



## **Influence of Aqueous Stem Bark Extract *Terminalia catappa* L. on Pregnant Wistar Rats and Brain g-6pdh Activities**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors NOA and UPI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** This study investigated the influence of an aqueous stem bark extract of *Terminalia catappa* on pregnant wistar rats and brain glucose-6-phosphate dehydrogenase (G-6PDH) activity.

**Study Design:** Forty-five adult female wistar rats at proestrus sexual cycle were caged overnight with sexually mature male rats of the same strain. The presence of sperm in the vagina lavage obtained the following morning confirmed coitus and was designated as day zero of pregnancy. The pregnant rats were randomly divided into five groups of nine each after confirmation of pregnancy.

**Place and Duration of Study:** This study was carried out in the Animal Holdings of the Department of Anatomy, University of Ilorin, Ilorin, between March 2014 and May, 2014.

**Methodology:** The Group A (control) animals were given feed and water liberally throughout the study. Group B received 400 mgkg/bwt of extract between 1-20 day, Group C received 400 mgkg/bwt between extract 1-6 day, Group D received 400 mgkg/bwt extract between 7-12 day

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and Group E received 400 mg/kg/bwt extract between 13-20 day of gestation. The rats were sacrificed on day 20 of gestation, foetal morphologic measurements were taken as well as adult brain G-6PDH activity assayed.

**Results:** There was no statistical difference between the control and experimental groups of foetal body weight, crown-rump length, tail length, foetal brain weight and placental weight. Also, the adult brain G-6PDH activity level was not statistically different when the control and experimental groups were compared.

**Conclusion:** The dose of extract at 400 mg/kg/bwt consumed during pregnancy, was considered as relatively safe, as it did not cause abnormalities in foetus or influence changes in brain G-6PDH activity in adult rats.

**Keywords:** *Terminalia catappa L*; Glucose-6-phosphate dehydrogenase (G-6PDH); brain.

## 1. INTRODUCTION

*Terminalia catappa* is a large, deciduous tree with smooth grey bark and whorled branches that form a canopy and is found mainly in tropical regions of Asia, Africa, and Australia measuring 25-40 m or 82-130 ft tall [1]. It is commonly referred to as tropical almond, India almond, sea almond [2]. The extract from various part of the plant is use in treatment of different ailment. The extract of the leaves shows antioxidant, anti-inflammatory and hepatoprotective actions [3]. It is also used for the prevention of diabetes [4,5], bacterial infection [6] and cancer [7].

The use of plants for medicinal purpose is no longer new to man, since the practice has existed for many centuries [8]. Many plants are known for medicinal purposes in Africa, but only a few have been described or studied. The safety concern of some of this medicinal plants has always been relegated to the background even by pregnant women; this has often led to many teratologic conditions.

The brain is the control centre for movement, sleep, hunger, thirst, and virtually every other vital activity necessary to survival. The brain control human emotions such as love, hate, fear, anger, elation, and sadness [9]. It also receives and interprets the countless signals that are sent to it from other parts of the body and from the external environment [9].

The brain is sensitive to oxidative stress injury, it has high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids and relatively low antioxidant capacity compared to other tissues [10,11]. The brain cells are damaged or destroyed by oxidized compounds called free radicals that are generated in the body by stress, exercise, oxidation of food and other chemical reactions that occur in the cell

[12]. As a result of depleted number of brain cells due to action of free radicals, neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and stroke occur. However, excessive free radicals can be removed by the action of Glucose-6 phosphate dehydrogenase (G-6PDH).

G-6PDH is a cytoplasmic enzyme that catalyses the first step in the pentose phosphate pathway, which produces NADPH [13]. NADPH is the principal intracellular reductant and its production is mainly dependent on glucose-6-phosphate dehydrogenase. NADPH is a coenzyme that is essential for the protection and repair of oxidative damage in cell. Even the entire antioxidant system and other processes requiring reduction rely on the supply of NADPH [14]. Brain G-6PDH protects against endogenous oxidative DNA damage and neurodegeneration in aging mice [15]. G-6PDH play embryoprotective role in developmental oxidative stress and chemical teratogenesis [16].

Despite many literatures supporting the medicinal use of *Terminalia catappa*, there exist claims among local folks of possible teratogenic effects when taken during pregnancy. That was the reason this study was aimed to investigate the influence of aqueous stem bark extract of *Terminalia catappa* on pregnant wistar rats and brain of G-6PDH activities.

## 2. MATERIALS AND METHODS

Forty-five female (having an average weight of 220 g) and ten male albino Wistar rats (having an average weight of 230 g) were procured from department of anatomy, Ladoke Akintola University of Technology. The animals were acclimatized for two weeks at the animal holdings of Anatomy department, University of Ilorin, Ilorin before the commencement of the

experiment. They were exposed to normal laboratory conditions of temperature, light and humidity. The rats were fed with Growers mash produced by Bendel feeds and water *ad libitum*. The animals were given adequate care in accordance with the Principle of Laboratory and Animal Care prepared by the National Academy of Sciences and published by the National Institute of Health [17]. All the rats were carefully assessed and screened at the end of the acclimatization period. The investigation was conducted in accordance with the principles and guidelines for animal research.

## 2.1 Plant Materials and Extraction

*Terminalia catappa linn* stem bark was obtained from Mission hill orchard, Ugwolawo, Kogi State and authenticated by botany department of university of Ilorin. The stem bark of *Terminalia Catappa* was properly washed in tap water and shade dried for two weeks. The dried stem bark of *Terminalia Catappa* was grinded into powder form using mechanical blender. The aqueous extract was prepared by maceration following the method of previous investigator [18].

## 2.2 Mating of the Female Rats

Vaginal smear test was carried out between 8:00 am and 9:00 am on daily basis prior to mating; this was done to know the phase of oestrous cycle of female rats before introducing the male rats [19]. The presence of predominantly round nucleated epithelial cells indicated the pro-estrus stage. Female rats that were in their pro-estrus phase were kept together with adult male rats between 4:00 pm and 8:00 am the next day. Mating was confirmed by the presence of spermatozoa in the vagina lavage on the following morning and the day was taken as day zero of pregnancy [20].

## 2.3 Experimental Design

Forty-five confirmed pregnant rats were randomly divided into five (5) groups of nine rats each;

- Group A:* (control): received only distilled water
- Group B:* received 400 mgkg/b.wt of extract between 1-20 days of gestation orally.
- Group C:* received 400 mgkg/b.wt of extract between 1-6 days of gestation orally.

*Group D:* received 400 mgkg/b.wt of extract between 7-12 days of gestation orally.

*Group E:* received 400 mgkg/ b.wt of extract between 13-20 days of gestation orally.

## 2.4 Termination of Treatment

On the 20<sup>th</sup> day of gestation, the rats were sacrificed by cervical dislocation; laparotomy was performed and the foetuses were excised. Foetal parameters such as foetal weight, tail length, crown-rump length and placental weight were measured. The crania of the adult rats were open by mid-sagittal incision and brain were removed, blotted dry with a filter paper and weighed. The adult brain was homogenized in 10 volumes of ice cold (0-4°C) medium containing 50 mM Tris-HCl, pH 7.4 and 300mM sucrose, using a homogenizing machine. The homogenates were centrifuged at 5000 rpm for 10 minutes; the supernatant was immediately used for G6PDH activity measurement.

G6PDH assay was done using the method of Beutler [20]. This method measures the rate of reduction of NADP<sup>+</sup> to NADPH when homogenate is incubated with Glucose 6-Phosphate (G6P). The assay mixture consist of 0.02M Glucose 6-Phosphate (0.1 ml), 0.02 mM NADP<sup>+</sup> (0.1 ml), (0.1M) MgCl<sub>2</sub>, Tris buffer pH 8.0 (0.68 ml) and homogenate (0.2 ml). The assay was monitored at the wavelength of 340 nm over a 5 minute time course on the spectrophotometer. A full scale reading of 1.0 absorbance unit was maintained for the spectrophotometer.

## 2.5 Statistical Analysis

Data was analysed using Analysis of Variance (ANOVA) and students t-test (Tukeys test) with the statistical software SPSS version 20.0 at 95% confidence interval. Values were reported as mean  $\pm$  S.E.M and  $p < 0.05$  was considered statistically significant.

## 3. RESULTS

Result gotten has shown in Table 1, indicated that there was no significance difference ( $p > 0.05$ ) in body weight of foetuses when control (Group A) was compared with the experimental groups. Even when the body weight of foetuses was compared between the experimental groups (Group B, C, D & E) there was no significant

difference ( $p>0.05$ ). Also from Table 1, the crown-rump length, tail length and placenta weight of the fetuses were compared between their control groups (Group A) and the experimental groups (Group B, C, D & E), the result indicates that there were no significant difference ( $p>0.05$ ).

The result of foetal brain weight as seen in Table 1 indicates no significant difference ( $p>0.05$ ) when foetal brain weight of experimental groups (Group B, C, D & E) were compared with the control as well as in between themselves.

Fig. 1 presents the glucose-6-phosphate dehydrogenase enzyme activity in the brain of rats administered extract *Terminalia catappa*.

The activity of glucose-6-phosphate dehydrogenase was not significantly different ( $p>0.05$ ) when experimental groups (Group B, C, D & E) were compared with the control group. Even when the level of G-6PDH was compared between the experimental groups there was no significant difference ( $p>0.05$ ).

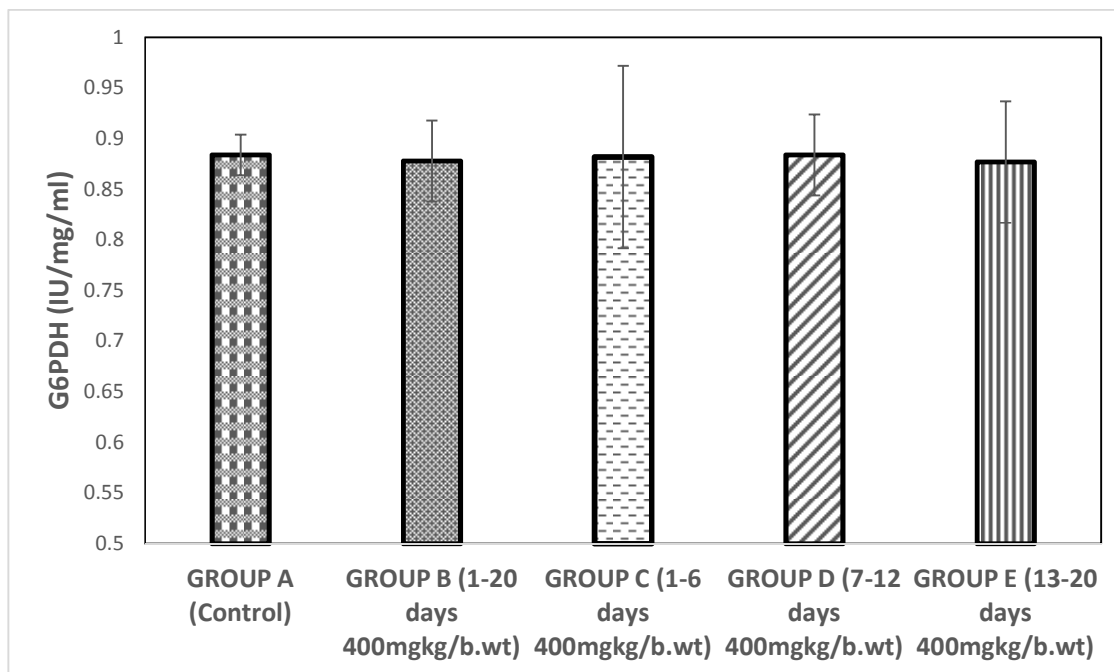
#### 4. DISCUSSION

Teratogens are substances that cause significant abnormalities either structural or functional following foetal exposure during pregnancy [21]. Several studies have demonstrated a correlation between intrauterine growth retardation and Teratogenesis. Parameters used in assessing

**Table 1. Foetal weight, crown-rump length, tail length and placenta weight foetal brain weight of rat fetuses**

Parameters	Group A	Group B	Group C	Group D	Group E
Foetal weight (g)	4.98±0.03	4.86±0.11	4.77±0.002	4.78±0.09	4.82±0.022
Crown-rump length (cm)	4.0±0.06	3.46±0.12	4.0±0.06	4.0±0.09	4.7±0.06
Tail length (cm)	1.07±0.03	0.97±0.07	1.03±0.09	1.07±0.07	1.10±0.06
Placenta weight (g)	0.26±0.02	0.26±0.02	0.28±0.01	0.28±0.02	0.28±0.02
Foetal brain weight (g)	0.81±0.04	0.78±0.04	0.80±0.06	0.79±0.09	0.79±0.06

Values presented as mean±SEM; n=8; No significant difference between parameters in control and experimental groups



**Fig. 1. Activities of glucose-6-phosphate dehydrogenase (G-6PDH) in the brain of pregnant Wistar rats following the administration of aqueous stem bark extracts of *Terminalia catappa***

foetal growth and morphological development include: body weight, crown-rump length, tail length and placental weight [22-26]. From the result of this study (in Table 1), the foetal body weight, crown-rump length, tail length, foetal brain weight and placental weight appeared statistically the same in both control and experimental groups. This possibly indicated that the extract administered to the rats may not be teratogenic as earlier suspected or the dose administered not potent enough to cause abnormalities in foetuses.

Enzymes are biocatalyst that regulates the rates at which all physiological processes take place [27,28]. The presence and maintenance of a complete and balanced set of enzymes is essential for the breakdown of nutrients to supply energy and chemical building blocks; the assembly of those building blocks result into proteins, DNA, membranes, cells and tissues [29].

G-6PDH is a cytoplasmic enzyme that catalyses the first step in the pentose phosphate pathway, which produces NADPH [13]. This enzyme act by removing free radicals that causes damage to cells. In the brain, low level of G-6PDH result in oxidative damage, depletion of brain cells which eventually causes neurodegenerative diseases [15]. G-6PDH is present in cells but not uniformly expressed in all cells, with basal activity varying up to 10 folds in different organs and tissues [30]. In this study, the result obtained (Fig. 1) indicates uniformity in the level of G-6PDH enzyme activity when control and experimental groups were compared. This indicates that the extract administered do not influence (increase or decrease) the level of G-6PDH activity in the experimental groups; since the level of enzyme activity in both the control and the experimental groups are the same. It could also mean that the dose 400 mg/kg/b.wt administered might not have been enough to influence (increase or decrease) the level of G-6PDH activity in experimental groups.

## 5. CONCLUSION

In conclusion, the present investigation demonstrates that the dose of aqueous stem bark extract of *Terminalia catappa* Linn. at 400 mg/kg/bwt consumed during pregnancy, is considered relatively safe, as it did not cause teratogenic abnormalities or influence the brain G-6PDH activity in adult.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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