion, the results of this study showed that exposure to petrol-contaminated diet and water causedsignificant decrease in liver glycogen, increased plasma lactate, triglycerides and caused changes in lipid profile . The present study reveals that exposure to petrol can predispose to dyslipidemia and alter glucose and lipidmetabolism. Therefore, exposure to petrol should be restricted or prevented.

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