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Phytochemical Screening and Acute Exposure of Methanolic Bark Extract of *Pterocarpus Tinctorius* in Wistar Rats

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ABSTRACT

Background: The bark extract of *Pterocarpus tinctorius (Fabaceae)* is traditionally used to treat diseases such as diabetes, gonorrhoea, hypertension, stomachache and bacterial gastroenteritis. However, little is known about toxicity and phytochemicals found in the bark of *Pterocarpus tinctorius*. Therefore, this study aimed at phytochemical screening and investigating acute oral toxicity of the stem bark of Pterocarpus tinctorius in Wistar rats.

Methods: Wistar rats (N=3 per group) were randomly assigned to four groups: negative control, 50, 300, and 2000 mg/kg methanolic sub-extract. A single dose was administered and female Wistar rats were observed for 14 days according to the Organisation for Economic Co-operation and Development guidelines for acute toxicity testing in rats. After dosing, rats were individually observed for their physical and behavioural changes throughout the study. The body weight of Wistar rats were monitored weekly and gross pathological changes of rat organs were observed. In addition, the biochemical markers and organ-body weight ratios of the kidneys and the liver were measured. Qualitative phytochemical tests were carried out to determine the types of phytochemicals present in the bark of *Pterocarpus tinctorius*.

Results: There were no signs of toxicity observed in all treatment groups and no abnormalities were observed on organs of rats. There was no significant difference in body weight, organ-body weight and biochemical markers with $p>0.05$, for the Wistar rats in all treatment groups compared to the negative control. Phytochemical tests showed the presence of alkaloids, phenolics, flavonoids, tannins, steroids, terpenoids, anthocyanins and saponins in the stem bark of *Pterocarpus tinctorius* as compounds that could be responsible to treat diseases in traditional medicine.

CONCLUSION

This study demonstrated that *Pterocarpus tinctorius* methanolic bark sub-extract is not acutely toxic to the liver and kidneys up to the dose of 2000 mg/kg body weight. It is highly recommended that toxicity studies on other organs of rats such as the heart, brain, pancreas and intestines are carried out.

Key Words: *Pterocarpus tinctorius*, toxicity, phytochemical screening, Wistar rats.

1. INTRODUCTION

The fact that herbal medicines are natural does not mean that they do not contain some toxic substances, which can be dangerous when consumed by humans [1]. Plants contain secondary metabolites that can exhibit both medicinal and toxic properties [2]. Some plants that are used in traditional medicine to treat human ailments and animal diseases are toxic [3]. Therefore, the common belief that anything natural is safe is not correct [1]. In plants, therapeutic effects often occur at lower doses, whereas overdose can induce poisoning [4]. Hence, there is no drug which is free from harmful effects [4]. The toxic effects of plants in both humans and animals may include depression, tremors, convulsions, paralysis, abnormal behaviour, death, inflammation, coagulation of blood, blindness, salivation, diarrhoea, gastrointestinal disor- ders (GIT), irritation, dermatitis, convulsions and abortifacient effects [5]. Thus, the safety of medicinal plants must be scientifically established before their use [6, 7]. This could be accomplished by performing acute, sub-acute, chronic, and sub-chronic toxicity studies [8]. Several studies have reported plants in the Fabaceae and other families that are safe to use [9-13]. Whereas, other plants have been reported to be toxic, for example; *Berlina grandiflora*, *Cylicodiscus gabunensis, Faidherbia albida, Glycine max, and Piptadeniastum africana* [14]. Furthermore, ninety-two plants belonging to 43 families were found to be toxic and most of them are members of Fabaceae family [5]. Hence, the claim that natural plant products are safe should be accepted only after the plant product passes through toxicity testing using modern scientific methods [15].

The genus *Pterocarpus* belongs to the Fabaceae family [16]. Some plants in the genus *Pterocarpus* are used in the treatment of diarrhoea, gastroenteritis, dysentery, gonorrhoea, and stomachache [16]. It is also reported that *Pterocarpus tinctorius* has antibacterial activity against *Shigella dysenteriae*, *Salmonella* Typhi, and *Escherichia coli,* hence, its traditional use in the treatment of bacterial gastroenteritis [17]. In the genus *Pterocarpus*, acute toxicity of the aqueous stem bark extract of *Pterocarpus soyauxii* Taub showed low toxicity in oral acute high dose administration with LD_{50} > 10 750 mg/kg [18]. *Pterocarpus santalinoides* have been reported to have hepatoprotective activity at doses of 50, 250, and 500 mg/kg body weight [19]. *Pterocarpus erinaceus* stem bark has been found to be nontoxic with LD_{50} > 5000 mg/kg body weight [6]. Though some toxicity studies have been conducted in the genus *Pterocarpus*, currently, data on toxicity of *Pterocarpus tinctorius* is scarce.

In China, qualitative phytochemical screening of the heartwood of *Pterocarpus tinctorius* showed the presence of stilbenoids, phenolics and flavonoids

[20]. However, same plant species can produce different compounds due to variation in genetic and environmental factors [21]. Furthermore, different organs of the same plant could synthesise different types of phytochemicals [22]. Thus, different parts of the same plant could be used to treat different diseases. Hence, it is imperative to conduct phytochemical screening and toxicity studies on different organs of plants like *Pterocarpus tinctorius* that are used in traditional medicine. In Africa and Zambia 87% and 70% of the population use Traditional Medicine respectively, [23, 24]. As a result, World Health Organisation and Ministry of Health have recognized the need to research on plants used in traditional medicine so as to provide information on the efficacy and safety of such plants [25, 26]. Therefore, this study has provided information on the phytochemicals present in *P.tinctorius* that could be responsible to treat several diseases in traditional medicine. It has also provided knowledge on potential toxicity effects of *Pterocarpus tinctorius* that could help safeguard the safety of the users. It is hoped that the results of this study will stimulate research that could help to scientifically validate the use of *Pterocarpus tinctorius* on the diseases to which it is effective in traditional medicine.

2. MATERIALS AND METHODS

2.1. Study site and Design

This is an experimental study involving phytochemical screening and acute toxicity testing of *Pterocarpus tinctorius* bark extract in Wistar rats. The fresh bark of *Pterocarpus tinctorius* was collected from Mulakupikwa village, Chinsali district of Northern Zambia (Easting 413636, Northing 8825840). A plant specimen was deposited, identified, and authenticated at the University of Zambia, School of Natural Sciences in the Department of Biological Sciences by a taxonomist.

2.2. Preparation of the extract

The method used to prepare the extract was adopted from [17, 27]. The fresh bark of *Pterocarpus tinctorius* was dried in the open air under shade to prevent direct sunlight from inactivating the chemical constituents. Following drying, bark samples were pulverized into powder using a mechanical grinder and stored in a polythene bag, then kept until extraction. The 100 g of *P.tinctorius* powdered bark was weighed using a sensitive digital weighing balance (Adam Nimbus Group, Stuttgart, and Baden-Württemberg, Germany). The powder was macerated in 700 mL analytical grade (99.9%) methanol (Sasol, Sandton, South Africa) in a 1L beaker on a magnetic stirrer for 24 hrs. After 24 hours of stirring, the extract was separated from the marc using gauze and the resulting liquid was suction-filtered through a Whatman No.1 filter paper using a Buchner funnel. The residue was re-macerated and the above procedure was repeated three times to exhaustively extract the compounds from the plant material. The filtrates obtained from the successive maceration were dried under reduced pressure using a rotary evaporator at 40^{o}C. The dried crude methanolic extract was then left in the desiccator for 24 hours to dry to a powder. Dried crude methanolic extracts (brown) were put in a labelled glass bottle and stored in the refrigerator at 4^{o}C until use.

2.3. Preparation of the methanolic sub-extract extract

Preparation of methanolic sub-extract is as described by [17]. The methanolic crude extract (5.1 g) was dissolved in the minimum amount of methanol and transferred into the separating funnel. First, 100 mL of methanol was added, and the mixture was well shaken. Thereafter, 100 mL of hexane was added to the separating funnel containing 5.1 g of methanolic extract dissolved in 100 mL of methanol to extract lipophilic or nonpolar compounds by hexane from methanolic crude extract. The mixture in the separating funnel was well shaken while releasing pressure. It was then allowed to stand until two clear layers were formed. The lower layer of methanolic extract (Brown) was then collected in the flat-bottomed flask by draining. The hexane layer (upper one, yellow) which remained in the separating funnel was also drained from the separating funnel into the flat bottomed flask. This process was repeated three times to allow the nonpolar compounds to be exhaustively extracted by hexane solvent. To extract the moderately polar compounds, 70 mL of chloroform was added to the separating funnel containing 100 mL of methanolic extract (Brown). The mixture was well shaken while releasing pressure and allowed to stand until two clear layers were formed. The chloroform layer (lower one) was drained from the separating funnel. This process was repeated three times to allow all the moderate polar compounds to be extracted by chloroform. After extracting the chloroform sub-extract, what remained in the separating funnel was the methanolic sub-extract (M1). The methanolic sub-extracts was evaporated at 40^{o}C using a rotary evaporator and stored in the freezer at 4^{o}C until use. Extraction procedure is shown in Figure 1.

2.4. Phytochemical screening of methanol crude extract and methanolic sub-extract

Standard qualitative procedures as described by [17,28] were used to detect the presence of saponins, tannins, anthraquinones, alkaloids, phenolics, steroids, terpenoids, flavonoids, glycosides and anthocyanins.

2.4.1. Preparation of the extract

Methanolic extract (0.6 g) was dissolved in 12 mL of water to prepare a concentration of 0.05 g/mL. 1 mL of 0.05g/mL was then subjected to perform qualitative phytochemical analysis for each group of phytochemicals as described below.

Preparation of the extract for phytochemical analysis

About 0.6 g of methanolic crude extract (M) was dissolved in 12 mL of water to prepare a concentration of 0.05 g/mL. 1ml of 0.05 g/mL was then subjected to perform qualitative phytochemical analysis for each group of phytochemicals as described below.

Test for anthraquinones (Borntrager's test)

About 5 mL of benzene and then 2.5 mL of 10% ammonia solution were added to 1 mL of 0.05 g/mL of the methanolic extract and shaken vigorously for 30 seconds. The presence of pink colour indicated the presence of anthraquinones.

Test for tannins (ferric chloride test)
Two millilitres of 2% solution of $FeCl_3$ were added to 1 mL of 0.05 g/ mL of the methanolic extract. The presence of black precipitate indicated the presence of tannins

Test for saponins (froth test)

About 1ml of 0.05 g/mL of the methanolic extract was added to 2 mL of distilled water and shaken vigorously. The formation of a stable persistent froth was taken as a positive test for saponins

Test for alkaloids (Dragendoff's test)

Approximately 2 mL of methanolic extract was added to 2 mL of 1% HCl and heated for 20 minutes. The mixtures were then cooled. 1 mL of Dragendoff's reagent (solution of potassium bismuth iodide) was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Test for phenolics (Ferric chloride test)

To 1mL of 0.05 g/ mL of the methanolic extract, 2 mL of 5% aqueous ferric chloride were added. The formation of blue colour indicated the presence of phenols in the methanolic extract.

Test for terpenoids (Liebermann's Burchard test)

Approximately 2 mL of chloroform and 3 mL of H_2SO_4 were added to 1 mL of 0.05 g/mL of methanolic extract. A reddish-brown colouration was taken as a positive test for terpenoids.

Test for flavonoids (Alkaline reagent test)

1ml of 0.05 g/mL of methanolic extract was treated with 1 mL of 10% NaOH solution. The formation of an intense yellow colour was an indication of the presence of flavonoids. To this, a few drops of 70% dilute hydrochloric acid was added and the yellow colour disappeared.

2.5. Experimental Animals

Female Wistar rats used in this research were obtained from the School of Medicine, Department of Physiology, University of Zambia, Ridgeway Campus. The rats were kept in the animal housing unit, School of Veterinary Medicine, University of Zambia. The rats were between 8-12 weeks old, non-pregnant and nulliparous and weighed between 170.5 - 280.5 grams. All rats were housed in polypropylene cages bedded with paper pellets. A maximum of 3 rats were housed in each cage. They were acclimatized for one week and kept under the normal 12 hour light/dark cycle at room temperature of 22°C (±3°C) with a relative humidity of 50-60%. The animals were allowed access to food and water *ad libitum* throughout the study period. The animals were fed on commercially obtained pellets. Good hygiene was maintained by constant cleaning and removal of litter and supplied feed from the cage on daily basis. Animal use and care guidelines set out according to the World Health Organisation were used in this study [29, 30].

2.6. Preparation of stock solution and dosage calculations

To prepare appropriate dosages for administration to experimental rats, the method described by [31] was adopted. A stock solution of 4000 mg/20 mL equivalent to 200 mg/mL

of the methanolic sub-extract (M1) was prepared. This was achieved by dissolving 4000 mg of the extract in 20 mL of distilled water. The dosage volumes for rats that received 2000 mg/kg body weight were calculated from 200 mg/mL. A part of this concentration (200 mg/mL) was serially diluted to 50 mg/mL and 10 mg/mL from which the dosage volumes of 300 mg/kg body weight and 50 mg/kg body weight were respectively calculated for each rat. The following formula was used to calculate the dosage volume for each animal:

$$\text{Volume to be given} = \frac{\text{D (desired dose)}}{\text{H (Amount on hand)}} \text{ X vehicle}$$

Where: D (Desired dose) = Dose to be administered X body weight of the animal

For example, if we consider administered dose of 2000 mg/kg for a rat weighing 0.2545 kg and if the amount on hand is 200 mg/mL. The volume was calculated as follows:

$$\text{Volume to be given} = \frac{2000 \text{ mg/kg X } 0.2545 \text{ kg X mL}}{200 \text{ mg}}$$

Therefore: the volume to be given = **2.6 mL**

2.7. Extract administration and clinical observation of animals

The acute oral toxicity method (limit test) was adopted from the Organisation for Economic Cooperation and Development (OECD) guideline 423, which stipulates the use of only three animals per group (OECD 423, Paragraph 23) [29]. Twelve (12) rats were randomly separated into 4 groups (3 rats per group) and labelled with permanent markers for ease of identification. One group of 3 rats acted as a control and received 1 mL of distilled water. The test was undertaken to determine the range of exposures where lethality was expected since the death of a proportion of animals is a major endpoint. The animals were fasted overnight for 12 hours and weighed. Test doses of methanolic bark extract (M1) were calculated in relation to the body weight of every fasted animal and administered via oral gavage by using syringes to the two groups at doses of 50 mg/kg and 300 mg/kg. Food was withheld for a further 4 hours after administration. No mortality was noticed in the animals within 24 hours at 50 mg/kg and 300 mg/kg. Hence, 3 rats received a limit dose of 2000 mg/kg body weight of the extract. Again no mortality was observed.

The animals were then regularly and individually observed for behavioural changes and general toxicity signs after dosing for the first 24 hours, with special attention being given during the first 4 hours whereby animals were observed for the first 30 minutes and every half an hour up to four hours. The signs observed included changes in skin and fur colour, mucus, and eye membrane. Attention was given to effects such as tremors, convulsions, salivations, diarrhoea, coma, and death were noted. Thereafter, the observation continued daily for 14 days. Individual body weights of the rats were taken before dosing on the first day (day 0), day 7, and day 14.

2.8. Observation of gross pathological changes, determination of weight and organ-body weight ratio of rats

On the 14th day, the rats were weighed to collect their weights. The method described by [32] was adopted to

observe gross pathological changes and to calculate the organ body weight ratio of the rats. The liver and kidneys were harvested and washed in saline and their wet weights were taken using a digital weighing balance. The relative organ-body weight ratios of the liver and kidneys were calculated by the formula:

$$\text{Relative Organ weight} = \frac{\text{Absolute organ weight}}{\text{Weight of animals}} \times 100$$

2.9. Collection of blood and analysis of biochemical parameters

The method and procedure used in the collection of blood samples were adopted from [33, 34]. At the end of fourteen days of oral administration of methanolic bark extract, the rats were anaesthetized using di-ethyl ether (CK Scientific Group). Approximately 2-4 mL of blood was collected in heparinized containers from each rat through the abdominal aorta and cardiac puncture. The blood was then centrifuged at 3500 rpm for 3 minutes at 4 degrees Celsius. A clean Pasteur pipette was used to carefully collect the serum and dispense it into a clean labelled specimen bottle. All biochemical parameters were analysed using the automated method with the automatic analyser "Beckman Coulter AU480 Chemical Chemistry analyser". Analysis of one sample by the machine took 20 minutes. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total protein were used as biochemical markers for liver function, while creatinine and urea were biochemical markers for kidney function [35].

2.10. Data analysis

Data was organised and presented using tables and graphs using SPSS version 22 (IBM Corporation, Chicago, USA). Weights, organ-weight ratio, and biochemical parameters values were expressed as mean±SD. Shapiro-Wilk test and histogram were used to check the normality of data. To test the assumption of homogeneity of variance, Levin's test was used. One-way analysis of variance (ANOVA) was used to analyse the means of biochemical parameters, the weight of rats, and organ-body weight ratios of organs of the kidneys and liver using SPSS version 22. To determine whether there was a significant difference between the groups and the control, a Turkey post hoc test was used since variance was homogeneity. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Phytochemical analysis

Phytochemical analysis of the methanolic crude extract and methanolic sub-extract of *P. tinctorius* was carried out and revealed phytochemicals such as alkaloids, saponins, terpenoids, steroids, tannins, phenolics, flavonoids and anthocyanin (Table 1)

3.2. Physical and Behavioral effects of *P. tinctorius* on rats

Acute oral toxicity effects of the methanolic stem bark extracts of *P. tinctorius* was also investigated in this study. There were no signs of toxicity or mortality observed in the treatment groups of rats receiving 50 mg/kg and 300 mg/kg of *P.tinctorius* methanolic sub-extract. Immediately after dosing

the rats at 300 mg/mL, they exhibited drowsiness during the first 30 minutes after receiving the dose. However, the rats recovered within 1 hour. The rats that were dosed at 50 mg/mL remained active and exhibited drowsiness after one hour and recovered within 30 minutes. While at the limit dose of 2000 mg/kg body weight, animals also exhibited drowsiness during the first 30 minutes after dosing but this was cleared in 2 hours. The results also demonstrated no observable signs of toxicity and death in experimental groups of rats even at the limit dose of 2000 mg/kg body weight. For the rest of the 14 day period of the study, no signs of toxicity observed and no animal died (Tables 2, 3 and 4)

3.3. Effects of *P. tinctorious* methanolic bark sub-extract on weekly mean body weights of rats

The body weights of rats were measured weekly until the end of the acute toxicity study. All the animals gained body weight over the 14-day test period in both the treatment groups and control (Table 5). Figure 8, show weight gain of rats over 14 day period. The mean weight increase of the treatment groups was non-significant when compared to the control ($p \leq 0.05$). The percentage weight gains were random in the treatment groups. However, the lowest overall weight gain was observed in the control group at 18.25% while the highest was observed in the 50 mg/kg body weight at 25.39%.

3.4. Effect of methanolic bark sub-extract of *P. tinctorius* on liver and kidney of rats after 14 days

The results show that the mean organ-body ratio of the liver in the control group (0.034±0.0014) was higher than all the treatment groups. However, there was no significant difference ($p \geq 0.05$) in the mean organ-body ratio of the kidney and the liver for all the treatment groups when compared to the control (Table 6).

3.5. Gross pathological changes

The control and treatment group organs were observed macroscopically to check for physical signs of abrasion. It was found that there were no abnormalities, necrosis, inflammation or changes in size or colour of the major organs of the rats such as the heart, spleen, kidney, liver, or small and large intestines.

3.6. Effect of a single dose of methanolic bark sub-extract of *P. tinctorius* on biochemical parameters

The results show the effects of *P.tinctorius* methanolic bark sub-extract administration at 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight on serum liver markers (ALT, AST, ALP and total protein) and kidney markers (creatinine and urea). The administration of *P. tinctorius* had no significant ($p > 0.05$) effects on the serum levels of ALT, AST, total protein, creatinine and urea at all doses. There were no significant differences ($p > 0.05$) in the outcome of serum levels analysed between the control and *P. tinctorius* treated rats at all doses. However, the 2000 mg/kg treatment group had the highest level of ALT, ASP and AST. Whereas the highest mean level (35.5667±1.2992) of creatinine was observed in the control group. While for Urea the lowest mean levels was 7.5±0.05 at 2000 mg/kg body weight (Table 7).

4. DISCUSSION

Phytochemical analysis showed the phytochemicals present in the methanolic bark extract of *Pterocarpus tinctorius*. The presence of tannins, saponins, terpenoids, flavonoids, and phenolics agrees with the findings of [36, 37], who have reported the same constituents in the genus *Pterocarpus*. However, this could be the first study to report the presence of anthocyanins in the genus *Pterocarpus*, which could be attributed to different biosynthetic pathways that are found in this genus [21]. Similarly, phenolics and flavonoids have also been reported to be present in the heartwood of *P. tinctorius* [20]. Nevertheless, this could be the first study reporting the phytochemicals present in the bark of *Pterocarpus tinctorius*. These phytochemicals present in the stem bark of *Pterocarpus tinctorius* could be responsible for the medicinal uses of the plant in traditional medicine to treat diseases such diabetes, bacterial gastroenteritis, hypertension, gonorrhea and diarrhea that have been reported in literature [38-40].

In this study, all doses including a maximum dose of 2000 mg/kg body weight of *P. tinctorius* methanolic bark sub-extract caused neither signs of toxicity nor mortality during the 14 days of the experiment. Throughout the 14 day period all animals were found to be healthy with no changes in their skin and fur colour, mucus and eye membrane, tremors, convulsions, salivations, diarrhea, coma and death. *P. tinctorius* methanolic bark sub-extract was found to be non-toxic up to 2000 mg/kg body weight, which is accepted as safe. This falls under category 5 of the Global Harmonization System (2000-5000 mg/kg body weight) (OECD, 2011). The results suggest that *P. tinctorius* methanolic bark extract could be safe as medicinal agent in traditional medicine at all doses tested although this was done in animals and not humans. Nevertheless, there is a strong correlation between toxicological results in rats and humans [41], while the correlation between humans and mice is weaker [42].

This could be the first study reporting the acute oral toxicity of *Pterocarpus tinctorius*. However, studies on other *Pterocarpus* species have been reported with similar findings, for example *P.erinaceus* stem bark extract LD_{50} has been found to be > 5 000 mg/kg body weight and considered nontoxic [7]. Similarly, the acute oral toxicity test of the aqueous stem bark extract of *Pterocarpus soyauxii* Taub have also been reported to have an LD_{50} > 10 750 mg/ kg body weight in rats and considered non-toxic [43].

The toxic nature of the drug could lead to abnormalities in body weight [44], hence, change in body weight is a sensitive index to study the detrimental effects of drugs and chemicals [45, 46]. In this study, there was progressive increase in body weight of rats for the treatment groups and the control. The percentage body weight increase was least for the control group compared to all the treatment groups. However, this increase was not statistically significant (p > 0.05), when the control group was compared to the treatment groups. Although, the percentage body weight increase at 300 mg/mL was lower than that of both 50 mg/kg body weight and 2000 mg/kg body weight. This increase in mean body weight of rats in the treatment groups was not dose dependent since the percentage body weight increase at a dose of 50 mg/kg body

weight was greater than that of 2000 mg/kg body weight. The results suggest that the increase in body weight observed in all the treatment groups could be due to the presence of secondary metabolites in *P. tinctorius* that can stimulate the eating habits of rats [46]. This could also imply that the compounds in the extract did not suppress appetite of the rats for food [47]. Decrease in body weight would indicate adverse effects of the plant extract and are often considered as the first signs of toxicity [1, 46]. In this study there was a normal gradual increase in percentage body weight for the treatment groups indicating positive health status of the rats. Hence, the plant extract did not interfere with the normal metabolism of rats which could lead to retard growth and reduction in body weight [48, 49]. Thus, the results support potential safety of *P. tinctorius* methanolic bark extract. In the genus *Pterocarpus* the stem bark extract of *Pterocarpus soyauxii* have been reported to increase body weight of rats [44].

In toxicological experiments, comparison of organ weights between control and treated groups has been accepted as sensitive indicator to predict the toxic effects of compounds [50]. In this study there was insignificant difference in the mean organ-weight ratios of the liver between the control and the treatment groups. It was also observed that increase in dose led to further reduction in the mean organ-body ratio of the liver. This suggests that *P. tinctorius* methanolic sub-extract (M1) could be non-toxic to the liver, since there was slight reduction in mean organ-body ratios of the liver for all the treatment groups compared to the control [9]. In the genus *Pterocarpus*, *Pterocarpus santalinoides* methanol leaf extract have been reported to reduce the mean weight ratio of the liver [51], and the results were statistically significant when the treatment groups were compared to the control. However, in our study the results were not statistically significant, suggesting that there was higher concentration of the constituents responsible for reducing the mean organ-body ratio of the liver in the methanolic leaf extract of *Pterocarpus santalinoides* than the methanolic bark sub-extract of *Pterocarpus tinctorius*.

This study demonstrated that increasing the dosage led to reduction in mean organ-body weight ratios of the right kidneys. Similar pattern was observed for both the left and right kidneys. However, for the left kidneys the mean organ-body weight ratio at 2000 mg/kg body weight was higher instead of being lower than that of 300 mg/kg body weight. This discrepancy could be explained by the difference in the intra species genetic variation of rats and cannot be attributed to the methanolic bark sub-extract of *P. tinctorius*. Generally, higher doses corresponded to lower mean organ-body weight ratio while lower doses corresponded to the higher organ-body weight ratio of both the left and right kidneys. Therefore, the results are possibly suggesting that reduction in mean organ-body weight ratios was dose dependent and that *P.tinctorius* methanolic bark sub-extract is non-toxic to the liver and the kidneys. This is because decrease in organ weight of the kidneys and liver is an indicator of non-toxic plants [9]. Likewise, in the genus *Pterocarpus*, methanolic leaf extract of *P. mildbraedii* have been reported to reduce organ-body weight ratio of albino rats [52], possibly suggesting that *P. mildbraedii* and *P. tinctorius* may have

similar compounds which could reduce the mean organ-body ratio of the liver and the kidney.

The liver and kidney are responsible for detoxification and excretion processes [33]. The nephrotoxic substances are .not only manufactured in the kidney but can also be transported from other parts of the body for excretion [53]. The liver is also a vital organ for metabolism and biotransformation of chemicals in the body [54]. Hence, it is susceptible to oral administered substances [53]. Thus, the liver and kidney are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity [55].

Creatinine and urea concentration are used to determine the effects of plant extract on the tubular and glomerular functions of the kidney [56, 57]. High serum urea and creatinine level is an indication of renal failure [58]. This is because as the kidneys become impaired, creatinine and urea levels in the blood will rise due to poor clearance by the kidneys [59]. In this study, there was insignificant random change of urea levels in the treatment groups and the lowest mean value was observed in the 2000 mg/kg while, the highest in the 50 mg/kg group. The results suggest that slight random changes observed in urea levels is not related to *Pterocarpus tinctorius* extract but could be attributed to the intra genetic variation of the rats. The insignificant changes in creatinine levels in the treatment groups were random and non-dose dependent. These random changes could be explained by the difference in the physiologic and genetic make-up of the rats. This could also suggest that *P. tinctorius* methanolic bark extract may possess mild nephroprotective activity at doses of 50 mg/kg, 300 mg/kg and 2000 mg/kg. In this study urea and creatinine levels were found to be within the normal reference range of 6.8-11.3 mmol/L and 20-60 mmol/L respectively [41, 60, 61]. Hence, *P.tinctorius* is non-toxic to the kidney up to the dose of 2000 mg/kg. Furthermore, the results indicate that the normal excretion of urea by the kidney was not adversely affected [58]. The findings of this study agrees with earlier reports in the genus *Pterocarpus* that the aqueous stem bark extract of *Pterocarpus soyauxii* Taub [43] and stem bark extract of *P. erinaceus [*6] are acutely non-toxic to the liver and the kidney due to non-significant difference mean levels of creatinine and urea in the treatments and control groups of the two studies.

The increase in serum activity of AST, ALT and ALP is usually an indication of damage to the liver cells [58]. These enzymes are mainly localized in the liver and are released into circulation upon damage to the hepatic cells [33]. Therefore, the increase in the activity and concentration of these enzymes reflect the extent of hepatotoxicity [62, 63]. This study demonstrated that there was a low mean level of alanine aminotransferase at 50 mg/kg and 300 mg/kg compared to the control group, while the highest mean level was observed at 2000 mg/kg. This could suggest that at doses higher than 2000 mg/kg, *P. tinctorius* could be toxic to the liver. However, at doses greater than 50 mg/kg and 300 mg/kg body weight but less than 2000 mg/kg body, *P. tinctorius* could protect the liver from damage. In this study, the mean level of aspartate amino transferase at 50 mg/kg was slightly lower than the control group. This result possibly suggests that *P.tinctorius* methanolic bark extract may have hepatoprotective properties at the dose of 50 mg/kg body weight.

However, the slightly higher mean levels of aspartate amino transferase observed at 300 mg/kg and 2000 mg/kg when compared to the control could imply that *P.tinctorius* bark extract may be toxic to the liver at doses greater than 2000 mg/kg body weight. The results for aspartate amino transferase support those of alanine amino transferase. However, for alanine amino transferase the mean levels were lower at 50 mg/kg and 300 mg/kg compared to the control. While for aspartate amino transferase the mean level was lower at 50 mg/kg but greater at 300 mg/kg when compared to the control. This difference could be due to the intra rat variation in the production of aspartate amino transferase and alanine amino transferase thus, cannot be attributed to *P. tinctorius* methanolic bark extract. Moreover, it is reported that alanine amino transferase is found in its highest concentration in the liver and it is more specific to the liver [64]. While aspartate amino transferase is found in the liver and it is also abundant in the cardiac muscle, skeletal muscles, brain, pancreas, lungs, leucocytes and red blood cells [65]. Hence, aspartate amino transferase is less specific to the liver.

Therefore, it is believed that the mean level of aspartate amino transferase, which was slightly higher than the control at 300 mg/kg, could be released into the blood stream even from the other organs of the body of rats apart from the liver, leading to slightly higher mean level of aspartate amino transferase.

In the current study, it was found out that there were non-significant lower levels of alkaline phosphatase at 50 mg/kg and 300 mg/kg compared to the control. However, the mean level of alkaline phosphatase was insignificantly higher than the control. This suggests that *P. tinctorius* is non-toxic to the liver and might possess mild hepatoprotective activity at 50 mg/kg, 300 mg/kg and doses lower than 2000 mg/kg.

An elevated level of total protein is an indication of liver damage and decreased level indicates starvation [1]. The results show that at 50 mg/kg and 300 mg/kg the mean total protein levels were slightly higher than the control group. However, the mean total protein level was lowest at 2000 mg/kg. This implies that the slight changes in the total protein content was random and non-dose dependent. The results further suggest that the random and slight insignificant changes observed in total protein content in the treatment groups were not related to *P. tinctorius* methanolic bark extract but, could be explained by other factors such as difference in the physiological process and genetic variation in rats. Hence, *P.tinctorius* methanolic sub-extract is non-toxic to the liver. In the genus *Pterocarpus*, random changes of total protein levels, which were not statistically significant for *P. santalinoides* have been reported [66, 67].

The biochemical reference ranges for Wistar rats for total protein, ALT, AST, and ALP are 55 to 77 g/L, 13 to 56 U/L, 65 to 203 U/L and 95-611 U/L respectively [68, 69]. In this study the biochemical ranges for total protein and ALT were within the normal range in all the treatment groups suggesting that the plant is non-toxic to the liver. On the other hand, the AST and ALP levels were found to be slightly outside normal reference range in the control group and all treatment groups. This could not be attributed to the plant extract but to the variables which may influence biochemical parameters such as

different analytical methods, number of samples analysed and the environmental conditions of the country [41, 68].

5. CONCLUSION

The results of this study demonstrated that *P. tinctorius* is acutely non-toxic to the liver and kidneys. There was no mortality and no significant changes observed in the physical and behavioral observations of rats. There was no significance difference (p>0.05) for body weight, organ-body weight and biochemical markers such as alanine amino transferase, aspartate amino transferase, alkaline phosphatase, creatinine, urea and total protein when treatment groups were compared to the control. Thus, *Pterocarpus tinctorius* could be considered acutely non-toxic to the liver and the kidney at single doses that are less than or equal to 2000 mg/kg body weight. Therefore, toxicity studies on other organs other than the liver and kidney are highly recommended. Furthermore, scientific research to validate the traditional medicinal use of *Pterocarpus tinctorius* on the diseases to which it is effective should be carried out.

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Table 1: Phytochemical profiles of *P. tinctorius* methanolic stem bark extract

Sno.	Phytochemicals	Methanolic crude extract (present/absent)	Methanolic sub-extract (present/absent)
1	Alkaloids	+	-
2	Phenolics	+++	+++
3	Tannins	+++	+++
4	Flavonoids	++	++
5	Terpenoids	++	-
6	Anthocyanins	++	++
7	Anthraquinones	+	-
8	Saponins	+	+
9	Steroids	+	-

+++ = intense, ++ = intermediate, +=low and - = absent

Table 2: Physical and behavioral effects of rats at 50 mg/kg for *P.tinctorius* methanolic stem bark sub-extract

Observation	0 min	30 min	1 hr	2hr	4hr	7th day	14th day
Skin colour	-	-	-	-	-	-	-
Fur colour	-	-	-	-	-	-	-
Eye colour	-	-	-	-	-	-	-
Sound response	-	-	-	-	-	-	-
Touch response	-	-	-	-	-	-	-
Urination	-	-	-	-	-	-	-
Defecation	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-
Drowsiness	-	-	+	-	-	-	-
Coma	-	-	-	-	-	-	-
Death	-	-	-	-	-	-	-

-= no change observed, + = change observed

Table 3: Physical and behavioral effects of rats at 300 mg/kg for *P.tinctorius* methanolic stem bark sub-extract

Observation	0 min	30 min	1 hr.	2hr	4hr	7th day	14th day
Skin colour	-	-	-	-	-	-	-
Fur colour	-	-	-	-	-	-	-
Eye colour	-	-	-	-	-	-	-
Sound response	-	-	-	-	-	-	-
Touch response	-	-	-	-	-	-	-
Urination	-	-	-	-	-	-	-
Defecation	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-
Drowsiness	-	+	-	-	-	-	-
Coma	-	-	-	-	-	-	-
Death	-	-	-	-	-	-	-

-= no change observed, + = change observed

Table 4: Physical and behavioral effects of rats at 2000 mg/kg for *P.tinctorius* methanolic stem bark sub-extract

Observation	0 min	30 min	1 hr.	2hr	4hr	7th day	14th day
Skin colour	-	-	-	-	-	-	-
Fur colour	-	-	-	-	-	-	-
Eye colour	-	-	-	-	-	-	-
Sound response	-	-	-	-	-	-	-
Touch response	-	-	-	-	-	-	-
Urination	-	-	-	-	-	-	-
Defecation	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-
Drowsiness	-	+	+	-	-	-	-
Coma	-	-	-	-	-	-	-
Death	-	-	-	-	-	-	-

-= no change observed, + = change observed

Table 5: Effect of a single dose of methanolic stem bark sub-extract of *P. tinctorius* on weekly mean body weights of rats

Extract dose	Initial weight	Day 7	Day 14	% weight increase	*p*-value
Control (1ml distilled water)	233.83±9.63	261.33±9.09	276.50±11.73	18.25	-
50mg/kg bw	178.5±4.62	202.0±2.02	223.833±4.23	25.39	0.182
300mg/kg bw	252.17±10.74	277.83±7.85	298.833±9.02	18.5	0.872
2000mg/kg bw	220.5±30.79	241.67±32.76	266.67±30.21	20.94	0.944

Values are expressed as mean ± standard error, Significant difference ($p \leq 0.05$) (n=3). There are no significant ($p > 0.05$) differences between the control and *P. tinctorius* treated rats in their body weight.

Table 6: Effect of methanolic stem bark sub-extract of *P. tinctorius* on the organ-weight ratio of liver and kidney of rats after 14 days

Treatment	Liver	*p*-value	Left Kidney	*p*-value	Right Kidney	*p*-value
Control	0.0343± 0.0014	-	0.0035± 0.0021	-	0.0038± 0.0003	-
50mg/kgbw	0.0367± 0.0001	0.249	0.0031± 0.0015	0.486	0.0032± 0.0019	0.419
300mg/kgbw	0.0344± 0.0004	1.000	0.0034± 0.0006	0.981	0.0033± 0.0002	0.473
2000mg/kg bw	0.0331± 0.0008	0.757	0.0032± 0.0003	0.758	0.0035± 0.0012	0.871

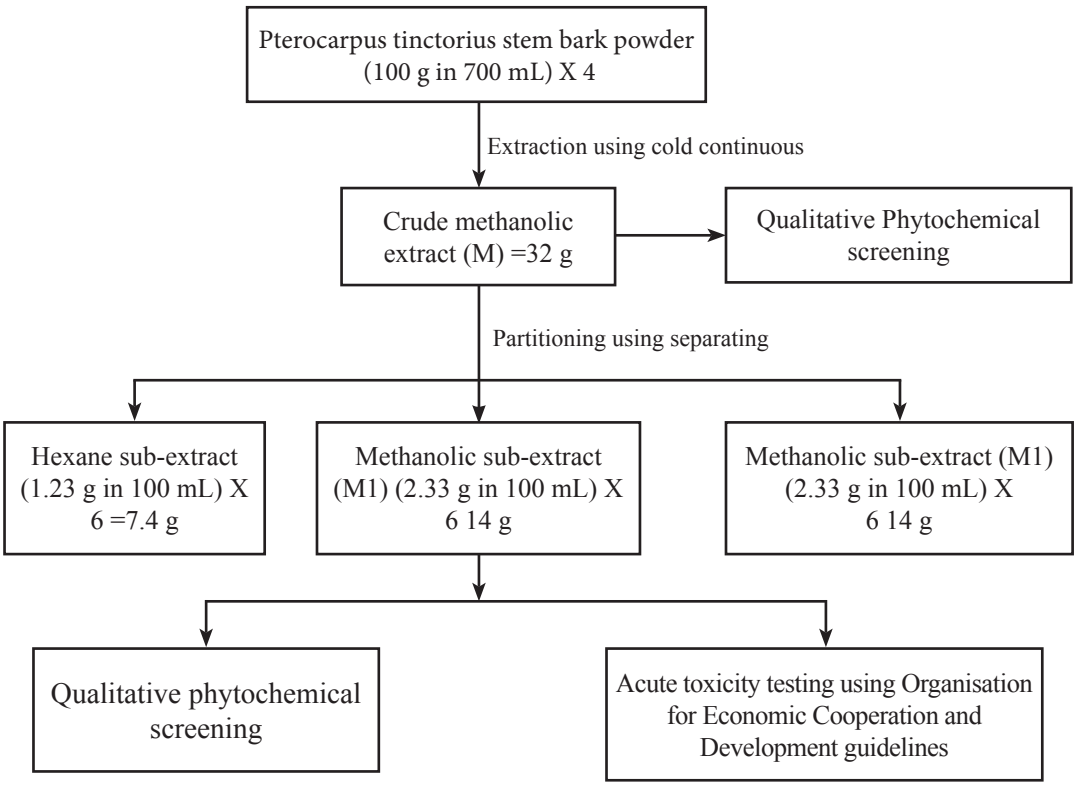
Values are expressed as mean ± standard deviation, Significant difference ($P \leq 0.05$) (n=3). There are no significant ($p > 0.05$) differences between the control and *P. tinctorius* treated rats in their Relative organ weights of the liver and the kidney

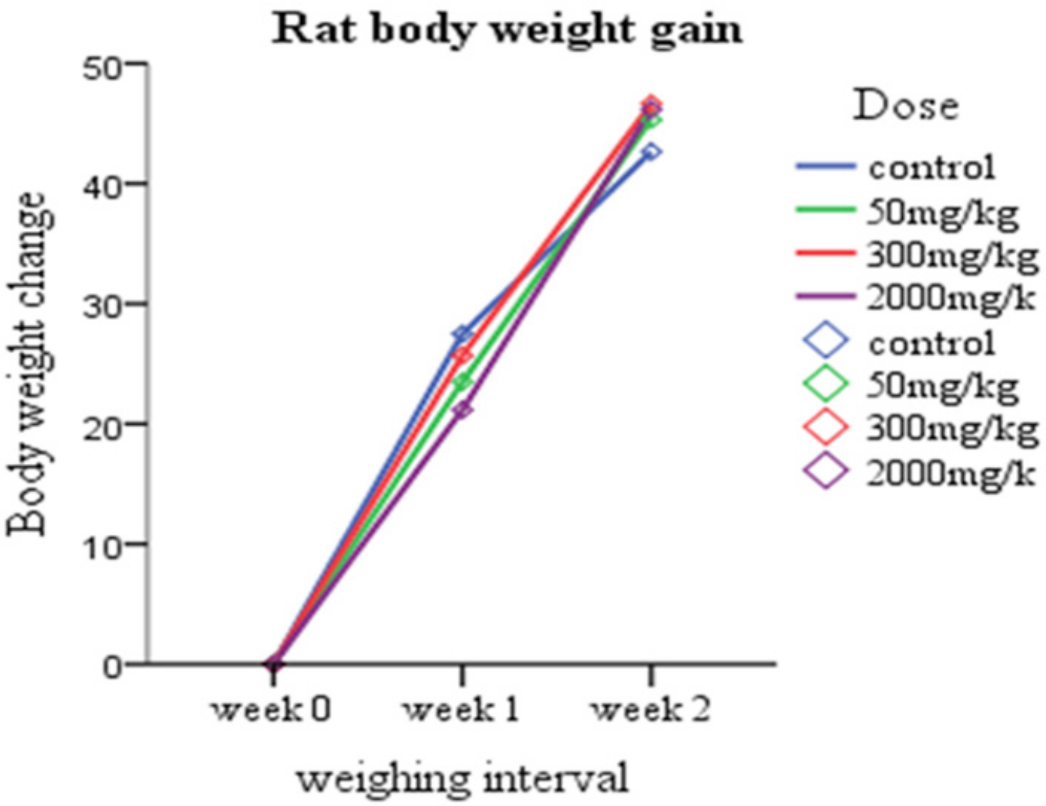
Table 7: Effect of *P. tinctorius* methanolic stem bark sub-extract on biochemical parameters of rats

Parameter	Control	50 mg/kg	*p*-value	300 mg/kg	*p*-value	2000 mg/kg	*p*-value
ALT U/ L	107.27± 4.44	83.07± 7.40	0.393	80.33± 10.93	0.312	118.80± 14.90	0.853
AST IU/ L	191.43± 16.83	160.33± 6.34	0.760	192.97± 3.98	1.000	206.37± 40.48	0.963
ALP U/ L	295.73± 16.38	267.63± 58.21	0.963	171.27± 26.33	0.490	300.20± 17.41	1.000
Creatinine Mmol/L	35.57± 1.30	28.63± 1.50	0.169	30.93± 2.80	0.450	28.87± 2.43	0.188
Urea mmol/L	8.33 ± 0.49	10.56± 1.30	0.410	9.580± 1.325	0.795	7.52± 0.05	0.929
T. P g/L	64.70± 1.08	68.80± 1.91	0.264	69.73± 1.22	0.143	65.97± 2.13	0.924

ALT: Alanine Transaminase, ALP: Alkaline phosphatase, AST: Aspartate Transaminase, TP: Total Protein. Values are expressed as mean ± standard deviation, Significant difference (P ≤0.05) (n=3). There are no significant (p > 0.05) differences between the control and *P. tinctorius* treated rats in their biochemical parameters tested.

Extraction procedure of Pterocarpus tinctorius stem bark extract




Rat body weight gain
Body weight change
50
40
30
20
10
0
week 0
week 1
week 2
weighing interval
Dose
control
50mg/kg
300mg/kg
2000mg/k
control
50mg/kg
300mg/kg
2000mg/k