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Afr. J. Biomed. Res. Vol. 23 (September, 2020); 421- 428

Research Article

Effect of *Taraxacum officinale* Leaf Extract on Liver Antioxidant Status in Streptozotocin-Induced Diabetic Male Wistar Rats

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ABSTRACT

The study aimed at evaluating liver antioxidant status in streptozotocin (STZ)-induced diabetic male Wistar rats following treatment with aqueous *Taraxacum officinale* leaf extract. The rats were divided into six groups of 6 rats each: Normal control, Diabetic control, Non-diabetic rats + 100 mg/kgbw extract, Non-diabetic rats + 200 mg/kgbw extract, Diabetic rats + 100 mg/kgbw extract and Diabetic rats + 200 mg/kg-bw extract. After the treatment period, the animals were sacrificed and the liver was collected, homogenized for biochemical analyses. Biochemical assays such as the levels of nitrite, lipid peroxidation product (LPO), reduced glutathione (GSH) and activities of antioxidant enzymes (superoxide dismutase, SOD and glutathione peroxidase, GPx) as well as Ferric Reducing Antioxidant Power (FRAP) were determined using established techniques. Lipid peroxidation and nitrite level decreased significantly ($p < 0.05$) in diabetic rats treated with 100 mg/kgbw and 200 mg/kgbw *Taraxacum officinale* extracts as compared to the untreated diabetic control. There was no significant difference in FRAP value in diabetic groups treated with 100mg/kgbw and 200mg/kgbw when compared with the diabetic control. Reduced GSH concentration as well as SOD activity increased significantly in diabetic groups treated with 100 mg/kgbw and 200 mg/kgbw *Taraxacum officinale* extracts as compared to the untreated diabetic control while GPx activity decreased significantly in the treated diabetic rats. The results indicate that *Taraxacum officinale* leaf extract could enhance liver anitioxidant status, hence, suggest its usefulness in ameliorating diabetic-induced liver injury.

Keywords: *Taraxacum officinale, antioxidant status, liver, streptozotocin, diabetic rats*

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Received: March, 2020; Accepted: July, 2020

Abstracted by:

Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Diabetes mellitus has been classified as a metabolic disease caused by excess blood sugar known as hyperglycemia. It is a disease condition resulting from unavailability or inability of insulin to mediate glucose uptake in the cells as a result of either impairment in insulin secretion, insulin action, or both (ADA, 2014). Diabetes occurs as type I, where the pancreas produces little insulin or no insulin at all leading to accumulation of glucose in the blood and type II, a condition whereby cells of the organs are unable to recognize the presence of insulin in the blood (Schaalan *et al.,* 2009). It has been reported that the generation of reactive oxygen species (ROS) increases in both type I and type II diabetes and that early stage of diabetes is closely connected with oxidative stress (Johansen *et al.,* 2005). Oxidative stress in diabetes mellitus might occur due to auto-oxidation of glucose, imbalance in redox reactions, reduction in GSH and vitamin E levels as well as decreased activities of antioxidant enzymes (Haskins *et al.,* 2003). Gluco-oxidation due to formation and accumulation of advanced glycation end-products (AGE) plays a major role in diabetic complications and in the mechanisms connecting uncontrolled hyperglycaemia with tissue damage in diabetic hepatopathy (Singh *et al.,* 2014). Other mechanisms of hyperglycemia-induced diabetic complications are through influx of glucose via the polyol pathway (Yan, 2018), protein kinase C (PKC) activation (Das Evcimen and King, 2007), increased-flux through the hexosamine pathway (Wang*et al.,* 2014) and NADPH-oxidase pathway (Giacco and Brownlee, 2010).

Antioxidants are molecules that are capable of donating one or more electrons to unstable free radical(s) and neutralize it,

thereby reducing its damaging effect to cells or tissues (Kumar *et al.,* 2009). Antioxidants interact with free radicals to stop the chain reactions that lead to damages in biological macromolecules such as DNA, lipids and proteins. Antioxidants are characterized into two; endogenous and exogenous antioxidants. Antioxidants produced within the organism are referred to as the endogenous and are referred to as enzymatic antioxidants such as glutathione peroxidase, catalase, glutathione reductase and superoxide dismutase. Non-enzymatic antioxidants are reduced glutathione (GSH), melatonin, uric acid, coenzyme Q (Birben et al., 2012). Exogenous antioxidants are said to be supplied from the diet, e.g. β-carotenoids, vitamin E, flavonoids, vitamin C, minerals like selenium, iodine, manganese etc (Biehlesr and Bohn, 2010; Carlsen, 2010). Different foods and supplements rich in naturally-occurring antioxidants, such as vitamins and phytochemicals such as polyphenols, carotene and flavonoids are known antioxidants that can protect the body against oxidative damages and enhance antioxidant status of an individual (André, 2010; Omar *et al.,* 2011).

Herbal medicine has formed the basis of scientific research to develop indigenous, inexpensive botanicals with readily available biologically active medicinal compounds with little or no side effect (Boadu and Asase, 2017). *Taraxacum officinale*, known as dandelion, is a medicinal plant that is known to grow wildly in nature in Asia, America, Europe and Africa (Molina-Montenegro, 2013). In the South-Western part of Nigeria, it is called 'efo-yanrin' and is mostly collected from the wild and only cultivated to a limited extent for home use and for local markets. In the South-South region of Nigeria, the Ijaws (Bayelsa State) of the Niger Delta called it 'Edule Imimi' and the leaves are traditionally used as remedy against several ailments such as liver dysfunction, anti-inflammatory conditions and diabetes mellitus (Sofowora, 1982; Idu and Ndukwu, 2006). *Taraxacum officinale* also functions as immunomodulator, insulin stimulant, anti-carcinogenic digestive stimulant, anti-carcinogenic, prebiotic, anti-inflammation, antineoplastic, demulcent, and hypoglycaemic (Koh *et al.,* 2010; Ahmed *et al.,* 2013). This study investigated the effect of *Taraxacum officinale* aqueous leaf extract on liver antioxidant status in streptozotocin-induced diabetic male Wistar rats.

MATERIALS AND METHODS

Preparation of plant material*:* Fresh leaves of *Taraxacum officinale* were collected within the months of March-April, 2019 from a local farm in Ede, Osun State, Nigeria. They were identified and authenticated by a Botanist in Biology programme of the Department of Basic Science, Adeleke University. The fresh leaves of *Taraxacum officinale* were chopped and washed with distilled water to remove extraneous materials. They were then dried for 7 days under room temperature in a well-ventilated laboratory and occasionally turned to avoid fungal growth while drying. The leaves were blended into powder. Then, 250g of the powdered leaves sample was soaked in 2500ml of distilled water. The content was thoroughly stirred and allowed to stand for 24 hours. The mixture was then filtered using a clean cotton material and refiltered with 0.1mm pores Whatsman filter paper. The filtrate was freeze-dried and the extract collected was kept at 4^{o}C until further use.

Phytochemical Screening

Cardiac Glycosides: Little quantity of dry powder sample of *Taraxacum officinale* leaf was treated with10ml of ferric chloride reagent, containing mixture of ferric chloride solution in 1:2v/v glacial acetic acid, 1ml concentrated H_2SO_4 was added to the solution. A blue-green color appearance indicated the presence of cardiac glycoside (Ajaiyeoba, 2002).

Tannin: The aqueous extract of crude dry powder of *Taraxacum officinale* leaf was treated with ethanolic ferric chloride solution. A greenish-blue colour indicated the presence of tannin (Segelman *et al*., 1969).

Steroid: The presence of steroid was confirmed in the crude dry powder of *Taraxacum officinale* leaf after treating the chloroform solution of powdered sample with acetic anhydride and few drops of concentrated H_2SO_4. A dark blue green color indicated the presence of steroids (Vaghasiya*et al.,* 2011).

Alkaloid: A little quantity of the dried sample powder was mixed with methanol. The mixture was filtered and the filterate was placed in a boiling water bath to evaporate. The residue mixed in 2N HCL was filtered and divided into three parts. The first part was treated with 1ml of Mayer's reagent, second part was with 1ml of Dragon's reagent and the third part with 1ml of Wagner's reagent. The presence of creamy, orange or brown precipitates respectively showed the presence of alkaloid (Saleh-Surmghi *et al.,* 1992).

Saponin: Frotting test was used to determine the presence of saponin. The crude dry powder sample of *Taraxacum officinale* was dissolved in enough amount of distilled water and shaken vigorously. It was allowed to stand for 10 min to observe the frost formation to indicate the presence of saponin.

Estimation of Total Phenolic Content: The total phenolic content in the extract was determined using spectrophotometer as described by Singleton *et al.,* (1999). The standard tannic acid was prepared by dissolving 10µg of tannic acid in 1ml of distilled water to obtain the stock solution. The stock standard was further serially diluted by pipetting 0µl, 200µl, 400µl, 600µl, 800µl and 1000µl and made up to 1ml with distilled water. Folin-Ciocalteu's phenol reagent prepared in 1:10 (v/v) ratio with distilled water was added by pipetting 1.5ml of the reagent to 200µl from the serial diluted standard and the samples. 1.5ml of 7.5% Sodium carbonate was then added across the test tubes and allowed to stand for 1hr 30min at room temperature. The blank was prepared according to the steps above with distilled water in place of the sample. The absorbance was read at 725nm against the blank and the standard calibration prepared by plotting the graph of absorbance against the concentration of tannic acid and the concentration of phenolic compound was obtained from the equation and expressed as µg/mlTAE; Where TAE = Tannic acid equivalent.

Estimation of Total Flavonoid Content: The total flavonoid content in the extract was determined using the method of Shen *et al.,* (2009) with rutin as the standard. The standard solution was prepared by dissolving 1mg of rutin in 1ml of distilled water to form the stock solution. This was serially

diluted into 0µl, 200µl, 400µl, 600µl, 800µl and 1000µl and made up to 1ml with distilled water. 200µl of the serially diluted standard and samples were pipette respectively into clean test tubes and added with 300µl freshly prepared 5% $NaNO_2$, followed by 300µl of 10% $AlCl_3$ and then 2ml of 4% NaOH. The reaction mixtures were then incubated at room temperature for 10min and the absorbance read in spectrophotometerat 500nm against the blank. The standard calibration graph was plotted as absorbance against the rutin standard concentration, while the concentration of flavonoids in the extract was obtained from the equation and expressed as µg/mlRE,

Where RE = Rutin equivalent.

Experimental Animal: Thirty-six (36) adult male Wistar rats within the weight of180-250g were purchased from Animal House Unit of the Department of Anatomy, Adeleke University, Ede, Osun State, Nigeria. The animal study was conducted in the Animal House, Department of Biochemistry, Adeleke University. Housing was done in wooden cages and placed on standard feed with free access to water. . The animals were acclimatized under normal conditions of 12h light/dark cycle for two weeks before commencement of experiment.

Induction of diabetes mellitus: Induction of diabetes mellitus was performed according to the method described by Hegazy *et al.,* (2013). Streptozotocin concentration (50mg/kg) was freshly prepared in 0.1M citrate buffer (pH 4.5) and injected intramuscularly into the experimental rats in ratio of 1ml to 200g body weight. The rats were allowed to stabilize for 3 days, before testing for diabetes. Diabetes was confirmed using glucose test strip stained with blood from tail tip puncture and analyzed in Acu-Chek glucometer. The rats with blood glucose level above 250mg/dl were considered as diabetic.

Experimental design

Thirty-six (36) male Wistar rats were randomly distributed into six groups of 6 rats each.

Group 1: Normal control rats.
Group 2: Diabetic control rats.
Group 3: Non-diabetic rats treated with100mg/kg Taraxacum officinale leaf extract.
Group 4: Non-diabetic rats treated with 200mg/kg Taraxacum officinale leaf extract.
Group 5: Diabetic rats treated with 100mg/kg Taraxacum officinale leaf extract.
Group 6: Diabetic rats treated with 200mg/kg Taraxacum officinale leaf extract.

The rats were orally treated for two weeks. At the end of experimental period, the rats were fasted overnight, anesthetized with diethylether and sacrificed. The liver was collected in universal bottles and stored in a refrigerator at a temperature of -20^0C for further use.

Preparation of samples: The samples were prepared according to the method described by Amujoyegbe *et al.*, (2012). The liver samples were homogenized using 10ml phosphate buffer per gramme of the sample. The homogenates were centrifuged at a 2500rpm for 20mins. The supernatants were collected and kept at – 4ºC for biochemical assays.

Ferric Reducing Antioxidant Power (FRAP) assay: FRAP assay was carried out using the method described by Benzie and Strain (1996). 2 litres of 0.1M acetate buffer; pH 3.4 was prepared using 3.24g of sodium acetate, 32ml of glacial acetic acid solution and 500ml of distilled water. The pH was adjusted to 3.4 and the volume made up to 2 litres using distilled water. 160ml of (tripyridyltriazine) TPTZ solution, was prepared using 0.496g of TPTZ in 160ml of 40mM HCL. Ferric chloride solution was prepared using 0.512g of ferric chloride in 160ml of distilled water. The reagent solutions were mixed together to make up the FRAP reagent. 2.0mM Standard Ascorbic acid solution was prepared using 0.017g of ascorbic acid in 50ml of distilled water as stock solution. Six-folds serial dilution of 0 µl, 50µl, 100µl, 200µl, 500µl and 1000µl were prepared and made up to 1ml using distilled water. 60µl from each dilution was pipetted into different labeled test tubes. Then 1800µl of the FRAP reagent was added and shaken to mix properly. The solution was incubated at 37ºC for 30mins and the absorbance was read at 539nm. FRAP in the samples were determined using the standard curve and were expressed in micromolar (µM) concentration.

Nitrite assay: The method of Giustarini *et al* (2004) was used to determine nitrite concentration. The Griess reagent used in the assay was prepared by dissolving 10ml of phosphoric acid in a distilled water making it up to 200ml. 0.2g of N-1-Naphthylethylenediamine dihydrochloride was dissolved in the 200ml solution of phosphoric acid and distill water. Then 2g of sulphanylamide was also dissolved in the solution to form the Griess reagent and stored in a dark cupboard prior to use. Sodium nitrite stock solution of 0.1mg of nitrite reagent in 10ml of distilled water was freshly prepared. It was diluted in 10 folds and further into 5 serial dilution of 0 µl, 250 µl, 500 µl, 750 µl and 1000 µl, then made up to 1ml. Clean test tubes were arranged in duplicate and labeled accordingly, then measured 200µl of the samples and different standard dilution into the corresponding test tubes. Also, 1800µl of the Griess reagent was added in all the test tubes and incubated at room temperature for 10min. The absorbance at 540nm was read, while the concentration calculated using standard curve expressed in mg/ml.

Lipid peroxidation assay: Lipid peroxidation assay was carried out according to the modified method of Iqbal *et al*., (1996). The reaction mixture contains 0.1M, pH 7.4 phosphate buffer, 100mM ferric chloride, 10% trichloroacetic acid and 0.67% thiobarbituric acid (TBARS). The assay was carried outin duplicate using 200µl of homogenates mixed with 0.58ml phosphate buffer, 200µlascorbic acid and 20µl ferric chloride against the blank which contain distill water in place of homogenate. The mixture was placed in a mechanical water bath for 1hr at the temperature of 37ºC. 1ml of TCA solution was added to stop the reaction process then followed by addition of 1ml of TBARS before placing in a boiling water bath for 20 min. The solution was further centrifuged at 3000rpm for 10min after cooling in a crushed ice bath for about 3 to 4 min. The supernatant was obtained and the

absorbance measured at 535nm using spectrophotometer against the reagent blank solution. The result of the assay was expressed in nMTBARS/min/mg tissue, using molar extinction coefficient $1.56x10^5M^{-1}cm^{-1}$. The value of MDA obtained can be calculated using the expression below:

$$MDA(\mu M) = A_{sample} / L \text{ X } £ \text{ X } DF$$

A_{sample} = Absorbance of the sample
L = light path = 1cm
£= molar absorptivity = $1.56 \times 10^5 M^{-1} cm^{-1}$
DF = dilution factor = 1

Reduced glutathione assay: The level of reduced glutathione in the liver was estimated using the method of Moron *et al.* (1979) with slight modification. DTNB reagent solution was freshly prepared by dissolving 0.6mM concentration of DTNB in 0.2M, pH 8.0 phosphate buffer, while 5% of trichloroacetic acid (TCA) was prepared in distilled water. The standard stock concentration of reduced glutathione (GSH) was prepared by dissolving 0.1mg of GSH in 1ml of distilled water. The assay was carried out in duplicate using 4ml of TCA solution in test tubes containing 1ml of the homogenates. The solutions were centrifuged at 4000rpm for 10min using a table top centrifuge. The supernatant (0.2ml) and the standards were pipetted in duplicate and added with 0.9ml phosphate buffer prepared, then, 2ml of DTNB solution was also added to all the test tubes. The absorbance was measured against the blank which contains distilled water instead of samples and the concentrations of GSH in the samples were extrapolated from the standard curve, expressed in µg/GSH/g sample.

Glutathione peroxidase (GPx) assay: The analysis of GPx activity was carried out using a modified method of Rotruck *et al*. (1973). Different chemicals for the analysis were prepared in solution of 0.2M, pH 8.0 phosphate buffer, 10mM Sodium azide, 4mM reduced Glutathione (GSH), 2.5mM H_2O_2, 10% Tricarboxylic acid (TCA), 0.3M Disodium hydrogen phosphate, 0.6mM DTNB in phosphate buffer and distilled water. The analysis was carried out in duplicate by mixing 0.1ml of the liver homogenate with 0.5ml phosphate buffer, 0.1ml Sodium azide, 0.2ml GSH, 0.1ml H_2O_2 and 1ml distilled water. The mixture was incubated at 37oC for 3min, then, added with 0.5ml TCA and centrifuged at 3000rpm for 10min. 0.1ml of the supernatant collected was mixed with 0.9ml Disodium hydrogen phosphate and 1ml of DTNB, then, the absorbance was taken at 412nm against the blank solution containing every other components except the homogenate.
The activity of the GPx is calculated using the expression:

GPx activity (µmol/mg sample) = $O.D_{412}$ X TV X df / 6.22 X 10^3 X EV

Where;
$O.D_{412}$ = Absorbance at 412nm, TV = Total volume, df = dilution factor, EV= Enzyme volume, 6.22 $X10^3$ = Extinction coefficient.

Superoxide dismutase (SOD) assay: The modified method of McCord and Fridovich (1969) was used to assay for the activity of superoxide dismutase. The reaction mixture contained 75mM, pH 8.0 Tris-buffer, 30mM EDTA and 2mM pyrogallol. 100µl of the liver homogenate was pipette into clean test tubes in duplicate, 2.5ml Tris-buffer was added followed by addition of 100µl of EDTA. The enzyme reaction was initiated by addition of 300µl of pyrogallol. The increased in absorbance is read at an interval of 30secs for 150secs against the blank containing 100µl of distilled water in place of homogenate. The maximum absorbance is read at wavelength of 420nm.

The differences in absorbance per minute were calculated using the expression of;

Change in Absorbance per minute ($\Delta Abs./min$) = $B_6 – B_2/2$.
Where, B_6 = Absorbance at 150secs and B_2 = Absorbance at 30secs.

The percentage activity of the enzyme is obtained as:

$$\% \text{ Activity} = 100 - 100 \text{ X } \frac{\text{Increased in absorbance of substrate)}}{\text{Increased in absorbance of blank}}$$

The activity of SOD was given in percentage (%)

Statistical Analysis
All the experiments were done in duplicates. The data were expressed as means ± SEM; n=6 per treatment. Statistical analysis was done using student t-test with Duncan post-hoc test (SPSS version 17). Significant differences were considered at $p < 0.05$.

RESULTS

Phytochemical screening
The presence of tannins, cardiac glycosides, alkaloids and steroids in *Taraxacum officinale* leaf extract were observed as shown in Table 1.

Table 1:
Phytochemical composition of *Taraxacum officinale* leaf extract.

Phytochemical constituents	***Taraxacum officinale* extract**
Cardiac glycosides	+
Tannins	+
Alkaloids	+
Saponins	-
Steroids	+

The positive sign (+) showed present and negative sign (-) showed absent

Total phenolic and flavonoid contents: Phenolic content in 100 mg/ml and 200 mg/ml extracts were 2.175 ± 0.032 µg/ml TAE and 3.097 ± 0.391 µg/ml TAE respectively while total flavonoid content in 100 mg/ml and 200 mg/ml extracts were 0.173 ± 0.001 µg/ml RE and 0.197 ± 0.002 µg/ml RE respectively (Table 2).

Table 2:
Total phenolic and flavonoid contents of 100mg/ml and 200mg/ml leaf extract of *Taraxacum officinale*

Sample	Concentrations	Total phenolic content (µg/ml TAE)	Total flavonoid content (µg/ml RE)
Taraxacum officinale leaf extract	100mg/ml	2.175 ± 0.032	0.173 ± 0.001
	200mg/ml	3.097 ± 0.391	0.197 ± 0.002

Ferric Reducing Antioxidant Power (FRAP) value in the liver: Administration of 100mg/kgbw and 200mg/kgbw plant extract in the diabetic rats resulted in insignificant decrease in FRAP value of the liver compared to the diabetic control while non-diabetic rats showed decrease FRAP value at 100mg/kgbw extract compared to the non-diabetic control (Figure 1).

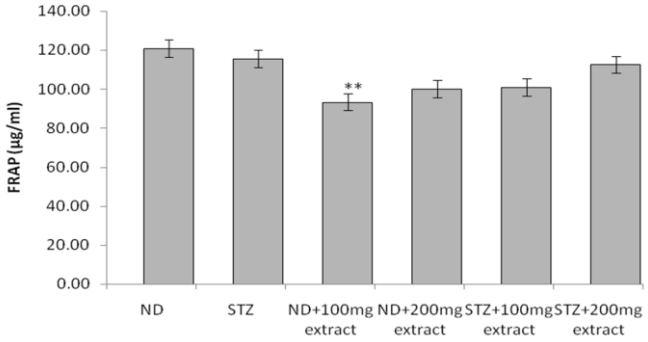

Figure 1:
Effect of *Taraxacum officinale* leaf extract on Ferric Reducing *Antioxidant Power (FRAP) in the liver of diabetic rats (p < 0.05).*

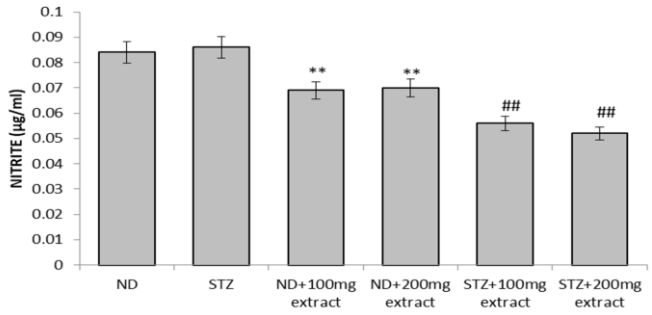

Figure 2:
Effect of *Taraxacum officinale* leaf extract on nitrite concentration in the liver of diabetic rats ($p < 0.05$).
*** indicates significant difference in comparison to non-diabetic (ND) control*
indicates significant difference in comparison to diabetic (STZ) control

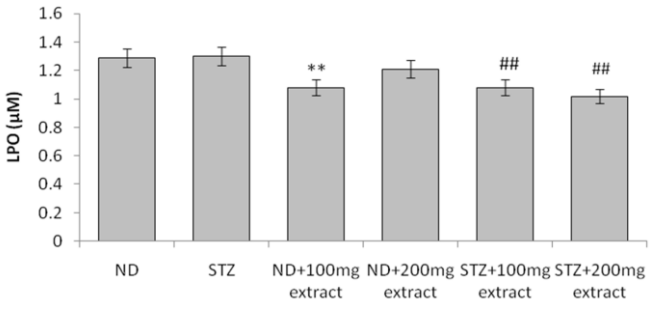

Figure 3:
Effect of *Taraxacum officinale* leaf extract on lipid peroxidation in the liver of diabetic rats ($p < 0.05$).
*** indicates significant difference in comparison to non-diabetic (ND) control*
indicates significant difference in comparison to diabetic (STZ) control

Level of nitrite in the liver: Nitrite level in the liver was significantly decreased in both diabetic and non-diabetic rats compared to their respective controls upon administration of the plant extracts as shown (Figure 2).

Level of lipid peroxidation in the liver: The diabetic control group showed no significant difference in the level of lipid peroxidation product compared to the non-diabetic control group as shown in Figure 3. In the non-diabetic rats, administration of 100mg/kgbw plant extract caused lipid peroxidation product to decrease significantly ($p < 0.05$) as compared to the non-diabetic control. In the diabetic rats, administration of 100 mg/kgbw and 200 mg/kgbw, lipid peroxidation product decreased significantly ($p < 0.05$) as compared to the diabetic control.

Concentration of reduced glutathione (GSH) in the liver: The GSH concentration in the liver is shown in Figure 4. In non-diabetic rats, administration of both 100 mg/kgbw and 200 mg/kgbw plant extract significantly decreased GSH concetration compared to the non-diabetic control. In the diabetic rats, administration of both 100 mg/kgbw and 200mg/kgbw extracts caused GSH concentration to increase significantly ($p < 0.05$) as compared to the diabetic control.

Activity of glutathione peroxidase (GPx) in the liver: The activity of GPx in the diabetic control rats was insignificantly increased as compared to the non-diabetic control. In the non-diabetic rats, administration of both 100 mg/kgbw and 200 mg/kgbw extracts caused GPx activity to increase significantly ($p < 0.05$) as compared to the non-diabetic control, while GPx activity decreased significantly in diabetic rats administered with both 100 mg/kgbw and 200 mg/kgbw of the plant extract as compared to the diabetic control (Figure 5).

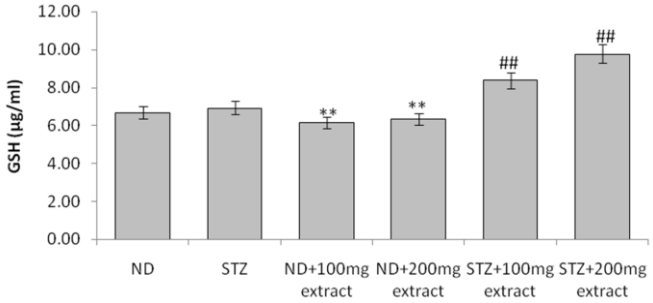

Figure 4:
Effect of *Taraxacum officinale* leaf extract on reduced glutathione concentration in the liver of diabetic rats ($p < 0.05$).
*** indicates significant difference in comparison to non-diabetic (ND) control*
indicates significant difference in comparison to diabetic (STZ) control

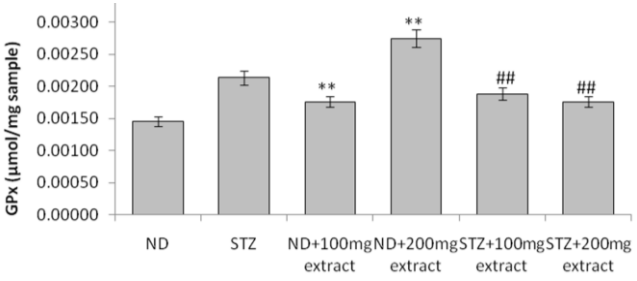

Figure 5:
Effect of *Taraxacum officinale* leaf extract on the level of glutathione peroxidase in the liver of diabetic rats ($p < 0.05$).
*** indicates significant difference in comparison to non-diabetic (ND) control*
indicates significant difference in comparison to diabetic (STZ) control

Activity of Superoxide dismutase (SOD) in the liver: The percentage SOD activity in diabetic control rats showed no significant increase as compared to the non-diabetic control. Administration of 200 mg/kgbw extract caused SOD to increase significantly ($p < 0.05$) in non-diabetic rats as compared to the non-diabetic control. In the diabetic rats, 100 mg/kgbw and 200 mg/kgbw extract increased SOD activity significantly ($p < 0.05$) as compared to the diabetic control (Figure 6).

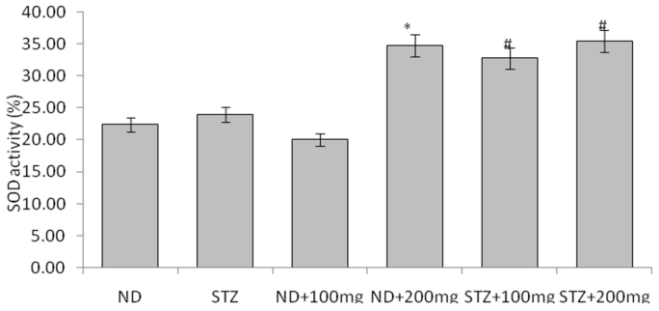

Figure 6:
Effect of *Taraxacum officinale* leaf extract on percentage SOD activity in the liver of diabetic rats ($p < 0.05$).
*** indicates significant difference in comparison to non-diabetic (ND) control*
indicates significant difference in comparison to diabetic (STZ) control

DISCUSSION

Taraxacum officinale leaf extract possesses phenols, flavonoids, tannins, cardiac glycosides, alkaloids and steroids that have the ability to reduce oxidative stress (Rajurkar and Hande, 2011). Ferric-Reducing Antioxidant Power (FRAP) evaluates the antioxidant potential of non-enzymatic defenses in biological fluid, where it provides a measure of antioxidant ability (Benzie and Strain, 1996). The mechanism of FRAP is based on electron transfer which is the ability to reduce Fe3+ to Fe2+ at suitable pH. This occurs in the presence of 2,4,6-trypyridyl-s-triazine (TPTZ) with formation of Fe2+ complex, followed by a colour change. The effect of *Taraxacum officinale* leaf extract on the liver of diabetic rats showed insignificant difference in FRAP value as compared to non-diabetic control group. This suggests that, diabetic rats respond to produce more antioxidants to curtail the oxidative species produced in the disease state. Treatment with 100 mg/kgbw extract showed marked reduction in the FRAP value in non-diabetic rats while 200mg/kgbw extract showed no difference in FRAP value when compared with the normal control. Lans et al. (2007) in their findings, discovered that the leaves of *Taraxacum officinale* pre-ingested by ruminants during pregnancy strengthened the liver by maintaining the FRAP level in preeclampsia induced animals. Our study indicated that in diabetic condition, the administration of 100mg/kgbw and 200mg/kgbw extracts showed no significant effect in FRAP value.

Nitrite in the body is reduced to nitric oxide, an unstable molecule, which can combine with oxygen to form a dangerous nitrogen radical known as peroxinitrite. Excess nitrite can also react with circulating proteins in the internal or external cellular matrix to form a modified protein structure of nitrosamine which can attack and cause dysfunction to cellular structure (Miles et al., 1996). The nitrite level was found to be non-significantly different in both diabetic and non-diabetic controls, but a significant decrease in nitrite level was observed in the liver of diabetic and non-diabetic rats treated with the plant extract when compared with the control groups. In this study, the extracts showed marked reduction of nitrite in the liver of both diabetic and non-diabetic treated rats. Colle et al. (2012) reported that the leaf extract of *Taraxacum officinale* decreased nitrite concentration. In the findings of Hu and Kitts (2005), it was reported that the activity of *Taraxacum officinale* flower extracts against RNS was attributed to the phenolic content. Ethanolic extract of *Taraxacum officinale* flowers has also been demonstrated to have suppressive effects on NO• production in macrophage cells (Jeon et al., 2008). Administration of 100mg/kgbw and 200mg/kgbw extract showed significant decrease in nitrite level in both non-diabetic and diabetic rats.

Lipid peroxidation is a function of interaction of polyunsaturated lipids with oxidative species (Ayala *et al*, 2014). This process involves the interaction of free radicals with the membrane lipids by taking electron from the lipids, resulting in damage to the cell membrane. Lipid peroxidation indicates the level of oxidative stress as it forms reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). (Ayala *et al*, 2014). The product of this reaction, i.e, MDA in the liver was found to be insignificantly different in both diabetic and non-diabetic control groups. Administration of 100mg/kgbw to non-diabetic rats significantly decrease lipid peroxidation product when compared to the non-diabetic control. In the diabetic condition, both 100 mg/kgbw and 200 mg/kgbw showed marked decrease in the lipid peroxidation product in the liver which was in contrast to the work of Choi et al. (2010), that supplementation with dandelion (*Taraxacum officinale*) leaf showed no significant reduction in TBARS level of fat-induced experimental rats. However, Hfaiedh et al. (2014) showed that Wistar rats pretreated with *Taraxacum officinale* had a significant decrease in the hepatic MDA content. In his work, MDA content was significantly increased in sodium dichromate-treated control group animals, while prior administration of *Taraxacum officinale* extract along with sodium dichromate showed a decrease in liver MDA content when compared with sodium dichromate-treated control rats.

GSH assay involved an optimized enzymatic recycling method and glutathione reductase where the sulphdryl group of the glutathione reacts with 5, 5 – dithiobis – 2-nitrobenzonic acid (DTNB) to form GSTNB disulfides mixture. The level of reduced glutathione (GSH) was not significantly different found to have increased in diabetic control group compared to non-diabetic group. This might be considered as a result of the body response to diabetic condition by producing more GSH to mob-out the oxidative species. This effect was found to GSH level increased significantly by in the administration of 100 mg/kg bw and 200 mg/kg bw extracts to diabetic group. due to body adjustment to balance the unhealthy situation. Non-diabetic groups treated with the plant extract showed no significant different in GSH compared with the normal control. The work of Choi et al. (2010) and El-gengaihi et al. (2016) have indicated that GSH level in the liver of

Taraxacum officinale treated rats increased significantly due to excess oxidative species production. Hence, it can be deduced that *Taraxacum officinale* leaf extract could increase GSH level in diabetic conditions.

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the reduction of superoxide (O2−) radical into molecular oxygen (O2) or hydrogen peroxide (H2O2). In this study, the activities of SOD were insignificantly increased in diabetic control as compared to non-diabetic control while diabetic rats treated with 100 mg/kgbw and 200 mg/kgbw extracts showed significant increase in % SOD activity as compared to diabetic control. Glutathione peroxidase (GPx) is responsible for catalyzing the reduction of hydrogen peroxide to water and oxygen. The GPx activity in the liver increased in diabetic control as compared with non-diabetic control in this study. The increase in GPx activity in diabetic control might be as a result of disease condition causing the body to synthesize more of the antioxidant enzymes to curtail oxidative species. Administration of 100 mg/kgbw and 200mg/kgbw extract in non-diabetic groups increased GPx activity significantly as compared to the normal control, indicating the potential of the plant extract to enhance the antioxidant system of the body in a healthy individual. Diabetic groups treated with the 100mg/kgbw and 200mg/kgbw plant extract showed decrease in GPx activity.

In conclusion, this study revealed that aqueous leaf extract of *Taraxacum officinale* can help to boost the antioxidant capacity in the liver in diabetic condition. Isolation of bioactive compounds in the plant is recommended to have better insights into the specific phytochemicals that exert health promoting potentials.

Acknowledgements: *We thank Adeleke University for providing the laboratory facilities for this research work. We also appreciate Prof. Oluwafemi Oguntibeju of Cape Peninsula University of Technology, South Africa for supporting this study*

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