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Original Research Article

Hypoglycemic Effects of the Methanolic Seed Extract of *Hunteria umbellata* ('Abeere') and Its Effect on Liver, Hematological and Oxidative Stress Parameters in Alloxan-Induced Diabetic Male Albino Rats

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A b s t r a c t

The study was conducted to determine the hypoglycemic effects of the methanolic seed extract of *Hunteria umbellata* (Abeere) and its effect on liver, hematological and oxidative stress parameters in alloxan-induced diabetic male albino rats. Adult male albino rats weighing between 130-160 g in five groups were used. The male albino rats were grouped into five groups. Group I rats were not induced with alloxan (normal control), Group II serves as the negative control and were given distilled water *ad labium*, Group III serves as positive control and was treated with glibenclamide, Group IV and V were treated with 100 and 250 mg/kg body weight of methanolic extract of *H. umbellata* seed respectively. The extracts were given to the animals orally for 14 days. The phytochemical constituents of the methanolic seed extract of *H. umbellata* indicates the presence of secondary metabolites like tannins, alkaloids, cardiac glycosides, reducing sugar, saponins, flavonoids and anthraquinones, The weight of diabetic untreated rats (Group II) were significantly (P<0.005) reduced when compared to other groups. The group of rats given glibenclamide, 100 and 250mg/kg bw of *H. umbellata* extract showed significant decrease (P<0.05) of blood sugar level compared to the untreated rats, indicating anti-diabetic effect. The extracts of *H. umbellata* significantly increased RBC, HGB and HCT. The extract may be hepatotoxic if consumed at high concentration. This is evidenced from the high plasma values obtained from the liver biomarker enzymes assayed. The extract significantly increased (P<0.05) the level of CAT and GSH in the liver homogenate induced with alloxan while the MDA values reduced significantly with the administration of *H. umbellata* seed extract. This signifies the antioxidant properties of the seed extract.

K e y w o r d s

Hunteria umbellata
Hypoglycaemic effect
Liver biomarker enzymes
Oxidative stress
Seed extract

Introduction

Hunteria umbellata (K. Schum.) Hallier belongs to the family Apocynaceae is a medicinal plant with a long standing use in the treatment of various ailments in Nigeria and Ghana (Adegoke and Alo, 1986). Among the Yoruba and Binis (Southwest Nigeria), it is locally known as "Abeere". Various parts of the plant have been used in herbal medicine for the treatment of Diabetes (Raman and Mallam, 1994; Longe and Momoh, 2014). Water decoction made from the dried seeds of *H. umbellata* is highly valued in the local management of diabetes mellitus, obesity, stomach ache, pains and swellings, hypertension and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009). In addition, the anti-obesity and hyperlipidaemic activities of *H. umbellata* have also been reported to be mediated via inhibitions of intestinal lipid absorption and *de novo* cholesterol and triglyceride syntheses (Adeneye et al., 2010). The seed of *H. umbellata* is relatively of less demand for medicinal application because of existing uncertainty about its value and possibly fear of a higher concentration of alkaloids and other toxic materials than the other parts of the plant (Adegoke and Alo, 1986). The present study was therefore initiated to determine the impact of *H. umbellata* seed on some biochemical parameters in male Albino rats.

Materials and methods

Collection and identification of *H. umbellata seeds*

The seeds of *H. umbellata* were gotten from Ikorodu market in Lagos State, Nigeria and authenticated by Miss Shokefun, a botanist from the Department of Science Laboratory Technology (Environmental Biology Unit), Lagos State Polytechnic Ikorodu, Lagos-Nigeria.

Preparation of methanolic seeds extract of *H.umbellata*

The seeds were air dried under shade in the Biochemistry laboratory. The dried seeds were pounded to coarse powder in a mortar and then to fine powder with a blender. Extraction was carried out by dispersing 200g of the grounded plant material in 1L of 70% Methanol and shaking was done with GFL shaker for 72 h. This was followed with vacuum filtration and evaporation at a temperature not exceeding 40^{o}C. The concentrate was heated over a water bath to obtain a solvent-free extract, which was stored in a refrigerator at 4^{o}C.

Qualitative phytochemical analysis of methanolic seed extract of *H. umbellata*

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures (Trease and Evans, 1995; Harborne, 1993; Sofowora, 1993).

Quantitative phytochemical analysis of the methanolic leaf extract of *H. umbellata*

Total phenolic content determination: The quantitative determination of total phenolic content using Folin-Ciocalteu (F-C) reagent involves oxidation in alkaline solution of phenols by the yellow molybdotungstophosphoric heteropolyanion reagent and colorimetric measurement of the resultant molybdotungstophosphate blue according to the method of Singleton and Rossi (1965) and modified by Dogÿan et al. (2005). The polyphenol fraction (extract corresponding to 1 g of dry plant material) was dissolved in 5 ml of double distilled water. An aliquot of 100 µl of this solution was diluted with double distilled water to 3 ml. Afterward, the obtained solution was added to 300 µl of double distilled water and 500 µL of the F-C reagent. After shaking, the mixture was incubated for 3 min at room temperature. Then 2000 µl of 20% Na_2CO_3 solution was added. The volume obtained was mixed vigorously, and held for 60 min in the dark at ambient temperature. The absorbance of the solution was then measured at 650 nm against a blank in a spectrophotometer. The sample was analysed in triplicate and the average content was noted for each measurement. The total phenolic content, expressed as mg of pyrrocatechol equivalents (PE) per g of dry weight of plant material (mg PE / g DW), was calculated through the calibration curve obtained using the equation given bellow:

$$\textbf{Absorbance} = 0.0828 \times C,\ R^2 = 0.9993$$

Where, C was the concentration (mg/l).

Flavonoid content determination: One hundred millilitres of 80% aqueous methanol was used to repeatedly extract 1 g of the defatted sample at room temperature. The solution was then filtered through

Whatman filter paper. The filtrate was evaporated to dryness in a crucible over a water bath and weighed to a constant weight (Oseni et al., 2013).

Alkaloid content determination: To about 1 g of the defatted sample, 80 ml of 10% acetic acid in ethanol was added. The beaker was covered and then allowed to stand for 4 h. The suspension was then filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered to obtain the alkaloid residue. This was dried and weighed (Oseni et al., 2013).

Determination of total proanthocyanidins: Determination of proanthocyanidin was based on the procedure reported by Sun et al. (1998). A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x was the absorbance and y was the catechin equivalent (mg/g).

Administration of alloxan

Male albino rats of about ten weeks old with weight range of 130-160g were made diabetic by injecting them with alloxan intraperitoneally with dosage of 100mg/kg body weight. Development of diabetes was confirmed after 72 h of alloxanisation by using "Accuchek Active Glucometer" (Roche Diagnostics) and blood glucose test strips.

Grouping of animals

The animals were grouped as follows:

Group I - control (non-diabetic rats).
Group II - Negative control (diabetic without treatment).
Group III - Positive control [diabetic + glibenclamide (standard drug)].
Group IV - Diabetic + 100mg/kg body weight (bwt) of *H. umbellata.*
Group V - Diabetic + 250mg/kg bwt of *H. Umbellata.*

Measurement of body weight

All experimental animals were weighed every 7 days for the entire 28 days treatment period, to monitor any weight losses or gains.

Determination of hematological parameters

The total red blood cell (RBC), hemoglobin concentration (HGB) ,white blood cell count (WBC), platelet count and other hematological parameters were determined in the blood using ADVIA 60 Closed Tube (CT) Automated Hematology System in Yaba, Psychiatric Hospital, Lagos, Nigeria.

Collection of blood samples for plasma preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. The blood was later centrifuged for 10 min at 3000rpm using a centrifuge. The clear supernatant was used for the estimation of liver biomarker enzymes.

Estimation of liver biomarker enzymes

Plasma enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) were determined using Randox diagnostic kits. The total protein (TP) was also determined using Randox diagnostic kits.

Estimation of oxidative stress parameters

Preparation of liver homogenate: The Liver tissues of some of the sacrificed albino rats were excised and the liver samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 50 minutes. The supernatant obtained was later used for assay of thiobarbituric acid reactive substances (TBARS) content, superoxide dismutase, catalase and reduced glutathione.

Estimation of Lipid peroxidative (LPO) indices: Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Niechaus and Sameulsson (1968).

Estimation of superoxide dismutase (SOD): The liver homogenate was assayed for the presence of SOD by utilizing the technique of Magwere et al. (1997), with slight modification. The assay was performed in 2.5ml of 50m M Na_2CO_3 buffer to which 0.05ml of the sample was added. 0.3 of the epinephrine stock solution was then added to the above before taking absorbance readings at 480nm for 3-5 min. A blank devoid of the samples (but having all the reagents) was used for background correction.

Estimation of catalase (CAT): The liver homogenate was assayed for catalase colorimetrically at 620mm and expressed as µmoles of H_2O_2 consumed/min/mg protein as described by Sinha (1972).

Estimation of Reduced glutathione (GSH): Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellman (1959).

Data analysis

Data analysis was done using the GraphPad prism computer software version 5.0. Students 't'-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value <0.05 was considered significant.

Results and discussion

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin (Mathur et al., 2010). The disease remains incurable and can only be controlled with drugs. Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depend on it as primary health care.

The presence of pharmacologically active phytochemicals like tannins, alkaloids, saponins, flavonoids and anthraquinone have been shown to be present in the methanolic seed extract of *Hunteria umbellata* (Table 1). The presence of these secondary metabolites in the extract may be responsible for the hypoglycaemic effect. The amount of total phenol, flavonoids, alkaloids and total proanthocyanidins present in the seed extract were 121.62 mg PE / g DW, 1.8%, 6.9% and 1.84±0.2 mg quercetin/g of dry plant material respectively (Table 2).

Table 1. Phytochemical screening of methanolic seeds extract of *H. umbellata.*

Test	Results
Tannins Ferric chloride test	+++
Cardiac glycosides Salkowski test	+++
Alkaloids Wagners test	+++
Flavonoids Lead acetate test	++
Reducing sugar Fehling A and B test	++
Saponins Frothing test	++
Anthraquinone	+

Present at low levels = +, Present at moderate levels = ++ and Present at high levels = +++

Table 2. Quantitative analysis of the phytochemical constituents of the methanolic seed extract of *H.umbellata*.

Phytochemical constituents	*H. umbellata* extract
Total phenol[a]	121.62
Flavonoids	1.8%
Alkaloids	6.9%
Total proanthocyanidins[b]	1.84±0.2

[a]mg of pyrrocatechol equivalents (PE) per g of dry weight of plant material (mg PE / g DW).
[b]Expressed as mg quercetin/g of dry plant material.

Most of the antioxidant potential of medicinal plants is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers (Hakkim et al., 2007). Studies have shown that phenolics and flavonoids constitute the main powerful antioxidant compounds (Ksouri et al., 2008). It has been shown that medicinal plants with hypoglycemic and antidiabetic effect usually contain high concentration of alkaloids and flavonoids (Oladele et al., 1995).

The untreated diabetic animals (Group II) have significantly lost weight ($P<0.05$) when compared to the healthy and treated animals (Fig. 1). This may be due to loss in muscle adipose tissue protein and fatty acids (Granner, 1996). Studies have also reported significant weight reduction in untreated diabetic animals (Ahmed et al., 2005; Momoh et al., 2014).

Fig. 1: Body weight of animals after treatment with glibenclamide, 100 and 250 mg/kg bwt of *H. umbellata* seed extract.

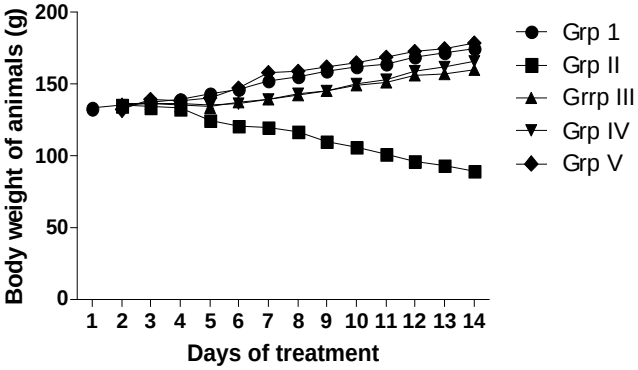

Fig. 2: Initial blood glucose concentration values in mg/dl for normal, diabetic untreated, diabetic treated with glibenclamide, 100 and 250 mg/kg bwt of *H. umbellata* seed extract.

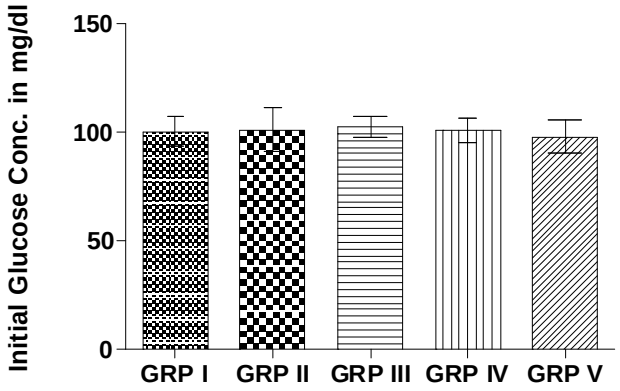

Fig. 3: Blood glucose concentration values in mg/dl after alloxan induction for all the experimental groups.

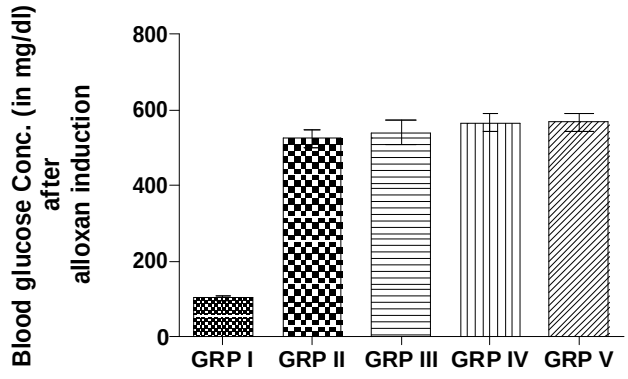

Fig. 2 shows the initial blood sugar level before induction of the control group and other groups. From the result obtained, all the animals used for the experiments are healthy. Group I animals were not induced with alloxan. Fig. 3 shows the blood glucose concentration after induction. The result obtained indicates that all the animals in Group II –V were diabetic after induction with alloxan. Animals in Group II and III serve has the negative and positive control (animals treated with glibenclamide) respectively while Group IV and V animals were treated with 100 and 250mg/kg bwt of *H. umbellata* respectively. Experimental findings shows that after 7 days of treatment, Group III –V animals blood glucose level significantly reduce (P<0.05) when compared to untreated diabetic rats (Fig. 4) and these hypoglycemic effect is similar to the work of Adeneye and Adeyemi (2009) who showed that 50-200mg/kg of *H.umbellata* significantly reduce the blood glucose level in glucose and nicotine-induced hyperglycaemic rats.

Fig. 4: Blood glucose concentration values in mg/dl after 7 days of treatment for all the experimental groups.

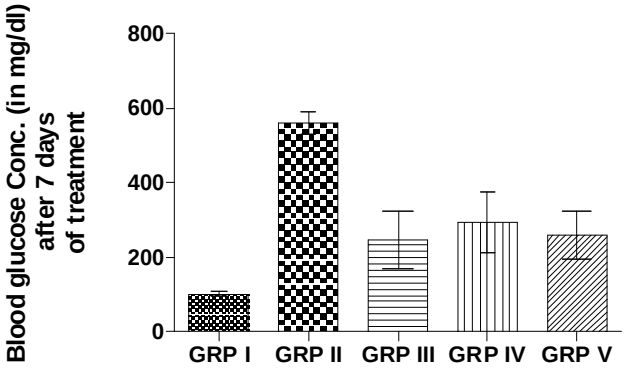

Fig. 5: Blood glucose concentration values in mg/dl after 14 days of treatment for all the experimental groups.

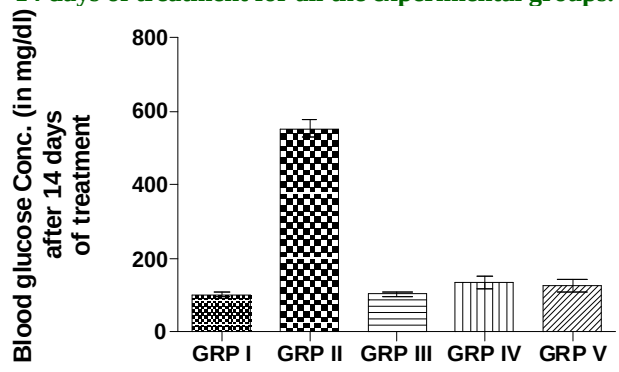

Fig. 5 shows the blood glucose concentration values in mg/dl after 14 days of treatment. Group III –V animals show significant (P<0.05) hypoglycemic effect after treatment with glibenclamide and methanolic seed extract of *H. umbellata* respectively. Hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Sexena et al., 2011). There were significant reductions (P<0.05) in WBC levels of

diabetic rats treated with methanolic seed extract of *H.umbellata* compared to the diabetic untreated animals. Leukocytosis observed in group II animals, may be due to bone marrow tumor, luekemia, tissue damage, and inflammatory disease of the animals with diabetes without treatment. In the present study, methanolic seed extract of *H. umbellata* treatment for 14 days produced significant elevations in RBC, PCV and HGB indicating the hematopoietic effect of *H.umbellata*. Thus, the significant elevations (P<0.05) in RBC, PCV and HGB strongly suggest that *H.umbellata* could be useful in the management of anemia. Other hematological parameters (MCH, MCHC, MPV and PCT) of the control groups and the treatment groups show no significant difference (Table 3).

Table 3. Effect of methanolic seed extract of *H. umbellata* on hematological parameters.

Hematological Parameters	Group I	Group II	Group III	Group IV	Group V
WBC ($\times10^9$/L)	9.9 ± 1.1*	14.6±5.2	11.3±1.4*	11.1±1.1*	10.2 ±1.5*
HGB g/dl	13.2 ± 1.2*	8.7 ± 1.4	12.7 ± 3.1*	13.1 ± 1.2*	13.9 ± 2.1*
RBC ($\times10^{12}$/L)	11.2 ± 2.6*	6.4 ± 1.3	9.4 ± 1.3*	10.3± 1.0*	10.9 ± 2.1*
HCT %	43.5 ± 2.3*	31.1 ± 10.2	39.8 ± 2.2*	40.2 ± 2.5*	44.1 ± 1.1*
MCH pg	16.7 ± 1.2	16.9 ± 2.1	17.4 ± 1.8	18.1 ± 0.1	18.3 ± 0.7
MCHC g/dl	29.1 ± 2.1	31.2 ± 1.3	31.1 ± 0.4	30.9 ± 0.8	32.2 ± 1.2
MPV fl	7.1 ± 0.6	7.3 ± 0.7	7.2 ± 0.1	7.5± 0.9	7.1 ± 0.8
PCT %	0.464 ± 0.0541	0.413 ± 0.065	0.450 ± 0.053	0.449 ± 0.052	0.481 ± 0.027

The values are the Means ± SD for five rats in each group. White blood count (WBC), Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean platelet volume (MPV) and Plateletcrit (PCT).

Table 4. The effect of methanolic seed extracts of *H. umbellata* on plasma total protein, total bilirubin and liver marker enzymes in alloxan–induced diabetic male albino rats.

Parameters	Group I	Group II	Group III	Group IV	Group V
AST (U/L)	6.20 ±2.20*	15.60 ±2.4	8.10 ± 2.50*	10.10 ±3.20	14.30 ±3.20
ALT (U/L)	5.90±2.40*	14.30±4.20	8.20±2.30*	11.10±3.10	15.10±3.10
ALP (U/L)	49.60±8.10*	92.10 ±11.30	63.40±7.20*	78.10±9.10	97.20±15.10
Total BIL (mg/dl)	0.42±0.12*	0.76±0.30*	0.51±0.10*	0.59±0..20*	0..61±0.30*
Total protein (g/dl)	7.80±0.30*	4.80±0.40	6.50±0.40*	6.60±0.40*	6.90±0.60*

* indicate significant difference (P<0.05) when comparing normal and treated group with negative control group.

Table 4 shows that there are significant increase (P>0.05) in plasma total bilirubin, AST, ALT and ALP in the untreated diabetic animals (Group II) and the diabetic animals treated with 250 mg/kg bwt of *H.umbellata* (Group V) compared to other animals in other groups. This shows that the albino rats in Group II and V had liver impairment or hepatocellular damage compared to other albino rats in other groups. This is an indication that prolong use of high dosage of abeere seed may be hepatotoxic and harmful to the body. The result of this research is similar to the work of Isaiah et al. (2007). They showed that there was an enhancement in the activities of alkaline phosphatase, aspartate transaminase and alanine transaminase in the rabbits exposed to 0.5ml of water extract of 'abeere' compared to the healthy animals, an indication of hepatic damage. Increase in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver damage (Mukherjee, 2003; Halim et al., 1997; Momoh et al., 2014). Nelson and Cox (2005) reported that a rise in plasma level of bilirubin suggests liver cell damage, since liver cells are responsible for removing bilirubin from serum.

Oxidative stress is the presence of reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity. ROS can damage macro-molecules (lipids, proteins, and DNA) thus altering the structure and function of the cell, tissue, organ, or system (Roberts and Hubel 2004). There was a significant increase (P<0.05) in the Malondialdehyde (MDA) levels in Group II animals compared to other animals in other groups. This is an indication of increased possibilities of lipid peroxidation and a consequent increase in oxidative stress (Table 5) in group II animals. In this study, there was a significant reduction (P<0.05) in activities of CAT and GSH in the liver homogenate of Group II albino rats compared to other

rats in other groups. These indicate hepatic injury from oxidative stress caused by the administration of alloxan in diabetic animals in Group II animals. From the analyses of the result obtained, the seed extract may be used to reduce oxidative stress since they cause low MDA values and high activities of CAT and GSH in diabetic animals treated with methanolic seed extract of *H. umbellata*. There were significant increase (P<0.05) in the activities of SOD in the liver homogenate of Group I, III and IV animals compared to Group II and V (Table 5) animals. The reduced level of activities of SOD in these groups may be due to the production of ROS in the liver homogenate of the animals in these groups, an indication of oxidative stress.

Table 5. Effect of glibenclamide and methanolic seed extract of *H. umbellata* on oxidative stress parameters in alloxan induced diabetic rats.

Oxidative stress parameters	Group I	Group II	Group III	Group IV	Group V
Lipid Peroxidation X 10^3 mm MDA/mg protein	*5.50±0.80	11.80±1.60	*6.80±1.50	*6.10±1.40	*6.60±2.40
Catalase (CAT) unit*/ mg protein	*29.50±8.50	14.30±7.60	*27.10±5.40	*36.30±10.62	*38.30±12.80
Superoxide dismutase (SOD) unit*/ mg protein	*9.20±3.20	5.20±1.40	*7.80±1.60	*7.10±1.30	*5.90±2.40
Reduced glutathione (GSH) mg/g protein	*0.37±0.08	0.14±0.07	*0.28±0.10	*0.35±0.04	*0.43±0.04
* indicate Significant difference (P<0.05) when comparing normal and treated group with negative control group.					

Conclusion

The findings of this research show that the methanolic seed extracts of *H. umbellata* contain some secondary metabolite, is not hematotoxic, it has hypoglycemic effect, and it may reduce oxidative stress and may cause hepatic damage if consumed for long at high concentration.

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