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EFFECT OF METHANOLIC LEAF EXTRACT OF *SOLANUM TORVUM* ON THE *IN VIVO* ANTIOXIDANT POTENTIALS OF ALBINO *WISTAR* RATS.

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ABSTRACT

Solanum torvum Swartz (family: Solanaceae) which is commonly called Turkey berry is an important medicinal plant. Knowledge of its phytochemicals has been employed in the prophylaxis and treatment of several diseases. Reactive oxygen species have been implicated in the pathogenesis of several diseases; they are however, destroyed by the *in vivo* antioxidants. This study evaluated the effects of the ethanolic leaf extract of *Solanum torvum* on the *in vivo* antioxidant potentials of albino *Wistar* rats; thus, delineating the strategy by which *Solanum torvum* exerts its antioxidant effects. This study was designed to evaluate the dose-dependent and time-dependent effects of the ethanolic leaf extract of *Solanum torvum* on the *in vivo* antioxidant potentials. The ethanolic leaf extract of *Solanum torvum* exhibited high antioxidant potentials which was time dependent ($p < 0.05$) and not dose dependent ($p > 0.05$). This suggests that a regular administration of *Solanum torvum* is of more clinical value than a plethoric administration.

Keywords: Malondialdehyde; Glutathione Peroxidase; Superoxide Dismutase; Catalase; Oxidative Stress.

INTRODUCTION

Solanum torvum is a bushy, erect and spiny plant. This evergreen plant is a broad-leaved shrub that invades a variety of ecosystems. The shrub usually has a single stem at ground level, but it may branch on the lower stem (Plate 1 and 2) (Yousaf *et al*., 2013).

Solanum torvum is considered a useful plant in the treatment of some ailments such as cough, liver and spleen enlargement. *Solanum torvum* has been found to possess essential amino acids, fatty acids, vitamins and minerals needed to support human biochemical processes (Otu *et al.*, 2017). *Solanum torvum* is useful in treatment of insufficient bloodstream launch, hemorrhoid, minus eyes, osteoporosis, flu, high uric acid, toxin accumulation in the body, low sexual desire in men and women, as well as cancer and erectile dysfunction (Source: (Onyeneke *et*

al., 2018). It is also used to treat asthma, diabetes, hypertension, liver diseases, tuberculosis, and anaemia (Yousaf *et al.*, 2013). With respect to phytochemicals, this plant species is a very good source of alkaloids, flavonoids, saponins, tannins, and glycosides (Chah *et al.*, 2000).

Oxidative stress may be indicated by markers associated with three key types of damage to macromolecules: lipid oxidation markers such as malondialdehyde (MDA), conjugated dienes or F2-isoprostanes, protein modification markers indicating protein fragmentation, nitrated proteins, carbonylated proteins or thiol group oxidation, and various markers associated with nucleic acid damage (Bartosz and Sadowska-Bartosz, 2015). These parameters not only have diagnostic value, but they may also be useful indicators of the need for antioxidant supplementation. Therefore, in recent years, great importance has been attached to the consumption of fresh vegetables and fruits, as they are a source of naturally-occurring antioxidants. Research suggests that increased intake of fruits and vegetables may be associated with a reduced incidence of disorders induced by reactive oxygen species (ROS), including cardiovascular disorders, cancer, neurodegenerative disorders, and inflammatory processes. Indeed, the consumption of antioxidants in the diet may have a significant effect on the prophylaxis and progression of various diseases associated with oxidative stress (Liquori *et al*., 2018; Pohl and Kong Thoo Lin, 2020; Alkadi, 2020).

Phenolic compounds extracted from different parts of *S. torvum* exhibited anti-oxidant activity (Loganayaki *et al*., 2010). Peroxide clearing studies indicated that aqueous extracts of *S. torvum* fruits had anti-oxidant activities (Re *et al*., 1999). This plant also exhibited some percentage of antioxidant activity and DNA-repair capability on oxidative DNA damage caused by free radicals (Abas *et al*., 2006). *S. torvum* is now amongst the important medicinal species used as analgesic and anti-inflammatory agents in different traditional medicinal systems (Ndebia *et al.*, 2007). Methanolic extracts have significant growth-inhibiting activity against bacteria commonly associated with pyogenic infections (Chah *et al*., 2000). Methanolic extract of the leaves of S. *torvum* also have very high activity against *Bacillus cereus* (Wiart *et al*., in 2004). Methanolic extracts from fruits and leaves have been described as potentially good sources of antimicrobial agents (David *et al.*, 1998). As part of its haemostatic properties, S. *torvum* has an antiplatelet aggregation effect (Henty, 1973; Nguelefacka *et al*., 2008). Ethanolic extract of S. *torvum* prevents and reverses the development of hyperinsulinemia and control the rise in systolic blood pressure (Mohan *et al.*, 2009).

Plate 1: Prickly berry fruits: *Solanum torvum*
Source: (Onyeneke *et al.*, 2018).

Plate 2: Prickly berry plant: *Solanum torvum*
Source: (Onyeneke *et al.*, 2018).

Malondialdehyde (MDA) is an end-product of the decomposition of arachidonic acid and larger PUFAs (Esterbauer *et al.*, 1991) through enzymatic or non-enzymatic processes. Lipid peroxidation usually involves hydrogen abstraction from a carbon and a concomitant insertion of oxygen resulting in lipid peroxyl radicals and hydroperoxides (Yin *et al.*, 2011). Higher levels of MDA are reported in patients of various categories including

lung cancer patients, complex regional pain syndrome patients, glaucoma patients and other degenerative diseases like aging, diabetes, cardiovascular diseases etc. (Singh *et al.*, 2014).

Glutathione peroxidase catalyzes the reduction of hydro-peroxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. Glutathione peroxidase is the most prominent primary intracellular antioxidant enzyme in mammalian cells (Weydert and Cullen, 2010). In the glutathione system, glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6-PD) do not act directly on reactive oxygen species (ROS), but they enable the glutathione peroxidase to function (Liu *et al.*, 2004).

Catalase catalyzes the conversion of two molecules of H_2O_2 to molecular oxygen and two molecules of water (catalytic activity). In humans, the highest levels of catalase are found in the liver, kidney, and erythrocytes. Catalase has been found to decrease oxidative stress (Boya *et al*., 1999; Favier, 2006).

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2). In fact, over expression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation (Zhao *et al.*, 2001). SOD as well as other *in vivo* antioxidants have been implicated in the etiology of hepatic dysfunction (Barbaro *et al.*, 1999; Czeczot *et al.*, 2006) and pleural effusion (Najeeb *et al.*, 2012).

MATERIALS AND METHODS

Test material:

The leaves of *Solanum torvum* plant were harvested from a garden beside the new pharmacy building of the University of Benin, Benin City, Edo state, Nigeria. A sample of the harvested plant was identified by a botanist and deposited in an herbarium at the Department of Plant Biology and Biotechnology (PBB), University of Benin, Benin City with the voucher number V_H 380. The leaves were transported to the Department of Biochemistry, air dried at room temperature for approximately two (2) weeks and pulverised at the laboratory in the Department of Pharmacognosy of the University of Benin, Benin City, Nigeria.

Experimental animals and housing

Adult female *wistar* rats with body weights between 122.00 ± 10.00g were used for this experiment and they were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and use of Laboratory Animals in Biomedical Research (Olfert *et al.*, 1993). The experimental rats were housed in a well-ventilated room, in steel and plastic cages with wire mesh tops in the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The experimental rats were fed daily with standard rat pellets and clean tap water for two weeks to allow them acclimatize. Twelve (12) of these rats were used for toxicity studies using Lorke's (1983) method. No mortality or clear signs of toxicity were observed. Forty (40) were subdivided into groups of 8 rats each: Aside the control group, the other groups were treated with 50mg/kg, 100mg/kg, 200mg/kg and 300mg/kg body weight respectively of the ethanolic leaf extract of *Solanum torvum.*

Tissue sample collection and preparation

At the end of each week, after an overnight fast two (2) rats from each group were sacrificed (using chloroform as anesthesia). The liver samples were thereafter homogenized (1g/10ml) using mortar and pestle and centrifuged at 4000 rev/min for 10 minutes to obtain the supernatant. The supernatant was separated into sterile plain containers and used for assay of the required biochemical indices.

Biochemical assays

Malondialdehyde (MDA) assay was carried out using the method described by Buege and Aust (1979), Glutathione peroxidase (GPx) activity by Nyman (1959) method, Catalase (CAT) activity by the method of Cohen *et al*. (1970), while superoxide dismutase (SOD) activity was by the method of Misra and Fridovich (1972).

Statistical analysis

SPSS version 23 computer program was used for statistical analysis. A p-value of less than 0.05 was considered statistically significant. The One-Way ANOVA was used to compare between the independent means. Duncan's test was used to measure the significant relationship between the quantitative variables. Data are represented as the mean ± standard error of mean (mean ± SEM).

RESULTS

Dose-dependent comparisons

Table 1. Levels of malondialdehyde (MDA) as well as activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) of various groups after treatments with ethanolic leaf extract of *Solanum torvum*.

Groups	MDA($x10^5$mg/dl)	GPx (U/ml)	CAT (U/l)	SOD($x10^2$U/mg)
Control	20.52 ± 3.88^a	0.06 ± 0.01^a	0.49 ± 0.20^a	1.59 ± 0.91^a
Group 1	16.17 ± 4.78^a	0.08 ± 0.02^a	0.41 ± 0.15^a	6.33 ± 4.00^a
Group 2	20.65 ± 6.25^a	0.06 ± 0.03^a	0.37 ± 0.15^a	4.71 ± 2.41^a
Group 3	13.60 ± 4.26^a	0.07 ± 0.01^a	0.26 ± 0.08^a	4.13 ± 2.85^a
Group 4	17.70 ± 5.31^a	0.04 ± 0.02^a	0.38 ± 0.19^a	6.50 ± 4.58^a

Values are represented as mean ± SEM (n=3). Values with different superscripts are significantly different ($p<0.05$) down the column.

There was no significant mean effect ($p > 0.05$) on the MDA levels at the various doses when ethanolic leaf extracts of *Solanum torvum* was administered (Table 1). Also, the activities of GPx, CAT and SOD did not differ significantly ($p > 0.05$) after the administration of the various doses of ethanolic leaf extract of *Solanum torvum*. The results obtained did indicate that both the levels of MDA and activities of the antioxidant enzymes are not dose dependent as there was no significant mean effect across the weeks.

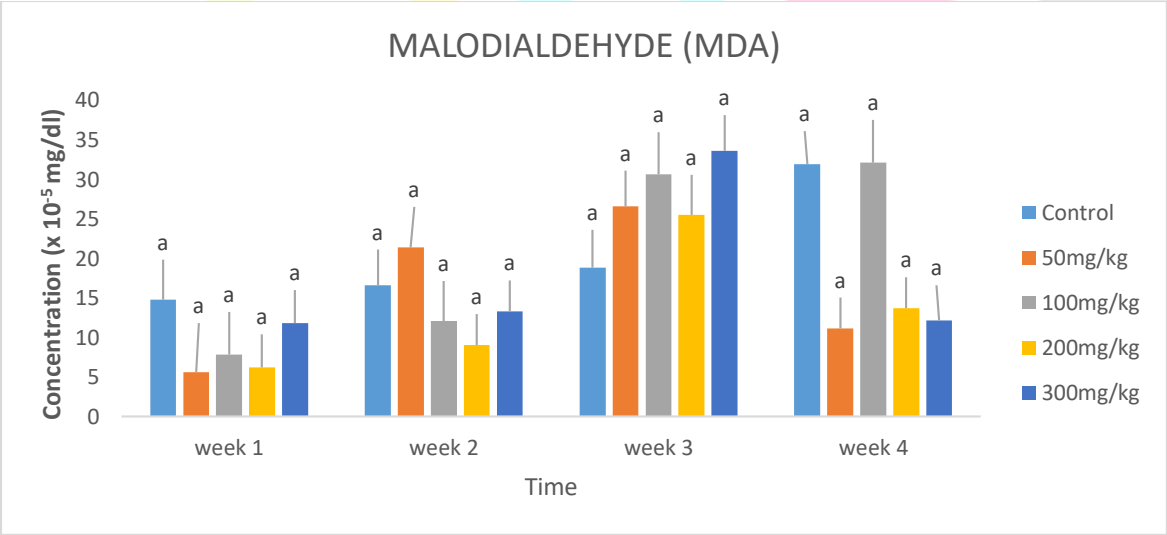

Fig. 1. Changes in MDA levels after treatment with various doses of the ethanolic leaf extract of *Solanum torvum*.

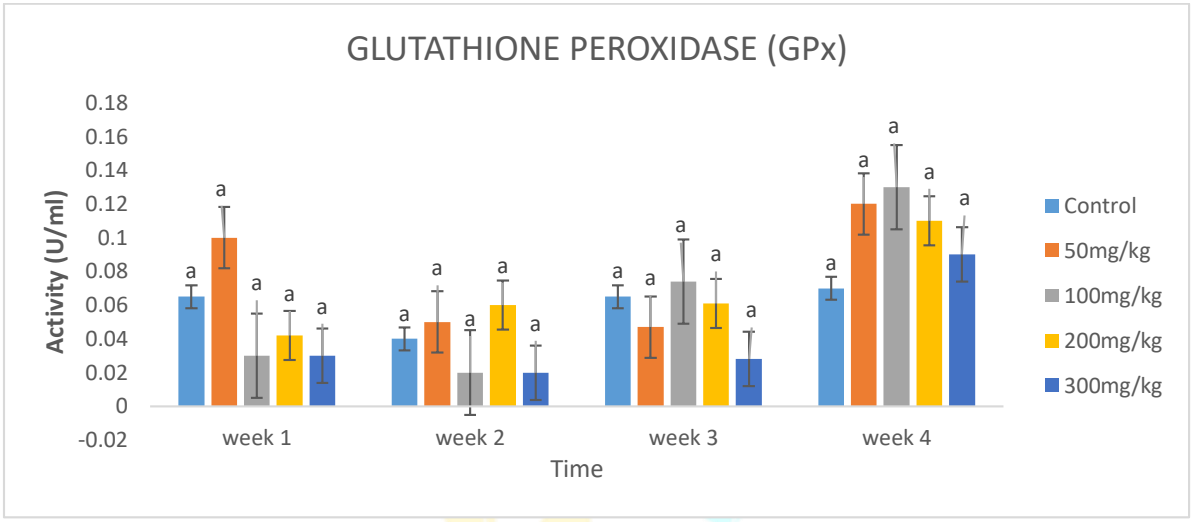

Fig. 2. Changes in glutathione peroxidase activity after treatment with various doses of the ethanolic leaf extract of *Solanum torvum*.

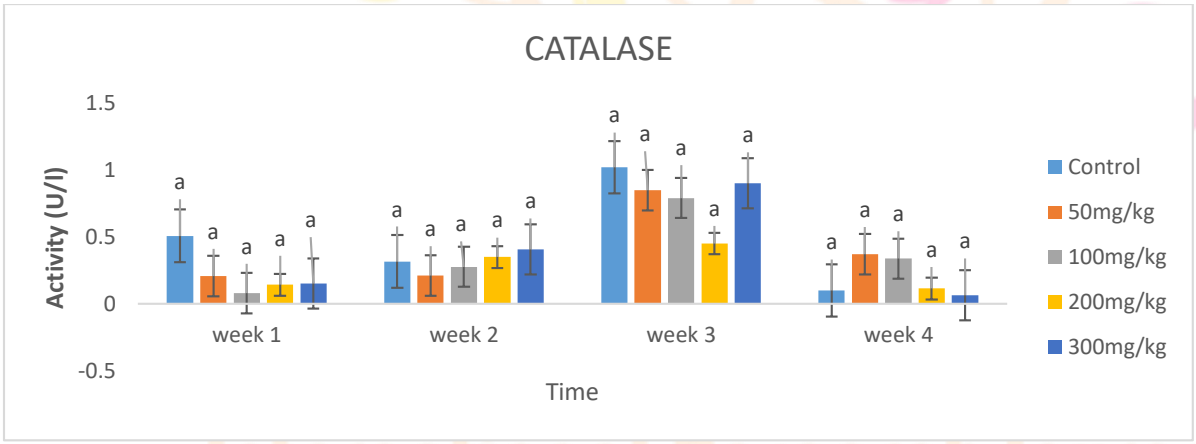

Fig. 3. Changes in catalase activity after treatment with various doses of the ethanolic leaf extract of *Solanum torvum*.

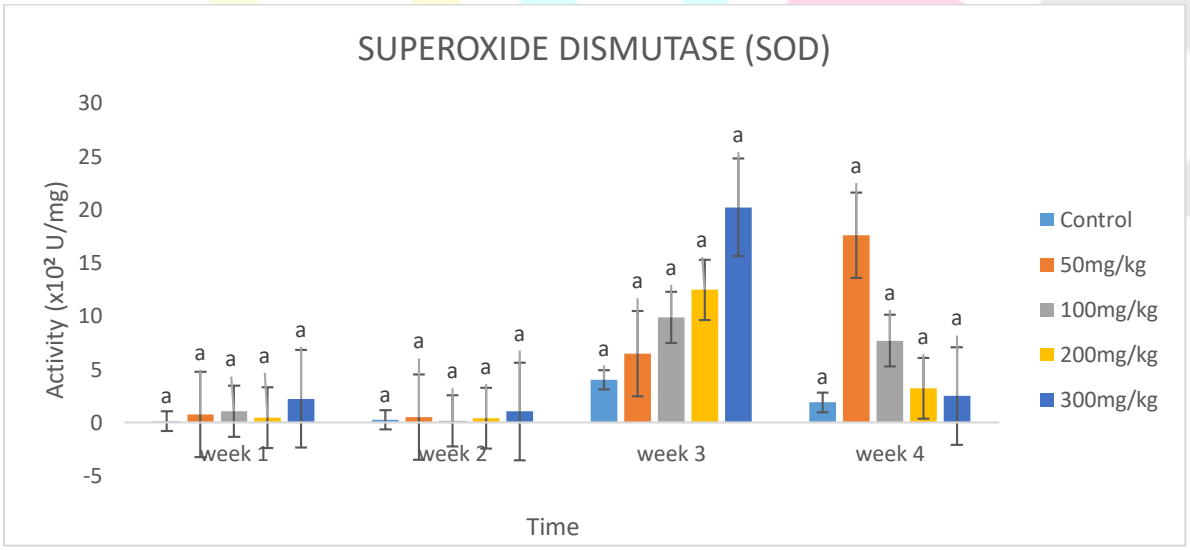

Fig. 4. Changes in SOD activity after treatment with various doses of the ethanolic leaf extract of *Solanum torvum*.

Time-dependent comparisons

Table 2. Levels of malondialdehyde (MDA) and activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) measured weekly for the various treatments of alcoholic leaf extracts of *Solanum torvum*.

Indices	Week 1	Week 2	Week 3	Week 4
MDA ($x10^{-5}$mg/dl)	9.23 ± 1.75^{a}	14.47 ± 2.12^{ab}	27.02 ± 2.5^{c}	20.18 ± 4.84^{bc}
GPx (U/ml)	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.06 ± 0.01^{a}	0.10 ± 0.01^{b}
CAT (U/l)	0.22 ± 0.08^{a}	0.31 ± 0.03^{a}	0.80 ± 0.10^{b}	0.20 ± 0.06^{a}
SOD ($x10^{2}$ U/mg)	0.93 ± 0.36^{a}	0.48 ± 0.16^{a}	10.61 ± 2.80^{b}	6.58 ± 2.94^{ab}

Values are represented as mean ± SEM (n=3). Values with different superscripts are significantly different ($p < 0.05$) across the row.

We observed significant ($p < 0.05$) increases in MDA, GPx, CAT and SOD activities after the administration of ethanolic leaf extract of *Solanum torvum* for four weeks (Table 2).

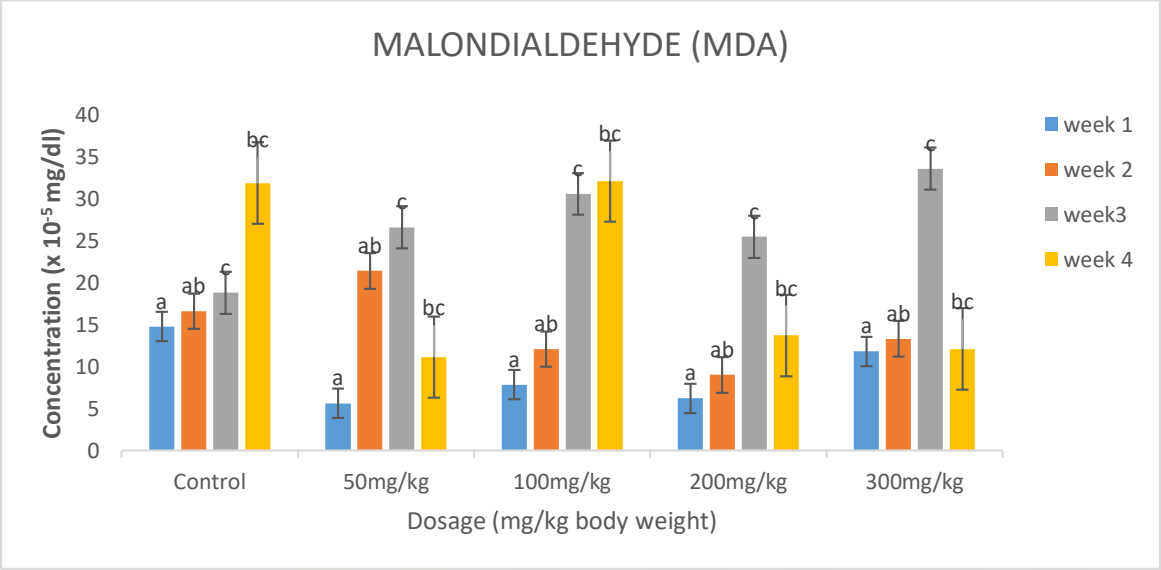

Fig. 5. Weekly changes in MDA levels after treatment with various doses of ethanolic leaf extract of *Solanum torvum*.

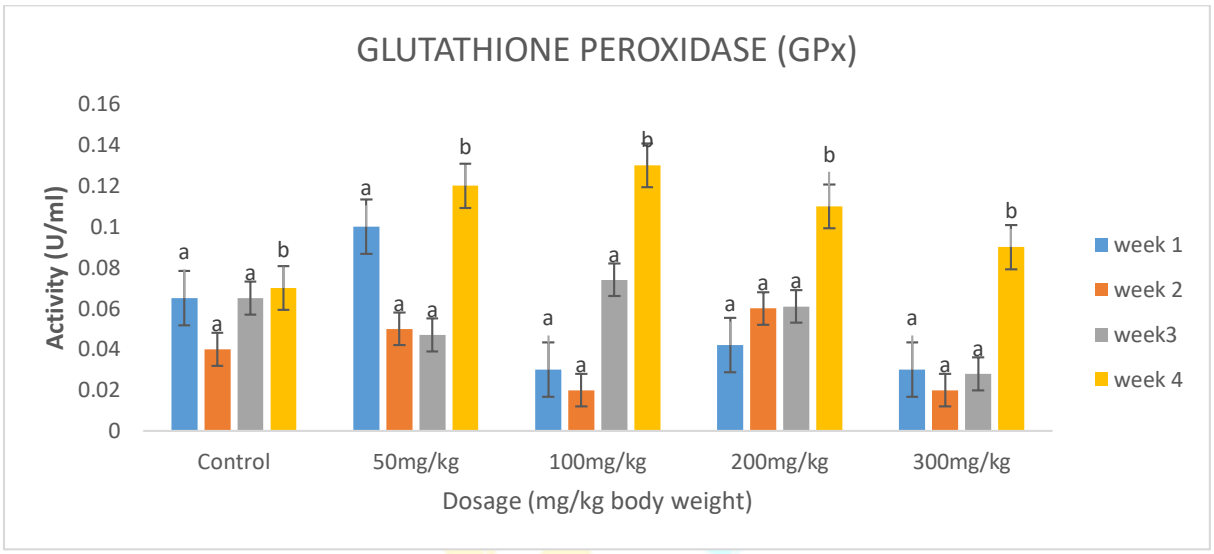

Fig. 6. Weekly changes in glutathione peroxidase activity after treatment with various doses of ethanolic leaf extract of *Solanum torvum*.

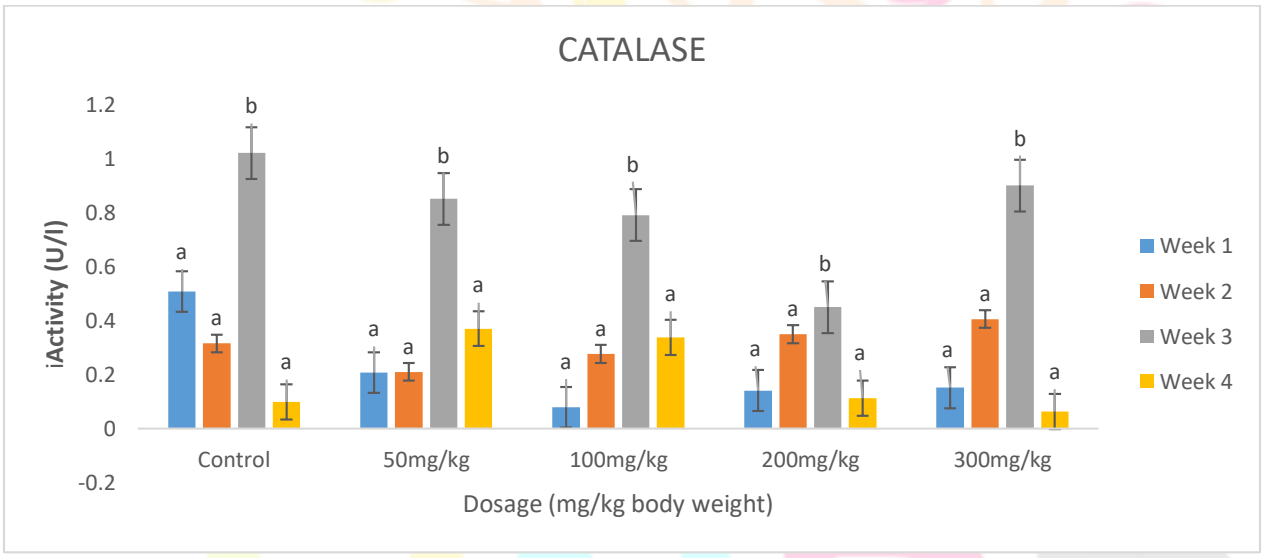

Fig. 7. Weekly changes in catalase activity after treatment with various doses of ethanolic leaf extract of *Solanum torvum*.

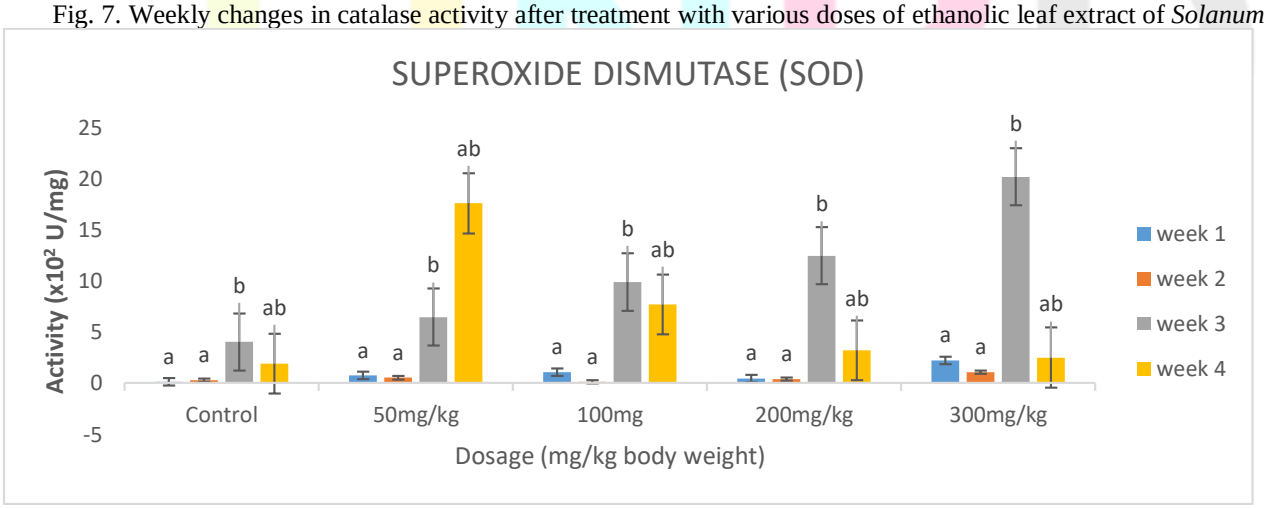

Fig. 8. Weekly changes in SOD activity after treatment with various doses of ethanolic leaf extract of *Solanum torvum*.

DISCUSSION

Knowledge of the phytochemistry of this epoch-making plant species has been greatly utilized in modern medicine and herbal preparations. A number of medicinally useful phytochemicals have been isolated, purified and employed in the prophylaxis and treatment of a myriad of health conditions. Compared to the *in vitro* antioxidant potentials, the *in vivo* antioxidant potentials are still in obscurity.

Elevation or reduction in the activities of the *in vivo* antioxidants are factors to consider in the etiology of many diseases. From the results, increasing doses of *Solanum torvum* generally decreases the concentration of MDA within the cells; this increase is however not significant ($p>0.05$). This mild decrease in MDA concentration probably resulted from a decrease in autoxidative degradation of fats and oils (Sinnhuber and Yu, 1958). Interestingly, when ethanolic leaf extract of *Solanum torvum* was administered over an appreciable period of time, the *in vivo* concentration of MDA increased significantly ($p < 0.05$).

The results for the dose-dependent comparisons show that the activity of glutathione peroxidase (GPx) is not significantly ($p>0.05$) increased *in vivo* by the administration of varying doses of the ethanolic leaf extract of *Solanum torvum*.

The catalase and SOD activities were also not significantly ($p > 0.05$) affected by the various doses. Catalase and SOD, however, showed a significant ($p<0.05$) time-dependent increase in their activities. The increase in catalase activity is of great advantage in the degradation of hydrogen peroxide which is a toxic molecule.

Catalase decreases oxidative stress which is important in development of several liver complications such as chronic hepatitis (Boya *et al*., 1999; Favier, 2006). Thus, *Solanum torvum* can be employed for the prophylaxis of hepatitis. In line with Wood *et al.* (2009), *Solanum torvum* administration may be a good remedy for the greying of hair because of its ability to activate catalase.

CONCLUSION

The results of this study showed that the alcoholic leaf extract of *Solanum torvum* could be safe even at a relatively high amount. Based on the two (time and dose dependent) comparisons, the results revealed that *Solanum torvum* would increase the *in vivo* antioxidant enzyme activities.

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