

HEPATO-PROTECTIVE EFFECT OF *Telfaria occidentalis* (Ugwu) LEAVES SUPPLEMENTATION IN PARACETAMOL INDUCED-TOXICITY IN RATS

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of Telfaria **ABSTRACT:** The *hepato-protective* effect occidentalis leaves supplementation on paracetamol induced liver toxicity in wistar rats was investigated. A total of forty Wistar (female and male) rats weighing between 150-250g were selected for the study. The animals were randomly divided into four groups of five animals each. Animals in group 1 and 2 received standard chow only while animals in group 3 and 4 received 5% and 10% T. occidentalis supplementation in their feed respectively for six weeks. Group 2, 3 and 4 were administered 3000 mg/kg bodyweight of paracetamol intraperitoneally after six weeks. The animals were sacrificed after 24hours of induction and the liver was harvested for analysis. Alanine transaminase, Aspartate Transaminase and Alkaline Phosphatase, total protein, total bilirubin and direct bilirubin were measured in the liver homogenate following standard methods. A significant decrease in the level of ALT, AST and ALP, Bilirubin and increased total protein in group 3 and 4 were observed when compared with group 2. This study showed that T. occidentalis leaves possess protective potential on the liver when supplemented in the diet thereby preventing deleterious effects that might arose from paracetamol overdose or overuse. The study concluded that the protective effect of the leaves on the liver is percentage supplementation (dose) dependent.

KEYWORD: Acetaminophen, Liver, Paracetamol, Telfaria occidentalis, Ugwu leaves.



INTRODUCTION

Acetaminophen, commonly known as paracetamol, is one of the most widely used oral analgesics and antipyretics. Paracetamol is used to treat conditions, such as headache, muscle aches, arthritis, backaches, toothaches, cold and fevers. Paracetamol is absorbed rapidly from the gastrointestinal tract reaching therapeutic levels (in plasma or blood) between 30 minutes and 2 hours when taken orally (Agrawwal et al., 2021). With an overdose, plasma or blood level reaches peak at 4 hrs (Agrawwal et al., 2021; Ye et al., 2018). Historically, paracetamol dose for adults is 650–1000 mg taken every 4–6 hrs, not exceeding 4 grammes/day; while in children, the dose recommended is 15 mg/kg every 6 hour, which is up to 60 mg/kg. In the year 2012, the US Food and Drug Administration suggested a maximum adult daily paracetamol dose of 3 g, not exceeding 650 mg every 6 hrs, as needed. McNeil Consumer Healthcare, manufacturer of Tylenol brand of paracetamol, has also implemented further reduction of the maximum recommended daily adult dose of its 500 mg tablet to 3 g, and 3.25 g for its 325 mg Tylenol tablet (Farrel, Germaine & Defendi, 2020). Ingesting more than 7.5–10 g of paracetamol over a 24-hour period increases the risk for developing paracetamol-induced hepatotoxicity (Offor et al., 2022).

When a therapeutic dose is administered, paracetamol is metabolized primarily (about 90%) through "first pass" metabolism in the liver via glucuronidation and sulfation (Ghanem et al., 2016). In addition, a small fraction (5–15%) of paracetamol is metabolized by CYP450 enzymes (mostly the CYP1A2, CYP2E1, CYP3A4, and CYP2A6 isoforms) (Offor et al., 2022). This reaction results in the production of N-acetyl-p-benzoquinoneimine (NAPQI), a reactive intermediate of very high toxicity. Glutathione, liver's natural antioxidant reduces NAPQI to nontoxic mercaptate and cysteine compounds, which are then safely excreted by the kidneys or bile. When there is an overdose of the paracetamol, metabolism is diverted into the CYP450 route, resulting in more accumulation of NAPQI (Ghanem et al., 2016). NAPQI decreases glutathione and directly damages cells in the liver, leading to liver damage and or hepatic necrosis.

The liver is a vital organ that plays a role in controlling biochemical and physiological activities including homeostatsis, growth, energy and nutrient supply, detoxification of drugs and other xenobiotics, and combating infections (Kalra et al., 2022). Therefore, it is very susceptible to being damaged by hepatotoxic agents (Bashir et al., 2021). Researches on plants and herbs that could potentially substitute the chemical-based drugs is very crucial as many medicinal plants have been found with hepatoprotective properties (Arman et al., 2022; Hassan et al., 2019; Ugwu & Suru 2021). One of the plants being investigated for its potential hepatoprotective activity is *Telfaria occidentalis*. *T. occidentalis* (fluted pumpkin) a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds is a member of the *Cucurbitaceae* family. *T. occidentalis* is one of the main vegetable crops cultivated in the Southern part of Nigeria, where it is known by different names such as "iroko" or "apiroko" in Yoruba, "ubong" in Efik, "ugu" in Igbo, "umeke" in Edo, and "umee" in Urhobo (Aworunse et al., 2018). However, it is commonly referred to as Ugwu leaves. It is thought to have originated from the South-Eastern region of Nigeria and spread by the Igbos, who have cultivated this crop since prehistoric times. It is used primarily in soups and herbal medicines.

It is used in ethnobotany as antidiabetic, antihypertensive, antitumouric, antioxidant, immunodulator, antibacterial, antihypercholesteremic, antiparasitic, anti-inflammatory and in the treatment of central nervous system-related disorders including convulsion (Aworunse et

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al., 2018; Nwozo et al., 2004; Oyewole & Abalaka, 2012; Igbenegbu & Abdul, 2014). Methanol extract of *T. occidentalis* has antidiabetic potential (Airaodion et al., 2019a) and aqueous extract of *T. occidentalis* leaves have been shown to exhibit hepato-protection against garlic-induced oxidative stress (Olorunfemi et al., 2005; Oboh, 2006) and ethanol-induced oxidative stress (Airaodion et al., 2019b). The leaves are rich in minerals, antioxidants and vitamins, such as thiamine, riboflavin, nicotamide and ascorbic acid and in treating anaemia due to its haematinic properties (Eseyin et al., 2014). Aisida et al. (2021) reported that the antibacterial activities of Nanoparticles found in *T. occidentalis* is effective against human pathogen pneumonia. Ogunmoyole et al. (2019) reported the hepatoprotective potential of extracts of *T. occidentalis* leaves, however its supplementation has not been reported in literature. Therefore, this study intends to evaluate the effect of supplementation of *Telfaria occidentalis* leaves on paracetamol-induced toxicity in male and female Wistar rats.

METHODOLOGY

Collection and preparation of plant materials

Fresh leaves of *T. Occidentalis* were purchased from a farm at Imota in Ikorodu local government area of Lagos State, identified and authenticated at Forest Herbarium Ibadan (FHI) and herbarium specimen deposited. The leaves were plucked from the stem and cleaned thoroughly with tap water to remove all debris and contaminants, oven dried at 40°C, pulverized using Warring blender and stored in an air tight containers for subsequent use. The pulverized sample was added to the rat feed as supplement.

Experimental Animals

Thirty Male and female Wistar strain rats averagely weighing 150 - 200 g were recruited for the study. They were purchased from Babcock University Ilishan, Ogun State animal facility, acclamatised for two weeks and fed with standard chow with free access to food and water. The rats were housed in different cages at 12 hours light and dark cycle, their cages were cleaned twice a week and water containers washed daily. The animals were used following guidelines for the care and use of laboratory animals by National Research Institute of Health (NIH, 2011). Ethical approval was obtained from Research and Ethics Committee, Caleb University, Lagos.

Grouping and Feeding

The rats were grouped into four. The 5% and 10% of *T. occidentalis* leaves were added as supplement to their feed (w/w) for six weeks. The rats feed and grouping were as follows:

Group 1 - Standard Chow only
Group 2 - Standard Chow + paracetamol (no supplement)
Group 3 - Standard Chow + 5% *T. occidentalis* leaves + 3000 mg/kg paracetamol
Group 4 - Standard Chow + 10% *T. occidentalis* leaves + 3000 mg/kg paracetamol

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The weights of the rats were recorded before supplementation, induction and before being sacrificed.

Preparation of Liver Homogenate

Twenty four hours after acetaminophen induction, animals were sacrificed under chloroform as an anesthesia. The liver was removed and rinse in 0.9% saline, 100 mg of liver was weighed and homogenized with 10 mL 0.05 M of potassium dihydrogen phosphate buffer, pH 7.4, using Teflon homogenizer, Thomas Scientific, India. The homogenate was centrifuged using refrigerated centrifuge at 10,000 rpm for 15 mins at 4°C. The supernatant was collected and used for analysis.

Determination of Biochemical Parameters

Alkaline phosphatase (ALP), Aspartate transaminase AST and Alanine transaminase (ALT) activity of liver homogenate were determined according to the method described by Reitman and Frankel (1957) using Fortress kit from United Kingdom. The total protein was determined by the biuret method as described by Tietz (1995). Total bilirubin and direct bilirubin activity in the homogenates were determined by the method of Modiffied Jendrassik and Grof by Mori (1978).

Statistical Analysis

Data obtained were presented as mean \pm standard deviation. The significant differences between groups were determined using one way analysis of variance (ANOVA) using statistical package for Social Sciences (SPSS) IBM version 21 followed by Duncan's multiple range tests where appropriate. The differences were considered significant at 95% confidence limit.

RESULTS AND DISCUSSION

	Group 1	Group 2	Group 3 (5% T.	Group 4 (10%
			occidentalis)	T. occidentalis)
Baseline	177 ± 0.02	177 ± 0.08	170 ± 0.08	170 ± 0.11
After supplement,	182 ± 0.11	183 ± 0.11	190 ± 1.2^{a}	198 ± 0.02^{a}
before induction (g)				
After induction,	182 ± 0.08	165 ± 0.11	180 ± 0.48^{b}	193 ± 0.24^{b}
before sacrifice (g)				

 Table 1: Effects of T. Occidentalis supplementation on body weight of female Wistar rats.

Data is represented as mean \pm SD.

Values represent significantly different from the control (Group 2) at p < 0.05.



Body Weight	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4 (10% T. occidentalis)
Baseline	217 ± 1.11	216 ± 1.30	218 ± 1.02	216 ± 2.11
After supplement,	220 ± 0.08	222 ± 0.12	230 ± 1.18^{a}	233 ± 1.04^{a}
before induction (g)				
After induction (g)	220 ± 0.11	205 ± 0.08	225 ± 1.11^{b}	230 ± 0.12^{b}

Table 2: Effects of T. occidentalis supplementation on body weight of male Wistar rats.

Data is represented as mean SD.

Values represent significantly different from the control (Group 2) at p < 0.05.

Table 3: Effect of T. occidentalis leaves supplementation on AST activity (UI) in paracetamol-induced toxicity in rats

	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4 (10% T. occidentalis)
Male	36.83±3.00	30.00± 6.19	3 1.53 ± 1.25	27.13 ± 5.02^{a}
female	33.67 ± 7.33	23.00 ± 2.31	$22.00\pm0.0^{\text{ a}}$	21.17 ± 2.95 ^a

Data represented as Mean \pm SD.

Values represents significantly different from the control (Group 2) at p < 0.05

Table 4: Effect of *T. occidentalis* leaves supplementation on ALT activity (UI) in paracetamol-induced toxicity in rats.

	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4
Male	67.00 ± 5.20	65.00 ±9.10	66.25 ± 8.78	62.25 ± 6.33^{a}
female	57.83 ± 5.83	89.17 ± 2.52	73.00 ± 8.19^{a}	64.0 ± 0.0^{a}

Data represented as mean \pm SD.

^aValues represent significantly different from the control (Group 2) at p < 0.05.

Table 5: Effect of T. occidentalis leaves supplementation on ALP activity (UI) in paracetamol-induced toxicity in rats

	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4 (10% T. occidentalis)
Male	50.60 ± 9.74	101.43 ± 49.04	73.56 ± 22.11	63.40 ± 22.56
female	66.23 ± 33.13	56.58 ± 37.26	42.32 ± 25.96^{a}	42.12 ± 4.14^{a}

Data represented as mean \pm SD.

^aValues represent significantly different from the control (Group 2) at p < 0.05.



Table 6: Effect of T. occidentalis	leaves supplementation	on total bilirubin	(mg/dL) in
paracetamol-induced toxicity in 1	rats		

	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4 (10% T. occidentalis)
Male	2.42 ± 0.12	1.52 ± 0.13	1.76 ± 0.35^{a}	1.09 ± 0.19^{a}
female	1.20 ± 0.45	1.12 ± 0.14	1.01 ± 0.16^{a}	0.94 ± 0.13^{a}

Data represented as mean \pm SD.

^aValues represent significantly different from the control (Group 2) at p < 0.05.

Table 7: Effect of *T. occidentalis* leaves supplementation on direct bilirubin (mg/dL) in paracetamol-induced toxicity in rats

	Group 1	Group 2	Group 3 (5% T.	Group 4 (10%
			occiaentalis)	1. occiaentalis)
Male	0.73 ± 0.14	0.72 ± 0.29	0.64 ± 0.18^{a}	0.30 ± 0.11^{a}
female	0.88 ± 0.15	0.51 ± 0.27	0.81 ± 0.17^{a}	0.29 ± 0.07^{a}

Data represented as mean \pm SD.

^a Values represent significantly different from the control (Group 2) at p < 0.05.

Table 8: Effect of T. occidentalis	leaves supplementation on	total protein (g/dL) in
paracetamol-induced toxicity in	rats	

	Group 1	Group 2	Group 3 (5% T. occidentalis)	Group 4 (10% T. occidentalis)
Male	19.77 ± 1.77	19.68 ± 1.76	21.69 ± 1.15^{a}	23.15 ±1.91 ^a
female	18.55 ± 4.14	18.63 ± 1.56	20.62 ± 1.03^{a}	20.81 ± 0.25^{a}
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Data represented as mean \pm SD.

^aValues represent significantly different from the control (Group 2) at p < 0.05.

There was a significant increase (p < 0.05) in body weight of animals after six weeks of *T*. *occidentalis* supplementation before inducing hepatotoxicity. After paracetamol induced toxicity, a significant decrease (p < 0.05) in body weight was observed in the control group (group 2). There was no significant difference (p > 0.05) in body weight of supplemented groups when compared with their weight before induction. However, there was a significant difference (p < 0.05) in body weight of supplemented groups (group 3 and 4) when compared with control (group 2). Similar response was recorded in male and female animals. Supplementation of *T. occidentalis* leaves resulted in a percentage dependent increase in body weight of experimental animals used in the study for both male and female (Table 1 and 2).

Hepatic damage is routinely monitored by measuring specific liver enzyme biomarkers such as AST, ALT and ALP in the liver of experimental animals (Arman et al., 2022). In healthy animals, these enzymes are located in the cytoplasm of hepatocytes, however, they are released into circulation following hepatic damage (Ogunmoyole et al., 2019). It is expected that levels of these enzymes should rise in the serum but decreased in the tissues where they were



originally localized prior to damage (Ugwu & Suru, 2021). Levels of AST was significantly increased (p < 0.05) in the tissue of the control animal as observed by the activity of the enzyme which was attributed to the toxicity caused by paracetamol (Table 3). There was significantly reduced (p < 0.05) AST activity in the liver tissue relative to the control animals. This was due to leakage of the enzyme from the hepatocyte, leading to a decrease in its activity. Arguably, leakage of an enzyme from a localized compartment should lead to a decrease in its amount while its level in the other compartment (recipient) should increase. The damage was higher in animals not administered with *T. occidentalis* supplementation. The same pattern of results was obtained for ALT (Table 4) and ALP (Table 5) in the liver homogenates of the experimental animals for similar reasons.

Total bilirubin levels of group 3 and group 4 (Table 6) was significantly decreased when compared with the control (group 2) for male and female respectively. However further significant reduction was observed in group 4 which indicated that increased supplementation suppressed paracetamol toxicity. The same pattern in activity was observed for direct bilirubin (Table 7). This finding was similar to the report of Ogunmoyole (2019) and Ugwu and Suru (2021).

The total protein concentration of the total protein concentration increases after supplementation with *T. occidentalis* (Table 8). However, total protein concentration of group 4 (10% supplementation) was significantly higher (p < 0.05) from the control group which corroborate the nutritional activity of *T occidentalis* and its ability to have protective effect on the liver as reported by Ogunmoyole et al. (2019), Airaodion et al. (2019), Aworunse et al. (2018) and Kayode et al. (2009).

CONCLUSION

Supplementation of *Telfairia occidentalis* leaves which can be taken orally or with meal can suppress the effects that might arose from paracetamol overdose or overuse. Hepatoprotective effect of *T. occidentalis* on paracetamol induced toxicity is hereby reported. However, it is observed that protective effect of the leaves on the liver is percentage supplementation (dose) dependent.

Author contributions

Adeyemi Maria, conceived and designed the experiments. Adeyemi and Olayemi, and Adigun contributed materials, analyse, and performed the experiments. Adeyemi wrote the manuscript while Osilesi Odutola proof read the manuscript.

Conflict of Interest

There are no conflict of interest whatsoever.

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