

Full Length Research Paper

Antioxidant and antikindling effect of *Tapinanthus globiferus* growing on *Ficus glumosa* in pentylenetetrazole induced kindled rats

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Tapinanthus globiferus growing on *Ficus glumosa* is a plant used locally for the treatment of epilepsy. It is found in West Africa on many tree crops. The present study aims to investigate the anti-kindling and antioxidant activity of the aqueous extract of *T. globiferus*. A total of 40 rats were divided into 4 groups (n=10). Groups 1 to 3 received 100, 200 and 400 mg/kg, respectively, of the extract orally, followed by 35 mg/kg of pentylenetetrazole (PTZ) *i.p* after an hour. Group 4 (control) was given 35 mg/kg of PTZ and normal saline and also observed for 30 min. This was repeated after every 48 h until all rats in the control group became fully kindled, that is, attained a racine score of 4 or 5 on three consecutive occasions. At the end of the experiment, the brain tissues of all rats were removed, homogenized and analyzed for antioxidant effect using lipid peroxidation, reduced glutathione, catalase, superoxide dismutase and uric acid tests. The extract was observed to significantly ($p < 0.001$) reduce the development of stage 5 kindling state as compared to the control group. The extract also significantly ($p < 0.05$) increased the activity of superoxide dismutase in the group treated with 400 mg/kg and also increased the activity of catalase in the 100 mg/kg treated group as compared to the control. The data obtained from this study suggests that the aqueous extract of *T. globiferus* growing on *F. glumosa* may possess bioactive compounds with antikindling and antioxidant effect and this may support its traditional use in the management of epilepsy.

Key words: Antikindling, *Tapinanthus globiferus*, antioxidant, epilepsy, pentylenetetrazole rats.

INTRODUCTION

Epilepsy is a chronic disease affecting up to 1% of the population, making it second to stroke as one of the most common serious neurological disorders (Carl, 2006). It is a disorder in which the balance between cerebral

excitability and inhibition is tipped towards uncontrolled excitability and characterized by recurrent unprovoked seizures (Gregory and Yehezkiel, 2001). The cause of most cases of epilepsy is unknown, although some

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people develop epilepsy as a result of brain injury, stroke, brain tumors, infection of the brain and birth defects (WHO, 2016). Therapy is symptomatic in that available drugs inhibit seizure but neither effective prophylaxis nor cure is available (McNamara, 2001).

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need (Akerere, 1988). Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity (Raza et al., 2003) and many such plants need to be scientifically investigated.

Chemical kindling is a phenomenon used for understanding the epileptogenic process involved in development of seizures and for studying molecules that have the potentials to prevent this process (Holmes, 2007). Kindling is a phenomenon ensuing from progressive intensity of convulsion activity due to repetitive administration of electrical or chemical sub-convulsive stimulators (Pavlova et al., 2004). If the stimulus causes generalized convulsion in experimental animal, it is accepted that kindling is completed and it is agreed that this abnormal excitable status remain permanent (Erdogan et al., 2006). Chemical kindling seizures induced with pentylenetetrazole (PTZ) are human absence epilepsy and myoclonic, generalized tonic-clonic and it is a model for drug resistance epilepsy (Ali et al., 2005).

The present study aims to investigate the antiepileptogenic potentials of TG used in parts of Sokoto and Kebbi states for the treatment of epilepsy. Through personal communication with traditional medicine men from these states, ethno-medical information on the use of the plant in the management of epilepsy was obtained.

MATERIALS AND METHODS

Plant collection and identification

The whole plant of *Tapinanthus globiferus* growing on *Ficus glumosa* (TG) was collected in Zuru Local Government Area, Kebbi State in the month of July, 2016. It was authenticated by an expert through a previously identified specimen of the plant deposited at the herbarium of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria with a voucher specimen (UDUH/ANS/0076).

Preparation of the extract

The whole plant was dried at room temperature and pulverized into powder using pestle and mortar. The powder was accurately weighed and subjected to aqueous extraction by maceration and subsequent filtering. The filtrate was thereafter dried in an oven at 45°C. After sufficient drying, the extract was scraped from the evaporating dish and weighed to ascertain the yield.

Experimental animals

Adult rats of both sexes weighing between 83 and 200 g were obtained from the Animal house facility of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were maintained in a well-ventilated room under ambient laboratory conditions of temperature and humidity and were fed on commercial feed and water throughout the course of the experiment. All experimental protocols were approved by the University Animal Ethics Committee. Forty rats were grouped into 4 groups of 10 rats (n=10). Group 1 served as the control which received normal saline, while groups 2 to 4 received TG extract at doses of 100, 200 and 400 mg/kg, respectively.

Phytochemical screening

The dried aqueous extract of the whole plant of TG was subjected to phytochemical screening tests for the detection of various constituents using the method described by Sofowora (2008) and Trease and Evans (2002).

Acute toxicity study

The acute toxicity studies of TG have been previously reported by Abubakar et al. (2016) to be ≥ 5000 mg/kg po.

Antikindling study

Pentylenetetrazole (PTZ) induced seizure in rats

The method described by Goddard et al. (1969) and Racine (1972) was employed for this study. 40 rats were divided into 4 groups, n=10 rats. Groups 2 to 4 were given 100, 200 and 400 mg/kg, respectively, of the extract orally, followed by 35 mg/kg of PTZ i.p after an hour and were observed for 30 min. The first group was treated with 35 mg/kg of PTZ and normal saline and was observed for 30 min. Seizure scores 30 min after each PTZ injections were defined as follows: (1) Phase 0: no response; Phase 1: ear and facial twitching; Phase 2: myoclonic body jerk; Phase 3: clonic forelimb convulsion; Phase 4: generalized clonic convulsions, turning onto one side position; Phase 5: generalized clonic-tonic convulsions (or death within 30 min).

Antioxidant activities of *T. globiferus* growing on *F. glumosa*

Determination of lipid peroxidation in PTZ-kindled rat brain homogenates

Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels, and its level was determined spectrophotometrically by use of thiobarbituric acid reactive substances (TBARS) method previously described (Ohkawa et al., 1979).

Twenty four hours after the 35 mg/kg PTZ challenge, the kindled rats were sacrificed by decapitation and the whole brain was removed and homogenized (100 mg/ml) in ice-cold 0.1 M phosphate buffer (7) (Ohkawa et al., 1979). One hundred and fifty microliter of the supernatant was diluted to 500 μ l with double deionized water. 250 μ l of 1.34% thiobarbituric acid were added to all the tubes, followed by addition of equal volume of 40% trichloroacetic acid. The mixture was shaken and incubated for 30 min in hot boiling water bath with a temperature $> 90^{\circ}\text{C}$. Tubes were allowed to cool to room temperature and the intensity of the pink-coloured complex formed was measured at 532 nm in a spectrophotometer using 0 concentration as blank.

Determination of reduced glutathione in the brain of kindled rats

The method described by Patterson and Lazarow (1955) was employed. The principle is based on the fact that glutathione reacts with an excess of alloxan to produce a substance with an absorption peak at 350 nm.

Enzymatic assay for catalase in the brain of PTZ kindled rats

The method of Beers and Sizer (1952) was employed. The principle involves:



The disappearance of peroxide is followed spectrophotometrically at 240 nm. One unit is equal to one μmole of hydrogen peroxide decomposed per minute under specified conditions of 25°C.

Enzymatic assay of superoxide dismutase in the brain of PTZ kindled rats

The method of Zou et al. (1986) was adopted, 6 tubes were arranged in rows of threes, to the first row, 0.10 ml of buffer, 0.83 ml of distilled water and 0.05 ml of sample brain homogenate were pipetted, respectively. To the second row, 0.15 ml, 0.83 and nil samples were pipetted, respectively. The test tubes were incubated at 25°C for 10 min, and then transferred into a cuvette and 0.02 ml pyrogallols were added. The content was mixed thoroughly by inversion and the increase in absorbance was measured at 430 nm using the maximum linear rate for both test and blank.

Determination of uric acid in PTZ-kindled rat brain

Uric acid level was determined using REDOX kit, following the protocol described by the manufacturer.

Determination of gain in body weights

The weight gain of the rats was assessed weekly and recorded until the day of sacrifice, the initial weight prior to initiation of PTZ administration was noted and compared with the final weight post-kindling.

Statistical analysis

Results were expressed as mean $\pm$ standard error of the mean (SEM) and percentages. Data analysis was performed using Graph Pad Prism statistical software (version 6.0). Comparison between groups was made using analysis of variance (ANOVA) and Kruskal Wallis test where necessary. When a statistically significant difference was obtained, a *post hoc* Dunnett's test or Dunnett's test was performed for multiple comparisons depending on the nature of data. Values of $p < 0.05$ were considered significant.

RESULTS

Percentage yield

Percentage yield of the extract was found to be 8.5% w/w.

Table 1. Phytochemical screening of *Tapinanthus globiferus* whole plant.

Constituent	Results
Carbohydrate	+
Tannins	+
Alkaloid	+
Saponnin	+
Flavonoid	+
Antraquinones	+
Proteins	+
Glycosides	+
Steroid	+
Triterpenes	-

+ = Present; - = absent.

Phytochemical screening

The phytochemical screening of *T. globiferus* growing on *F. glumosa* revealed the presence of carbohydrates, tannins, alkaloids, saponins, flavonoids, anthraquinones, proteins, glycosides and steroids with absence of triterpenes as shown in Table 1.

Effect of *T. globiferus* growing on *F. glumosa* in pentylenetetrazole induced kindled rats

The result of forty eight hourly treatments of rats with 35 mg/kg of pentylenetetrazole on alternate days showed a progressive increase in convulsion response. The control group and 100 mg/kg extract treated group reached the Racine score of 5.0 on the 10th day. In the 200 and 400 mg/kg treated group, the extract of *T. globiferus* significantly ($p < 0.001$) decreased the development of kindling, and the maximum seizure score was 1 in the 400 mg/kg treated group and 3 in the 200 mg/kg treated group respectively, as shown in Figure 1.

Effect of *T. globiferus* on weight gain in pentylenetetrazole induced kindled rats

The results of 48 hourly administration of aqueous extract of *T. globiferus* to rats indicate a dose dependent increase in the mean weight gain which is statistically insignificant ($p > 0.05$) as shown in Table 2.

Effect of *T. globiferus* aqueous extract on antioxidant enzymes in the brain of pentylenetetrazole induced kindled rats

The extract at a dose of 400 mg/kg significantly ($p < 0.05$) increased the activity of superoxide dismutase (Figure 5).

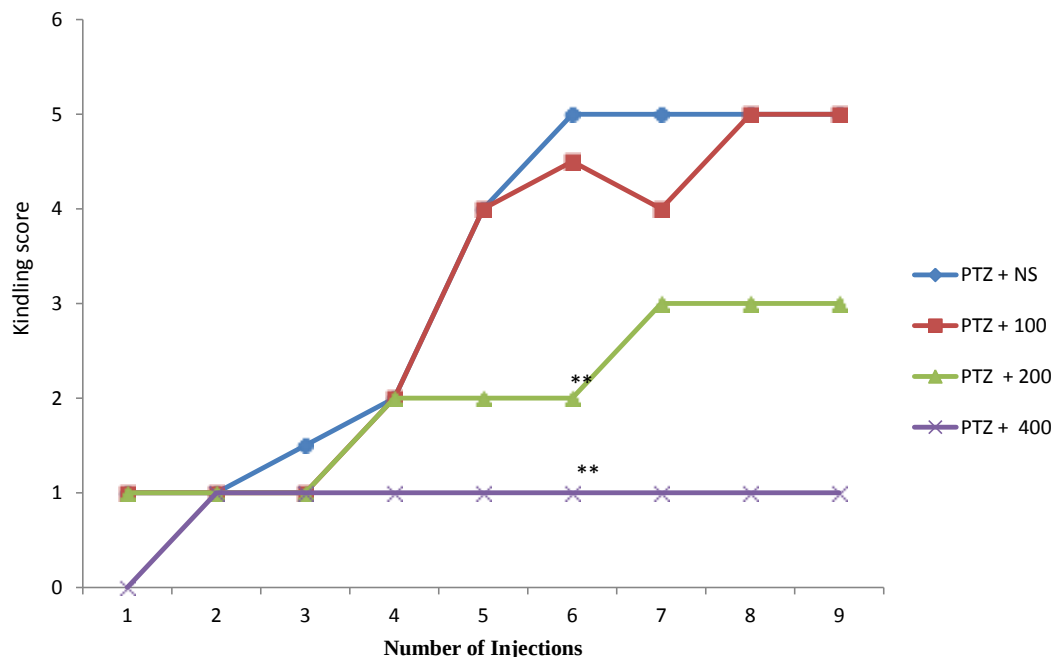


Figure 1. Effect of *T. globiferus* on pentylentetrazole induced kindled rats. Result presented as median scores; n=10; ** p < 0.001 as compared to the control group. Kruskal-Wallis test, followed by Dunn's post hoc.

Table 2. Effect of *Tapinanthus globiferus* on weight gain in pentylentetrazole induced kindled rats.

Treatment (mg/kg)	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	132.0 ± 10.5	177.4 ± 11.2	45.4 ± 0.7
100	126.5 ± 10.6	179.4 ± 9.7	52.9 ± 0.9
200	128.7 ± 8.9	182.1 ± 10.2	53.4 ± 1.3
400	131.4 ± 9.0	180.9 ± 11.4	49.5 ± 2.4

Values are expressed as mean ± SEM, n=10.

In addition, there was a significant ($p < 0.05$) increase in the activity of catalase (Figure 6) in the 100 mg/kg treated group relative to the control. Figures 2, 3 and 4 show the effects of the plant extract on the activities of reduced glutathione, lipid peroxidation and uric acid, respectively, in the brain of control and pentylentetrazole kindled rats. There is an insignificant increase ($p > 0.05$) in the activities of these enzymes.

DISCUSSION

Phytochemical screening of *T. globiferus* revealed the presence of constituents such as alkaloids, saponins, tannins, glycosides, protein, steroids and flavonoids. Alkaloids have been reported to function as amoebicides, expectorant, anesthetic, analgesic and anti-helminthes (Fabricant and Farnsworth, 2001). Mistletoe alkaloids are

sequestered by the parasite from the host tree (Gill and Onyibe, 1990). Flavonoids and tannins detected in the *T. globiferus* growing on *F. glumosa* leaves have been implicated to play significant roles in the metabolism of lipids (Abolaji et al., 2007). Anticonvulsant effect of saponins and flavonoids has been reported by Shibata (2001).

The effect of the aqueous extracts (200 and 400 mg/kg) of *T. globiferus* on pentylentetrazole induced kindling indicated antiepileptogenic activity. The ability of this compound to abolish seizure induced by pentylentetrazole may be due to the extracts ability to antagonize one of the several mechanisms of pentylentetrazole action which includes: interaction with γ -aminobutyric acid (GABA) neurotransmission, central noradrenergic activity and/or blockage of glutamatergic neurotransmission mediated by N-methyl D-aspartate (NMDA) (Khan et al., 2013).

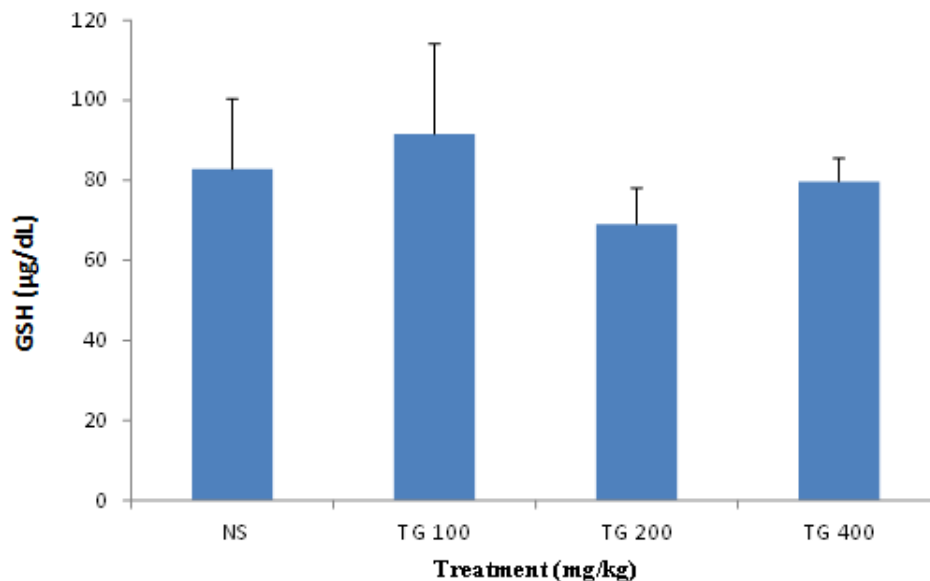


Figure 2. Effect of *T. globiferus* aqueous extract on reduced glutathione (GSH), n=10, TG = *T. globiferus*, NS= normal saline.

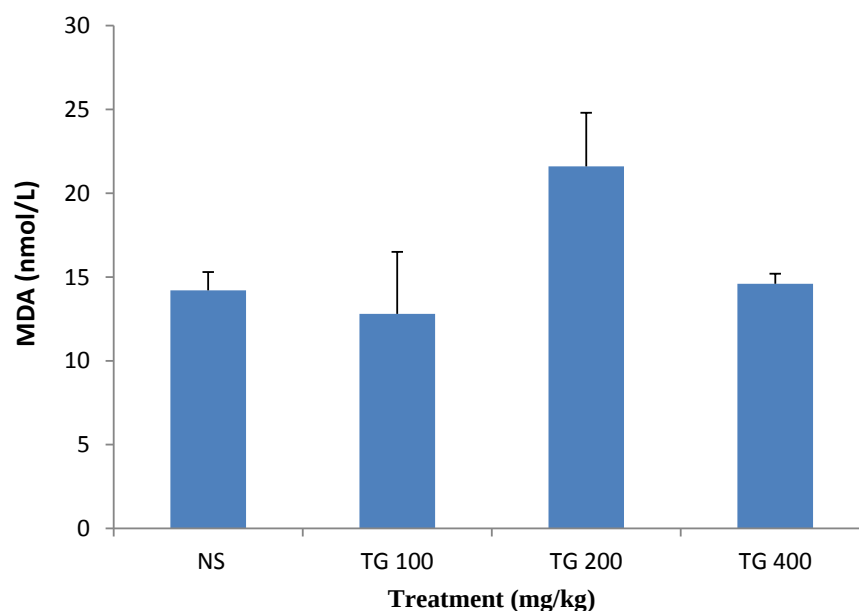


Figure 3. Effect of *T. globiferus* aqueous extract on lipid peroxidation, n=10, TG = *T. globiferus*, NS= normal saline.

Pentylentetrazole induced kindling resulted in mortalities in the control and 100 mg/kg treated group and this may be due to little or no protection received from the extract as compared to the group that received 200 and 400 mg/kg of the extract in which there was no mortality. *T. globiferus* shows antioxidant effect in the superoxide dismutase and catalase assay, this

corroborates the findings of Adekunle et al. (2012) that the leave extract may serve as a good antioxidant due to its high iron chelating capacity. The relationship between antioxidant and anticonvulsant activities has been previously reported (Patrick, 2011; Rahmati et al., 2013). Epilepsy is accompanied by reversible convulsions which induce production of reactive oxygen species (ROS) in

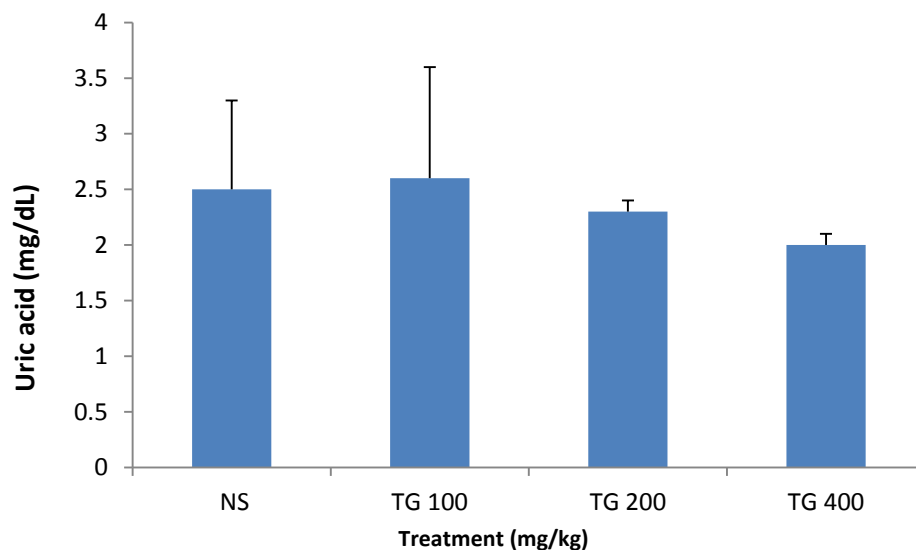


Figure 4. Effect of *T. globiferus* aqueous extract on uric acid. n=10, TG = *Tapinanthus globiferus*, NS= normal saline.

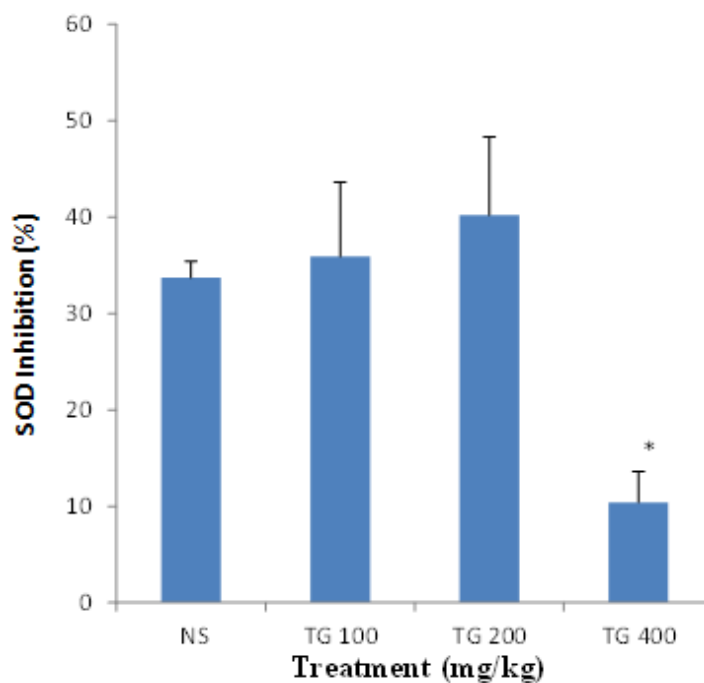


Figure 5. Effect of *T. globiferus* aqueous extract on superoxide dismutase (SOD), n=10, *p < 0.05 as compared to the control group. One way ANOVA followed by Dunnett's post hoc. TG = *T. globiferus*, NS= normal saline.

the brain, and these free radicals are reported to mediate convulsion development (Rahmati et al., 2013). Free radical causes lipid peroxidation at polyunsaturated sites on biological membranes and tissue injury which leads to

cell membrane destruction and cell dysfunction (Gupta and Sherma, 1999). The ability of the plant to abolish epileptogenesis may be due to its anticonvulsant and antioxidant activity. The aqueous extract of *T. globiferus*

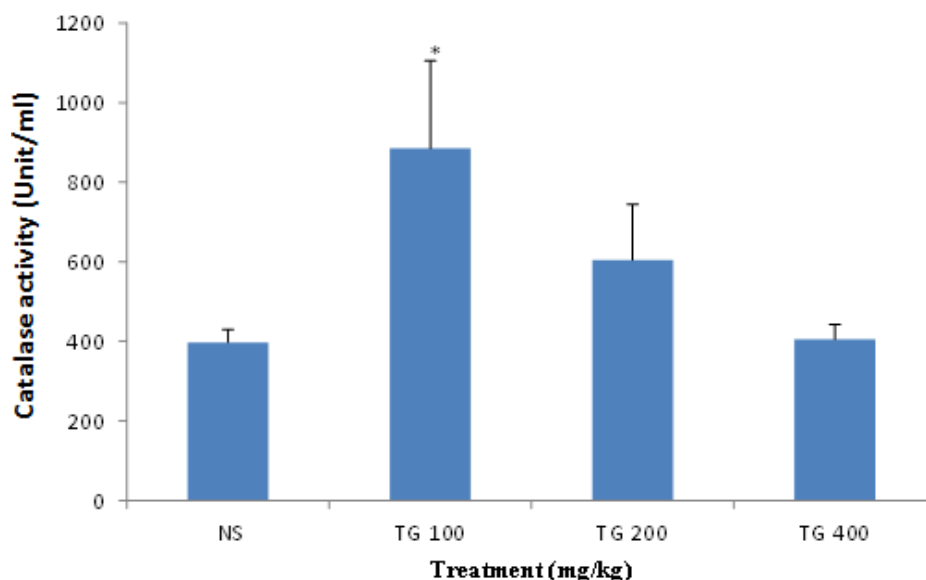


Figure 6. Effect of *T. globiferus* aqueous extract on catalase enzyme. $n=10$, $*p < 0.05$ as compared to the control group. One way ANOVA followed by Dunnett's *post hoc*. TG = *T. globiferus*, NS= normal saline.

did not cause significant changes in the weight of rats after the eighteenth day of treatment ($p > 0.05$). This effect may be due to the absence of noxious compounds in the aqueous extract of *T. globiferus* which may cause anorexia. Recent study on the antikindling effect of plants showed a significant reduction in weight in the extract of untreated group post-kindling (Abubakar, 2017).

Conclusion

The result of this study suggests that the aqueous extract of *T. globiferus* growing on *F. glumosa* may possess bioactive compounds with antioxidant and antikindling activity and this may support its traditional use in the treatment of epilepsy.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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