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ORIGINAL ARTICLE

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# Sub-chronic oral propylene glycol administration sustained normal liver function and histology: A suitable alternative vehicle for hydrophobic test-compounds

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## ABSTRACT

**Background:** An undocumented controversy exists on the suitability/unsuitability of propylene glycol as a solvent or vehicle for hydrophobic test-compounds in animal experimentation(s). This is partly due to the paucity of evidence that aims to probe its histological effects beyond Hematoxylin and Eosin assessment.

**Objective:** To determine the potential suitability/unsuitability of oral propylene glycol as a vehicle for hydrophobic test-compounds by assessing its histological and biochemical effects in female Wistar rats.

**Methods:** The study recruited 10 Wistar rats which were divided into two groups of five rats each as follows: Group 1 received distilled water (2 ml/kg, po) for four consecutive weeks while group 2 received propylene glycol (2 ml/kg, po) for the same period of time.

**Results:** Propylene glycol caused no alteration in liver function as indicated by the plasma levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and hepatic total protein level when both groups were compared ($p > 0.05$). No significant difference between both groups was also recorded in the oxidative stress status (glutathione and catalase) of their liver homogenates. Histological examinations using Hematoxylin and Eosin (H&E) (general histoarchitecture) as well as special stains such as Gordon and Sweet (reticular fiber formation), Masson's trichrome (collagen fibers), and periodic acid-Schiff (glycogen content) did not reveal any feature of pathology. Quantification analyses of their liver sections, using image J, further revealed no significant difference ($p > 0.05$) in their histoarchitectural features.

**Conclusion:** Propylene glycol is a suitable alternative vehicle for hydrophobic test-compounds in experimental studies, as its sub-chronic administration sustained normal liver function and histology.

## ARTICLE HISTORY

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## KEYWORDS

Distilled water; propylene glycol; hydrophobic test-compounds; liver function; Wistar rats

## Introduction

The experimental models of clinical conditions (involving the use of rodents and other animals) remain an important aspect of scientific research, even in the light of recent advancement in the field of medical sciences. Scientific explorations, evaluations, and tests of hypotheses are limited IN human subjects/participants due to ethical reasons. It is, therefore, expedient to adopt experimental models of clinical conditions for a better/more robust scientific study of the prognosis of a condition as well as to develop novel approaches in determining better treatment or management alternatives. In this light, the scientific evaluation of plant-derived medicines remains an important aspect of scientific research, as it can inspire novel drug development. However, due to the vast available protocol for extraction processes which also involves the use of different chemicals such as acetone, methanol, ethanol, and water (to mention a few), the final yield obtained may differ in both physical and chemical properties. Furthermore, the solubility of the yield in a solvent is a function of their hydrophilic or hydrophobic nature.

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Distilled water and normal saline solutions are common solvents that are used as a vehicle for plant-derived medicines in scientific experimentations. Their use as a solvent, however, is only possible for hydrophilic substances. Also, some plant-derived agents may form a suspension rather than a homogeneous mixture in vehicles of distilled water and normal saline, thereby giving a false interpretation of the agent's potency or therapeutic efficacy due to under-evaluation of the administered amount.

Propylene glycol, miscible with water and other solvents, is a synthetic organic compound that is relevant in several industrial processes including food processing, making of electronic cigarettes, de-icing of aircrafts, and liquid sweetener [1,2]. It is expedient to have an adequate documentation of its biological effects via in-depth experimental studies. Generally, a period of study below 2 days and ranging from a few hours to minutes is considered *acute* while that between 2 and 29 days is referred to as a *sub-acute* condition [3,4]. A period/condition between 30 and 90 days is *sub-chronic* while that which exceeds 90 days is referred to as *chronic* [3,5–7]. Through the processes of detoxification, the liver performs important functions of homeostasis in the body. The capacity to perform these detoxification processes makes it one of the body's vital organs [8–10]. If the liver becomes exposed to the deleterious effects of chemical agents, the result is a consequent biological accumulation of toxic metabolites or toxins leading to terminal illness [9,11].

There is an undocumented controversy, particularly in the field of "Anatomy and Cell Biology" regarding the paucity of evidence that aims to probe the histoarchitectural effects of propylene glycol beyond H&E assessment; considering the fact that propylene glycol is not a physiological solution. Available literature on the histological effects of this vehicle tends to focus on H&E staining technique and male Wistar rats [12–14]. Apparently, more comprehensive information can be obtained regarding the effects of a test-compound when histological assessments are made with special stains. Furthermore, female Wistar rats were recruited for this study in order to provide additional information to the existing body of scientific knowledge. Apparently, sex-dependent differences or similarities will provide a better scientific foundation to judge and, possibly, extrapolate the biological effects of the vehicle for the benefit of humanity (male and female inclusive). The novelty of this study lies in the assessment of the histological effects of propylene glycol beyond H&E staining technique, using female Wistar rats.

## Materials and methods

### *Chemicals and biochemical kits*

Propylene glycol was procured from BioVision Inc. (USA) while the assay kits were purchased from Randox Laboratory Ltd. (UK).

### *Fluid administration*

Both Propylene glycol and distilled water were administered at 2 ml/kg so that each 100 g rat received 0.2 ml of either fluid (in order to avoid the deleterious effects of fluid overload).

### *Experimental protocol*

All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals [15] and approved by local institutional Research Committee. Ten female Wistar rats of about 3 months of age (130–150 g) were recruited for this study. They were purchased from the Animal Holdings Unit of the College of Health Sciences, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria, where the study was carried out. They were housed in plastic cages under natural light and dark cycle and allowed access to standard laboratory rat chow (ACE Feed PLC, Osogbo, Nigeria) and water *ad libitum*.

The rats were divided into two groups of five rats each as follows: Group 1 received distilled water (0.2 ml/ 100 g) orally for four consecutive weeks while group 2 received an equivalent amount of propylene glycol via oral route for the same period of time. Thereafter, the rats were euthanized and their blood samples were collected into separate Ethylene diamine tetraacetic acid (EDTA) bottles by cardiac puncture.

Blood samples were centrifuged at 4,000 rpm for 15 minutes at −4°C using a cold centrifuge (Centurium Scientific, Model 8881). The plasma obtained was decanted into separate plain bottles using sterile syringes. About 1 g of the liver of each rat was excised and kept in a cooler for the preparation of tissue homogenate that was used to assess the indicators of oxidative stress while the other portion of the liver was fixed in 10% formal-saline solution for histological examination.

### *Measurement of body and organ weight*

Weekly body weight was assessed using Hanson digital weighing balance (Hanson, China) while

organ weights were weighed with the aid of Camry sensitive weighing balance (Camry, China). The percentage weight change and relative liver weight were determined using the formulae below [9,16,17]:

Percentage weight change (PWC) (%)

$$= \frac{(\text{Final body weight} - \text{Initial body weight})\ \text{g}}{\text{Initial body weight (g)}} \times 100\%$$

Relative liver weight (RLW) (%)

$$= \frac{\text{Weight of whole liver (g)}}{\text{Final body weight (at the point of sacrifice) (g)}} \times 100\%$$

### *Assessment of the biomarkers of liver function*

Liver function biomarkers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assayed in the plasma of each rat using Randox standard laboratory kits as described by the manufacturer's instruction. However, using the Biuret method as described by Tietz [18], total protein was assayed in the liver homogenate.

### *Assessment of oxidative stress indicators*

At a pH of 7.4, about 1 g of each tissue (liver) was used to prepare 10% homogenate in phosphate buffer (100 mM). The tissues were homogenized using an electric homogenizer (S1601001). Thereafter, the homogenates were centrifuged at 3,000 rpm for 20 minutes and the supernatants were collected for the assessment of the following indicators of oxidative stress and lipid peroxidation.

Reduced glutathione (GSH) level was determined by the method of Beutler et al. [19] while the activity of catalase (CAT) was by the method of Sinha [20].

### *Histopathological examination*

The remaining portion of the liver for each rat was fixed in a 10% formal-saline solution. Thereafter, they were dehydrated in graded alcohol and embedded in paraffin wax. Sections taken (7–8 µm thick) were stained using H&E technique (for general histoarchitectural appraisal); Periodic acid-Schiff (PAS) technique (for histochemical study); Gordon and Sweets' silver staining technique (to appraise reticular fibre formation); as well as Masson's trichrome staining technique (to appraise collagen fibres).

Photomicrographs of each slide were taken with the aid of a Leica DM 750 microscope, interfaced with Leica ICC50 digital camera at objectives of ×10 and ×40. The representative photomicrographs were transported to "Image J" software for quantificational analyses.

### *Statistical analysis*

Data obtained were expressed as mean ± standard error of mean. Students' *t*-test was used to determine differences in the variables between the two groups and the level of significance was set at $p < 0.05$. Data were analyzed using Graph Pad Prism 5.03 (Graph Pad Software Inc., CA).

## Results

### *Effects of sub-chronic administration of distilled water and propylene glycol on percentage weight change (%) and relative liver weight (%) of Wistar rats*

No significant difference was recorded in the percentage weight change of group 1 (23.86 ± 3.21) when compared with group 2 (24.43 ± 2.28) ($t$ = 0.145; $p$ = 0.889) (Fig. 2). The relative liver weight for both groups also showed no significant difference (Group 1: 4.72 ± 0.24, Group 2: 4.66 ± 0.18) ($t$ = 0.200; $p$ = 0.847) (Fig. 3).

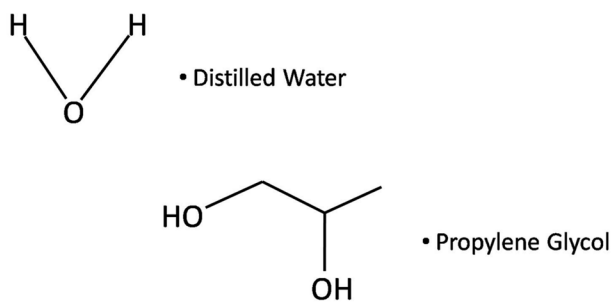


**Figure 1.** Chemical structure of distilled water and propylene glycol.

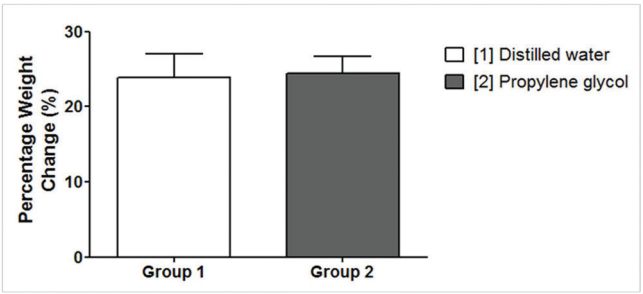


**Figure 2.** Effects of sub-chronic administration of distilled water and propylene glycol on the percentage weight change of Wistar rats. Each bar represents mean ± standard error of mean at $p < 0.05$.

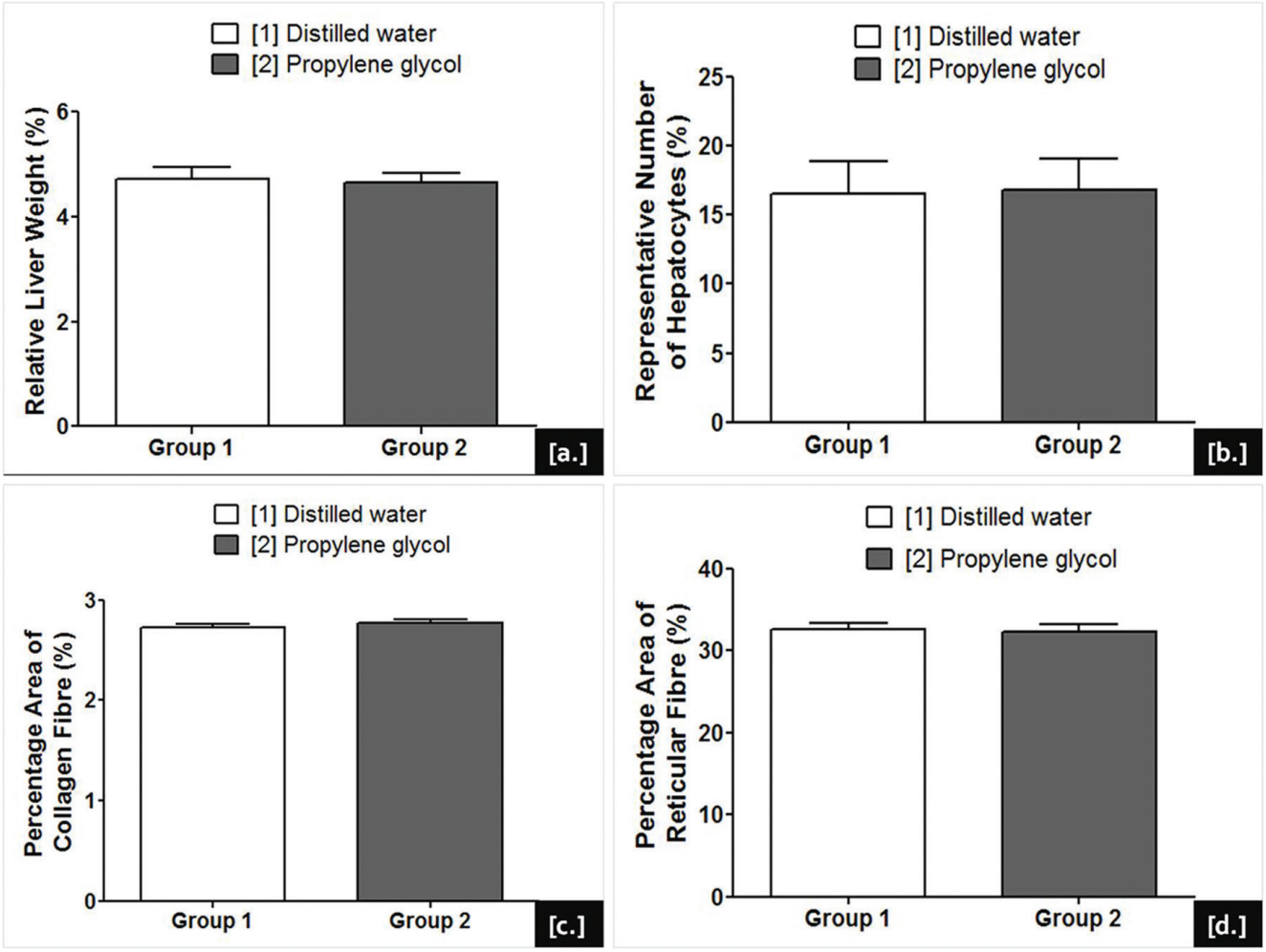


**Figure 3.** Effects of sub-chronic administration of distilled water and propylene glycol on the relative liver weight and some quantification analyses of liver histoarchitecture of Wistar rats. Each bar represents mean ± standard error of mean at $p < 0.05$.

**Table 1.** Effects of sub-chronic administration of distilled water and propylene glycol on biomarkers of liver function in Wistar rats.

| Groups | AST (U/l) | ALT (U/l) | ALP (U/l) | Total protein (mg/ml) |
|---|---|---|---|---|
| [1] Distilled water | 60.30 ± 12.08 | 45.81 ± 0.53 | 12.91 ± 0.56 | 5.99 ± 0.27 |
| [2] Propylene glycol | 62.57 ± 13.22 | 44.89 ± 1.03 | 13. 89 ± 0.45 | 5.80 ± 0.24 |

### *Effects of sub-chronic administration of distilled water and propylene glycol on plasma AST (U/l), ALT (U/l), and ALP (U/l) levels of Wistar rats*

As shown in the representative table (Table 1), no significant difference was recorded in the plasma levels of AST, ALT, and ALP of both groups 1 and 2 ($p > 0.05$).

### *Effects of sub-chronic administration of distilled water and propylene glycol on liver total protein (mg/ml) levels of Wistar rats*

The liver total protein level showed no significant difference when group 1 was compared with group 2 ($t = 0.526$; $p = 0.613$) (Table 1).

### *Effects of sub-chronic administration of distilled water and propylene glycol on liver GSH (U/ml) and CAT (µmol/minute/mg protein) activities of Wistar rats*

The activity of GSH and CAT in groups 1 and 2 were observed to be within a physiological range at the end of the study ($p > 0.05$) (Table 2).

**Table 2.** Effects of sub-chronic administration of distilled water and propylene glycol on some hepatic indicators of oxidative stress in Wistar rats.

| Groups | GSH (U/ml) | CAT (µg/minute/mg protein) |
|---|---|---|
| [1] Distilled water | 27.81 ± 0.93 | 3.32 ± 0.26 |
| [2] Propylene glycol | 27.80 ± 1.59 | 3.28 ± 0.15 |

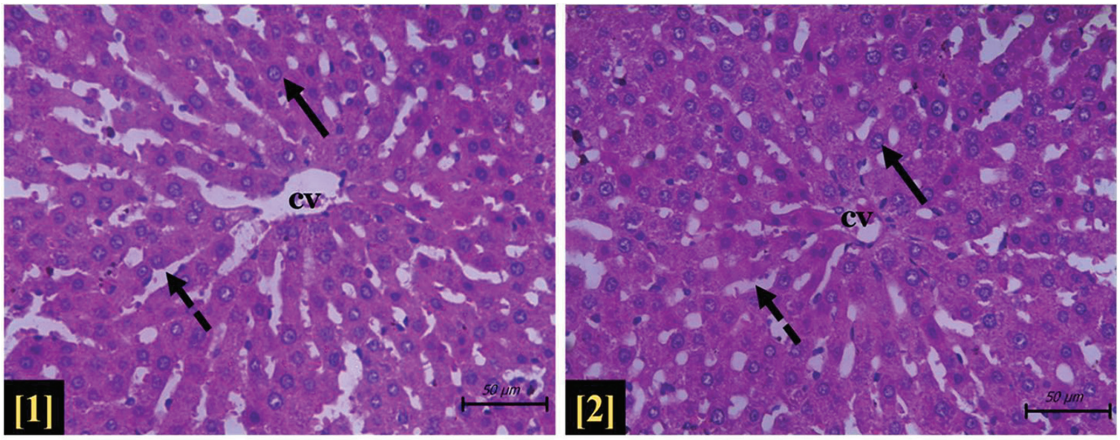


**Figure 4.** Histological effects of distilled water and propylene glycol on the liver of Wistar rats: Assessment of general histoarchitecture. H&E stain, ×400 (scale bar = 50 µm). [1] = distilled water; [2] = propylene glycol; CV = centrilobular vein; black arrow = apparently normal (polyhedral) hepatocyte; black dashed arrow = apparently intact hepatic sinusoid.

### *Histological effects of sub-chronic administration of distilled water and propylene glycol on the liver of Wistar rats*

#### Effects on general histoarchitecture (Hematoxilin—Eosin staining technique)

The general histoarchitecture of both groups showed similar features as revealed by their representative micrographs. This was characterized by apparently intact polyhedral hepatocytes, radially disposed and organized into cords toward the centrilobular vein (CV). Their CVs were also well defined, having apparently intact hepatic sinusoids (Fig. 4).

The quantification analysis and comparison of both groups' number of hepatocyte showed no significant difference (Group 1: 16.50 ± 2.42, Group 2: 16.83 ± 2.26) (t = 0.010; p = 0.923) (Fig. 3).

#### Effects on reticular fibre formation (Gordon and Sweet's silver staining technique)

The features of an apparently intact reticular fiber formation were similar in both groups. Each representative micrograph revealed evidence of apparently intact dark-stained reticular fibers, lining the wall of the sinusoids. The reticular fibers also clearly surrounded the wall of the individual hepatocyte, spanning across the perisinusoidal space and CV (Fig. 5).

Also, the quantification analysis and comparison of both groups' percentage area of reticular fibres showed no significant difference (Group 1: 32.57 ± 0.81, Group 2: 32.35 ± 0.92) ($t$ = 0.180; $p$ = 0.862) (Fig. 3).

#### Effects on collagen fibres (Masson's trichrome staining technique)

Each representative micrograph for both groups showed similar features of an apparently normal lobular histoarchitecture with scanty collagen fibers (light-green) which were present at the portal tract as well as the walls of the CV (Fig. 6).

The quantification analysis and comparison of both groups' number of hepatocyte showed no significant difference (Group 1: 2.73 ± 0.03, Group 2: 2.77 ± 0.04) ($t$ = 0.800; $p$ = 0.447) (Fig. 3).

#### Histochemical effects (periodic acid-schiff staining technique)

The PAS stain of each representative micrograph also showed similar features of PAS-positive substances (glycogen) in the parenchymal cells of the liver. Both micrographs demonstrated features of apparently normal hepatic lobular histoarchitecture (Fig. 7).

## Discussion

This study investigated the sub-chronic effects of oral propylene glycol administration on liver function homeostasis in a Wistar rat model. The study demonstrated that sub-chronic administration of oral propylene glycol had no deleterious hepatic effects as revealed by the biomarkers of liver function, liver antioxidant indicators, and micrographic evidence as demonstrated by special stains.

An observational study of daily food consumption from the feeding trough suggests that sub-chronic oral administration of propylene glycol

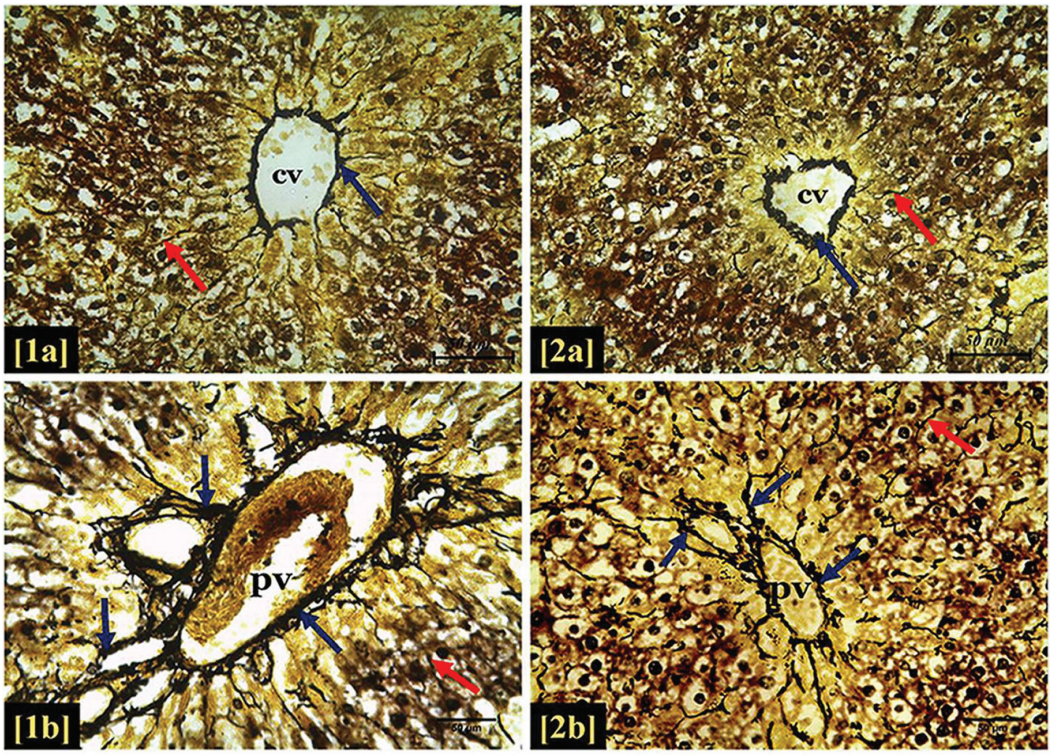


**Figure 5.** Histological effects of distilled water and propylene glycol on the liver of Wistar rats: Assessment of reticular fiber formation. Gordon and Sweet's silver stain, ×400 (scale bar = 50 µm). [1] = distilled water; [2] = propylene glycol; CV = centrolobular vein; PV = portal vessel; dark-blue arrow = portal space; red arrow = apparently delicate reticular fibres surrounding individual hepatocyte. Plate [a] shows the centrilobular zone while plate [b] demonstrates the portal area.

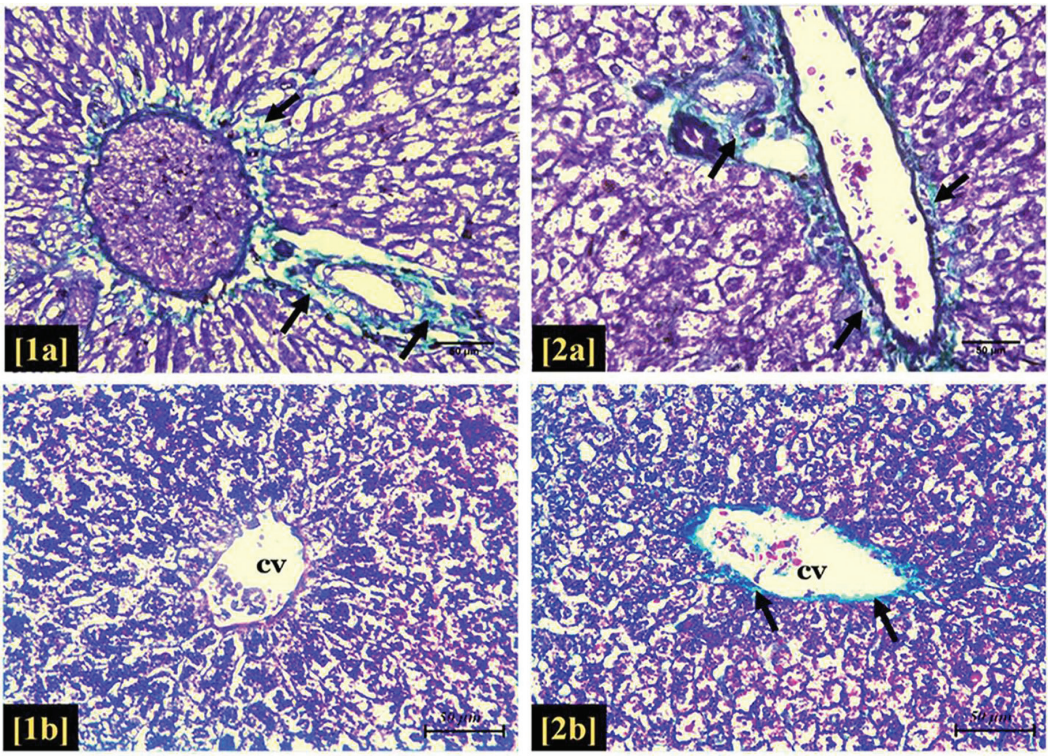


**Figure 6.** Histological effects of distilled water and propylene glycol on the liver of Wistar rats: Assessment of collagen fibre formation. Masson's Trichrome Stain, ×400 (scale bar = 50 µm). [1] = distilled water; [2] = propylene glycol; CV = centrolobular vein; black arrow = apparently normal centrolubular zone with few (green-stained) collagen fibres; Plate [a] demonstrates the portal areas while plate [b] shows the centrilobular zones.

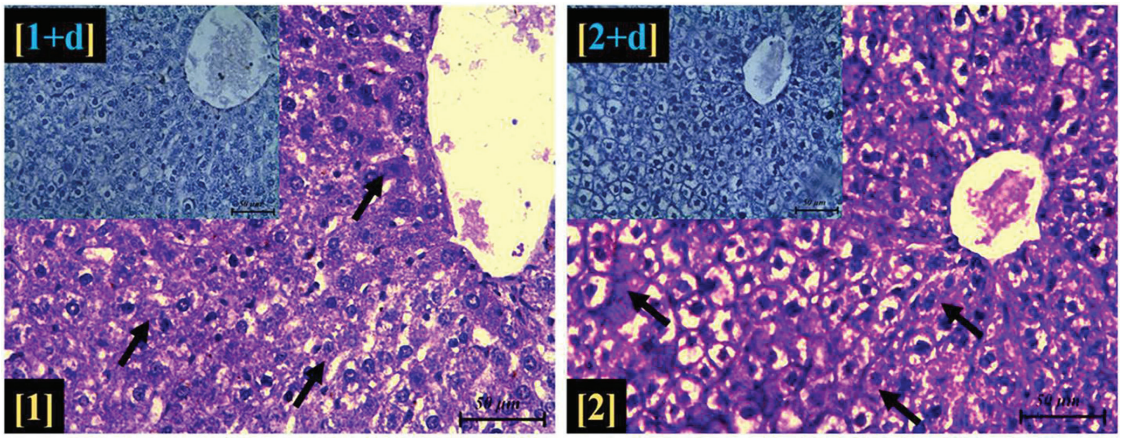


**Figure 7.** Histochemical effects of distilled water and propylene glycol on the liver of Wistar rats. PAS stain, ×400 (scale bar = 50 µm). [1] = distilled water; [2] = propylene glycol; black arrow = magenta-stained intracytoplasmic PAS-positive substance (glycogen); Insets ([1+d] and [2+d]) = light micrographs of PAS stain in combination with diastase, showing digestion of PAS-positive content (glycogen) of the hepatic lobule.

did not cause any abnormal derangement in feeding pattern, as feeds were observed to be adequately consumed on a daily basis. Consequently, no significant difference in body weight change was observed when compared with the control. Striking a balance between food intake and the expenditure of energy, under the modulatory influence of the hypothalamic center, is a strong determinant of the tendency to gain or lose weight [21]. Subject to further verification, this study suggests that propylene glycol administration has no deleterious influence on the hypothalamic center for food consumption and energy expenditure. Consequently, its sub-chronic administration does not result in abnormal derangements in feeding pattern and, thus, body weight change.

In experimental research, when morphological changes are absent, a sensitive indicator of the effects of a chemical compound is the relative organ weight of an animal [9,22,23]. It is, therefore, implied that the maintenance of a relative liver weight within a physiological level following sub-chronic propylene glycol administration is indicative of the potential suitability of this solvent as an oral vehicle for test compounds in experimental research/studies.

The release of AST, ALT, and ALP (basic biomarkers of liver function) into the circulatory system is usually associated with hepatocyte injury [9]. This makes the assay of these biomarkers of clinical importance, as abnormal levels in circulation reflect an injury or damage to the liver. The maintenance of physiological levels of these enzymatic activities, associated with sub-chronic propylene glycol administration, reveals the fact that this solvent did not potentiate membrane fragility of the hepatocytes as well as abnormal leakage of hepatic enzymes into the circulation.

Since protein synthesis can be affected by a chemical agent or a test compound, its stimulation is an important index for the determination of chemically-induced hepatic dysfunction or injury [9,24]. Also, the process involved in liver regeneration can be alluded to the stimulation of protein synthesis [24]. Therefore, sustained hepatic total protein level, as recorded in this study, is an indicator of a non-deleterious effect of sub-chronic propylene glycol administration on hepatic protein synthesis. Consequently, its sub-chronic administration sustains normal liver regeneration process. This fact was supported by the representative micrographs which revealed normal appearing general liver histoarchitecture.

Significant reductions in the activities of hepatic indicators of oxidative stress, such as GSH and CAT, can be suggestive of an existing oxidative stress due to the generation of free radicals [9]. This would have been attributed to a reduction in the ability of the liver to sustain the production of hepatic GSH and CAT levels, and (or) the increased use of these lines of defense (by the liver) in scavenging existing free radicals in an attempt to restore homeostasis of the antioxidant system [9]. Contrariwise, this study demonstrated an associated maintenance of normal hepatic antioxidant system following sub-chronic administration of propylene glycol in Wistar rats.

The evidence of an apparently normal general liver histoarchitecture following sub-chronic exposure to propylene glycol, as revealed by the representative micrograph, further supports its

suitability as an alternative vehicle for hydrophobic test compounds. The mesh-like framework of the liver (reticular fibers), which provides the supporting connective tissue of the organ, was also, micro-graphically, demonstrated to be apparently intact at the end of the study. The physiological collagen fiber deposition, demonstrated by quantificational analysis, suggests that sub-chronic propylene glycol administration does not induce functional impairment of the liver. Also, micrographic evidence of apparently normal hepatic PAS-positive materials (glycogen content) demonstrates a possible non-deleterious influence (or non-modulatory effect) of propylene glycol on the activities of insulin and glucagon. These micrographic evidence, supported by their respective quantification analysis, point to the fact that oral propylene glycol administration (2 ml/kg) at a sub-chronic level has no deleterious effect on the liver function.

Although this study's organ of focus is the liver, the reason being that it is a *critical* vital organ that performs a *homeostatic function* through *detoxification processes*, literature exist on the use of propylene glycol as a positive control in animal experimentations that recruited male Wistar rats [12–14]. Evidently, this sex-similarity in the biological effects of propylene glycol provides a better scientific foundation for the experimental exploitation of this suitable hydrophobic solvent for the benefits of humanity (male and female inclusive). Propylene glycol is also well used as a solvent in many pharmaceuticals for the topical formulation of drugs including lorazepam and diazepam which are insoluble in water [25]. The aforementioned scientific information, together with the findings of this study, should (to a large extent) allay the undocumented controversy about the suitability/unsuitability of propylene glycol as a solvent for hydrophobic test-compounds. Nevertheless, it is recommended that further studies on the sub-chronic and chronic effects of this fluid on (other) vital organs be carried out using special stains and more specialized techniques in order to have comprehensive information about its biological effects. These should also ensure the recruitment of a larger number of experimental animals.

## Conclusion

This study concluded that, at a sub-chronic level, oral propylene glycol is a suitable and non-toxic alternative vehicle for hydrophobic test-compounds, as its administration sustained normal liver function and histology.

## Acknowledgments

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