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Role of Terminalia Chebula Fruits Extract in the Antioxidant Status of Diabetic Rats

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ABSTRACT

Terminalia chebula is a traditional medicine belonging to the genus Terminalia, family Combretaceae. It was recorded to have antidiabetic activity. The present work was subjected to investigate the antioxidant status of diabetic rats due to the aqueous extract of *Terminalia chebula* fruits and/or metformin treatment. Malondialdehyde as indicator to lipid peroxidation process, glutathione level and superoxide dismutase activity were measured in rat liver. Free radical capacity and glucose levels were determined in blood samples. Results revealed different responses of oxidant and antioxidant levels in non-diabetic and diabetic rats via *T. chebula* extract and/or metformin treatment. Decrease glucose levels in all diabetic treated groups compared with diabetic rats. *T. chebula* extract treatment significantly lowered glutathione levels except of its combined treatment with metformin in diabetic rats where very high significant increase occurred. Superoxide dismutase levels exhibited significant increase in all metformin treated groups with no significant increase in diabetic treated group with metformin. Significant decrease in free radical capacity was observed but no significant effects occurred in diabetic group and in *T. chebula* extract administrated rats. In conclusion, the combined treatment with *T. chebula* extract and metformin has been effective more than the either single treatment on diabetic rats.

Keywords: Antidiabetes, traditional medicines, metformin, oxidative stress, free radicals.

1. INTRODUCTION

Free radicals such as reactive oxygen species (ROS) are produced as byproducts in aerobic metabolism, and have been implicated in the pathogenesis of many diseases such as diabetes mellitus[1-4]. ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle[5, 6]. Most living species have efficient defense systems to prevent themselves against oxidative stress induced by ROS[7]. Thus, antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction[8]. The previous studies have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases[9]. There are adverse effects of synthetic antioxidants, therefore researchers have channelled their interest in isolating natural antioxidants[10] which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation. *Terminalia chebula* is a traditional medicine belonging to the genus Terminalia, family Combretaceae. Many studies have demonstrated that *Terminalia chebula* exhibits a wide range of biological activities, including cardioprotective[11, 12], antioxidant[13], free radical scavenging[14] and hypolipidemic properties[15]. Compounds that scavenge for free radicals and have membrane stabilizing potential are reported to be effective in ameliorating the progress of biochemical tissue injury[16].

The present study provides screening the ability about the free radical scavenging capacity of *Terminalia chebula* on diabetic rats and its antioxidant activity with a reference of anti-diabetic drug, metformin.

Material and methods

Animals:-

Male adult albino rats weighing 150–200 g were obtained from united company for chemicals of medical preparation (UCCMA). Animals were housed in polypropylene cages and maintained under standard laboratory conditions with controlled light-dark cycle, room temperature at about 20-30 ºC and humidity from 50% to 75%. Balanced animal food and tap water were available. The food was obtained from UCCMA.

Treatments:

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Aloxan was obtained from El-Gomhoria Egypt Company, dissolved in saline and adjusted for a single intra-peritoneal dose 120 mg/kg body weight[17].

Metformin (MF) is an oral anti-diabetic drug. The tablets was crushed and suspended in water to prepare oral doses of 100 mg/kg body weight[18].

Terminalia chebula aqueous extract (TAE): *T. chebula* fruit were supplied from the Agriculture Research Center, Giza. It had no previous treatment with any pesticides. The dried fruits of *T. chebula* were powdered to get a coarse powder. The dried powder (250 g) was taken in 500 ml distilled water, mixes well and leaves in 40 ºC in incubator for 24 hours before use. The mixture put in refrigerator for use at doses of 500 mg/kg body weight[19, 20] daily for two weeks.

Instruments

The biochemical assay was achieved using Helios γ UV/VIS Spectrophotometers (England). ESR signals were recorded by using a Bruker EMX spectrometer (X-band) product of Bruker, Germany.

Experimental design:-

Rats were divided into the following groups (eight rats were assigned for each sacrificing):

-Control: no treated group.

-Diabetic rats: animals received a single dose 120 mg/kg body wt of alloxan via intra-peritoneal injection.

-TAE: animals administered with 500 mg/kg body wt TAE daily for two weeks.

-MF: animals administered with 100 mg/kg body wt MF daily for two weeks.

-MF+TAE: animals administered with 100 mg/kg body wt MF and 500 mg/kg body wt TAE daily for two weeks.

-MF treatment: diabetic animals treated with 100 mg/kg body wt MF daily for two weeks.

-TAE treatment: diabetic animals treated with 500 mg/kg body wt TAE daily for two weeks.

-MF+TAE treatment: diabetic animals treated with 100 mg/kg body wt MF and 500 mg/kg body wt TAE daily for two weeks.

Blood glucose was measured after 72 hours of aloxanization by one touch glucometer to confirm diabetes.

Biochemical analysis:-

Lipid peroxidation levels were ascertained by the formation of malondialdehyde (MDA). Sample preparation was performed as described by Sardar et al.[21]. 0.5 ml of liver homogenate was treated for determination of MDA as described by Yoshioka et al.[22]. Glutathione (GSH) concentration in liver was estimated according to Beutler et al.[23]. Determination of superoxide dismutase (SOD) in liver was according to Marklund and Marklund[24]. Total free radicals capacity (FRC) assay by electron spin resonance technique (ESR) in blood samples were measured according to the method of Heckly[25, 26].

Statistical analysis:-

Data were analyzed by unpaired two-tailed Student's t–test[27]. The results were presented as mean ± S.E. *p* value ≤0.05 was considered significant.

RESULTS

The results indicated that the antioxidant status of control (non diabetic rats) and diabetic rats had been affected by TAE and/or MF treatment.

With high levels of blood glucose in diabetic rats, significant elevation in MDA level and decreasing in GSH content was observed. Whereas, the change in SOD and FRC were not significant as showed in Table 1.

Table (1): Effect of TAE and/or MF on the antioxidant status and glucose levels in control (non diabetic rats) and diabetic rats (mean ± SE):

Groups	MDA (nmol/g)	GSH (mg/g)	SOD (U/g)	FRC (Radical/ g)	Glucose
Cont	52.12 ± 2.77	461.1 ± 22.2	14.77 ± 0.82	4.101 ± 0.17	119.4 ± 2.154
TAE	57.64 ± 3.51	392.0 ± 19*	13.10 ± 0.68	4.005 ± 0.19	127.5 ± 5.916
MF	48.35 ± 1.10	950.8 ± 27.2***	19.09 ± 0.66**	2.658 ± 0.06***	105.4 ± 1.802***
MF+TAE	56.96 ± 2.381	193.5 ± 2.34***	18.92 ± 0.97*	2.325 ± 0.05***	117.4 ± 2.639
Diabetic (D)	61.18 ± 2.496*	244.8 ± 12.5***	15.93 ± 0.56	3.851 ± 0.18	519.8 ± 28.26***
D+ TAE	65.79 ± 2.03***	224 ± 8.8***	12.08 ± 0.38*#	3.618 ± 0.22*	298.1 ± 22.42***#
D+MF	57.91 ± 1.265	388.2 ± 21.2*#	16.14 ± 0.58	2.903 ± 0.16***#	171.3 ± 7.375***#
D+MF+ TAE	41.17 ± 2.587*#	851.2 ± 68.0***#	17.75 ± 0.49*#	2.627 ± 0.09***#	164.6 ± 2.878***#

* p≤ 0.05, ** p≤ 0.005 & *** p≤ 0.0005 *vs* control group.

p≤ 0.05 *vs* diabetic rat group.

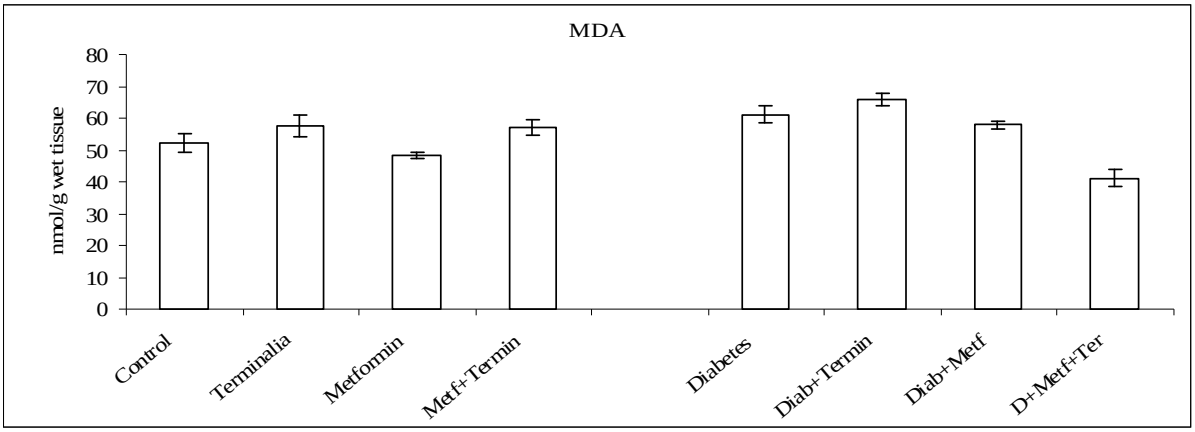

Fig.1: Effect of TAE and/or MF on liver MDA levels in control (non diabetic rats) and diabetic rats: no significant effect of TAE and/or MF on MDA was found in the control. Diabetic treated group with TAE resulted in very highly significant increase in MDA levels while no significant effect occurred with MF treatment. The combined treatment lowered significantly MDA levels. Diabetic rats show significant increase in MDA level.

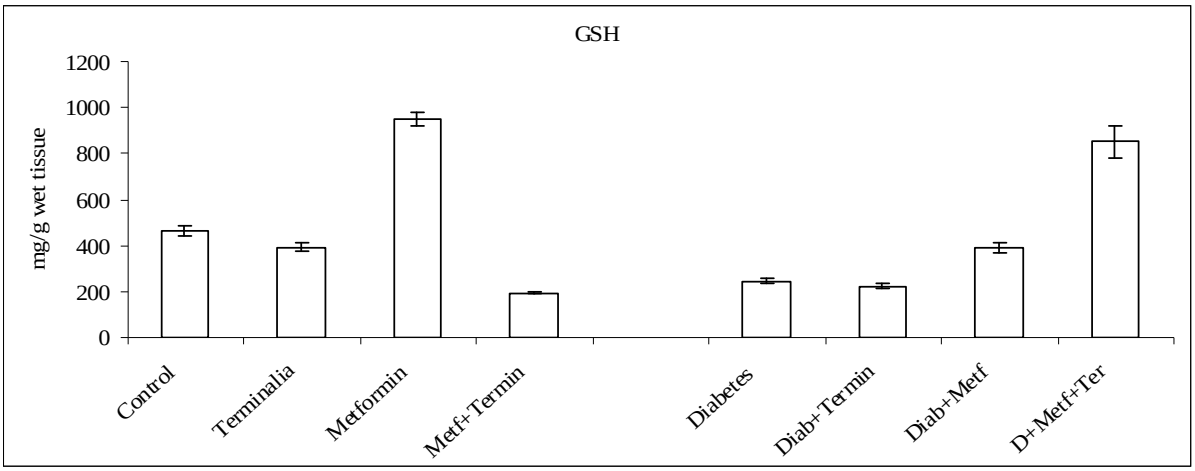

Fig.2: Effect of TAE and/or MF on liver GSH levels in control (non diabetic rats) and diabetic rats: lowering in GSH levels was observed in non-diabetic rats after administration with TAE or TAE combined with MF, where MF administration led to highly elevation in GSH. Diabetic rats showed very high significant decrease in GSH levels. Compared with diabetic rats, MF treatment improved the GSH concentration. Very high significant increase in GSH levels was observed after the combined treatment with TAE and MF compared with control or diabetic rats.

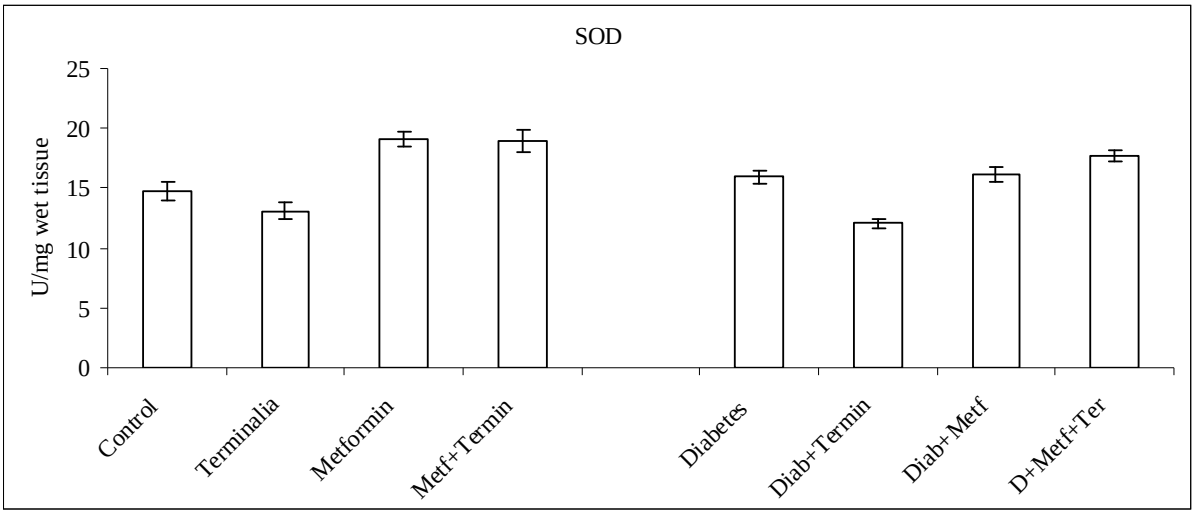

Fig.3: Effect of TAE and/or MF on liver SOD levels in control (non diabetic rats) and diabetic rats: SOD activity had not been affected in diabetic rats or after administration of TAE, while MF as single administration or combined with TAE significantly increased SOD activity. There was decrease in SOD in diabetic treated group with TAE but the combined treatment with MF caused increase in SOD activity compared with control or diabetic rats.

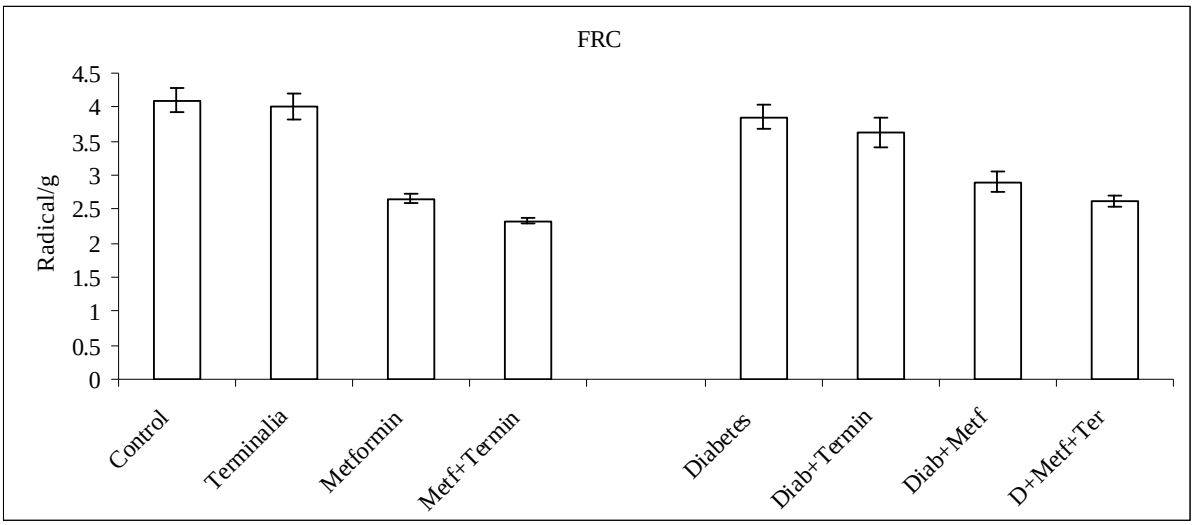

Fig.4: Effect of TAE and/or MF on FRC in blood in control (non diabetic rats) and diabetic rats: no significant effect occurred in diabetic rats or in TAE administrated group. A very high significant decrease was observed after MF treatment and after its combined treatment with TAE against control and diabetic rats. TAE caused decrease in FRC in diabetic rats.

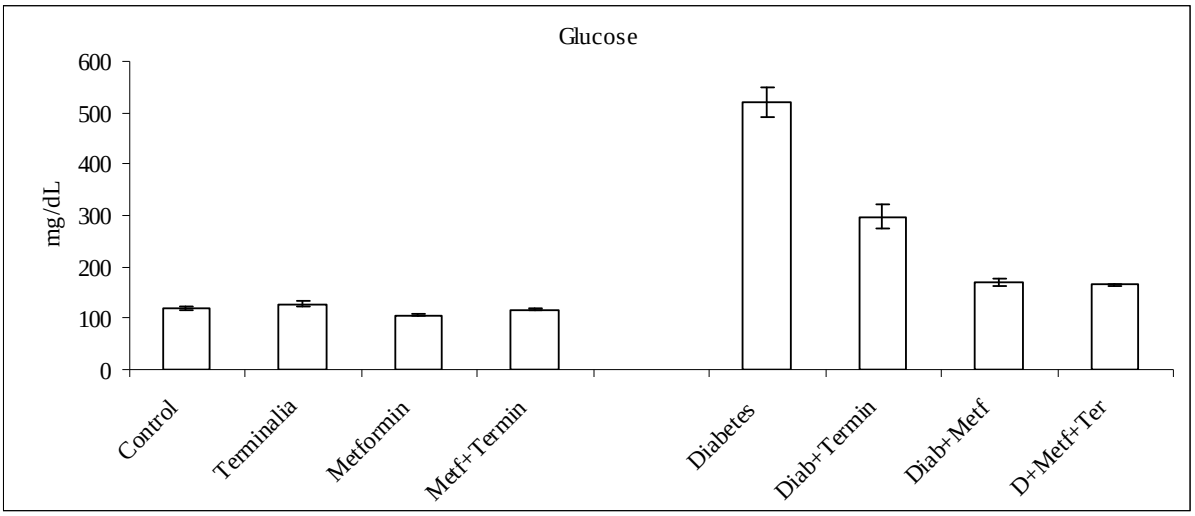

Fig.5: Effect of TAE and/or MF on blood glucose levels in control (non diabetic rats) and diabetic rats: very high significant increase in glucose levels in diabetic treated groups with TAE and/or MF compared with control. Compared with diabetic rats, very high significant decrease in glucose levels occurred in diabetic treated groups with TAE and/or MF. Single administration with MF for control highly significantly decreased glucose level but no significant effect after TAE administration.

DISCUSSION

Results of the present work revealed that treatment of alloxan induced diabetic rats with aqueous extract of *T. chebula* significantly reduced the hyperglycemia. This result agrees with that of Rao and Nammi[28] who demonstrated the dose dependent hypoglycemic effect of chloroform extract of *T. chebula* seeds on streptozotocin-induced diabetic rats. Also, Murali et al.[29] found that oral administration of aqueous extract of *T.chebula* reduced the elevated blood glucose by 43.2% whereas, Lee et al.[30] demonstrated the antihyperglycemic effect of aqueous extract of *T. chebula*.
There is growing evidence that oxidation stress is increased in diabetes due to the overproduction of ROS, and decreased efficiency of antioxidant defenses[31]. Glycation is a reaction which occurs during diabetes[32]. This reaction begins with adduction of a reducing sugar to an amino group in protein, and finally proceeds to form advanced glycation end products. Advanced glycation end products can mediate their effects via specific receptors, thereby activating diverse signal transduction cascades which can lead to the generation of ROS [32]. Pazdro and Burgess[33] reported that oxidation of lipids, proteins, macromolecules and DNA occurs during the development of diabetes. El-Missiry et al.[34] reported that alloxan administration significantly increased lipid peroxidation products.

In the present study, diabetic rats had been suffered from oxidative stress by increasing of liver MDA level. Also the hepatic GSH concentration was decreased significantly in the diabetic rats with respect to the control which is in agreement with the previous studies[35-37, 20]. Maritim et al,[35] reported that the high levels of MDA in the diabetic rats might contribute to the impaired glutathione defenses.

Aqueous extract of *T. chebula* have been characterized by high phenolic content[38]. In non-diabetic rats, TAE as a natural product had been able to maintain MDA, SOD, FRC and glucose levels with slight significant decrease in GSH levels. In the present study, despite of the high phenolic content of TAE, the liver concentration of GSH was highly decreased after its administration to diabetic rats. TAE as single exposure (500 mg/kg body wt for two weeks) can not restore the oxidative stress effect of alloxan in diabetic rats whereas it reduced blood glucose level but not restores its normal level. ROS radicals are toxic to cells, particularly the cell membrane in which these ROS are involved in lipid peroxidation[39]. In the mitochondria, the excessive levels of glucose lead to an overdrive of the electron transport chain, resulting in the formation of excess superoxide anions[40]. Treating mice with total extracts of medicinal plants as *T. chebula* increased the activity of antioxidant enzymes as SOD and GSH, where these enzymes are modulated in various diseases by free radical attack, thus maintaining the balance between the rates of radical generation and scavenging[8]. The results of the present study showed that treatment of diabetic rats with TAE and/or metformin reduced significantly glucose levels compared with its level in diabetic rats, whereas the combined effect of them inhibited lipid peroxidation process and FRC while elevation in GSH level and SOD activity was observed.

Cells are normally protected against oxidative damage by multiple enzymatic mechanisms and by antioxidant molecules. The SOD is the first and most important line of antioxidant enzyme defense systems against ROS and particularly superoxide anion radicals[41]. SOD catalyzes the breakdown of $O_2^{\cdot}$ to O_2 and H_2O_2, and thus prevents the formation of OH, and thereby, has been implicated as an essential defense against the potential oxygen toxicity[8].

In the present work, SOD activity showed no significant change in diabetic rats, while it increased significantly in normal rats treated with metformin alone, or metformin with aqueous extract of *T. chebula* compared to control group. Meanwhile, SOD activity decreased significantly in diabetic rats treated with aqueous extract of *T. chebula* alone compared to the untreated diabetic group. This decrease in SOD activity may be attributed to the induction of diabetes in rats with alloxan which uniformly results in an increase of ROS that extrapolated antioxidant capacity of SOD. In response to oxidative stress, SOD behaves in two different ways. Initially, the body reacts during a moderate oxidative stress by over expression of SOD (e.g. exercise). If the stress persists and produces a massive toxic of oxygen active species, SOD will be destroyed and its concentration will drop. Paradoxically, an excessive concentration of SOD may be dangerous because, in this case, it is the basis of an overproduction of hydrogen peroxide (paradoxical effect of antioxidants)[42].

Maritim et al.[35] recorded that nonenzymatic glycation may also alter the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals, exacerbating oxidative stress in diabetes. In this respect, lysine residue in the active site of the SOD is non-enzymatically glycosylated (glycation) in the presence of hyperglycemia, decreasing SOD activity[43].

Cheng et al.[14] indicated that no antioxidant activity of casuarinin, chebulanin, chebulinic acid or 1,6-di-O-galloyl-β-D-glucose, isolated and purified from *T. chebula*, had been reported. When evaluated for antioxidant activity, an interesting result was observed among the tested pure compounds. It was found that tested pure compounds, in some terms, were specific in their antioxidant activity. For example, although casuarinin was active in scavenging free radicals, its anti-superoxide formation and anti-lipid peroxidation activities were moderate or weak. For chebulanin, the inhibitory effects on the peroxidation of lipids and the formation of superoxide radicals were more significant than that on the scavenging of free radicals.

In this work, a highly significant decrease in free radical capacity was observed after Metformin treatment alone or in combination with an aqueous extract of *T. chebula* for normal and diabetic rats compared to control group.

Hyperglycemia can promote an important oxidative imbalance, favoring the production of free radicals and reduction of antioxidant defenses. At high concentrations, ROS/RNS can lead to damage of major components of the cellular structure, including nucleic acids, proteins, amino acids and lipids. Such oxidative modifications in diabetes condition would affect several cell functions, metabolism and gene expression, which can cause other pathological conditions[44].

In conclusion, the combined treatment with aqueous extract of *T. chebula* and metformin has been effectiveness more than the either single treatment on diabetic rats whereas the treatment by aqueous extract of *T. chebula* alone have not able to recomposed the adverse effect of diabetes on the antioxidant status. The synergistic action of the combination of *T. chebula* and metformin need further study.

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