B-4355
[1-9]

RESEARCH ARTICLE Indian Journal of Animal Research

# The Anti-oxidant and the Anti-diabetic Effects of *Terminalia chebula* and *Withania somnifera* in Subclinically Diabetic Dogs

Devi Gopinath[1], Umesh Dimri[2], Y. Ajith[3], P.M. Deepa[4], M.I. Yatoo[5], A. Gopalakrishnan[6], E. Madhesh[7]

10.18805/IJAR.B-4355

## ABSTRACT

**Background:** The present study evaluated the antioxidant as well as the anti-diabetic potential of *Terminalia chebula* (Haritaki) and *Withania somnifera* (Ashwagandha) in subclinically diabetic dogs.

**Methods:** A total of thirty subclinically diabetic dogs were divided into five groups of six animals each; Group I (Positive control), Group II (*T. chebula* extract), Group III (*W. somnifera* extract), Group IV (*T. chebula* and *W. somnifera* extracts at 1:1 ratio) and Group V (N- acetylcysteine). Six apparently healthy dogs were kept as negative control (Group VI) for the study. The animals were evaluated based on diabetic biomarkers (Random blood glucose, fasting blood glucose, glycated hemoglobin, serum fructosamine and serum insulin) and oxidative stress markers (lipid peroxidation, reduced glutathione, superoxide dismutase and catalase activities) on days 0, 15 and 30 of the study.

**Result:** Significant oxidative stress was observed in the subclinically diabetic dogs and the supplementation of N- acetylcysteine, *T. chebula* and *W. somnifera* effectively curbed the oxidative impairments and further progression of diabetes. The improvement in oxidative, as well as diabetic biomarkers, was predominant in Group V animals, followed by Group IV, Group II and Group III. It may be concluded that therapeutic modulation of redox mechanisms may aid in the clinical management of subclinical diabetes mellitus, however, the mechanisms involved should be further explored.

**Key words:** Canine diabetes, Herbal, Oxidative sress, Subclinical, *Withania somnifera*.

## INTRODUCTION

Diabetes mellitus (DM) is an emerging metabolic syndrome of greater importance in dogs because of its multi-systemic terminal effects and cachexia. The characteristic features of clinical canine diabetes mellitus are polydipsia, polyuria, polyphagia and weight loss similar to the human syndrome. The prime factors associated with the increased incidence of DM in dogs were obesity, altered food habits, lack of exercise, genetic manipulations and inbreeding (Temelkova-Kurktschiev and Stefanov, 2012; Raffan *et al.,* 2015). Earlier studies reported the prevalence of DM among dog populations in the range of 0.0005% to 1.5% (Wilkinson, 1960; Mattheuws *et al.* 1984) whereas, recent studies from India revealed the existence of clinical/subclinical DM among dog populations in alarming levels, that is 1.8% to 7.2% (Das and Lodh, 2015; Devi *et al.,* 2016). Geriatric female dogs were at high risk than their male counterparts may be due to their hormonal peculiarities leading to insulin resistance (Marmor, *et al*., 1982). In dogs, clinical DM was expressed as elevated blood glucose concentrations (180-220 mg/dl) which is above the renal threshold and the major contributing factor was untreated pancreatitis (Nelson and Reusch, 2014; Rand, 2020).

Prior to clinical manifestation of DM, there is a prediabetic stage often called as subclinical diabetes in humans as well as dogs. According to Buysschaert and Bergman (2011), prediabetes is the presence of Impaired Fasting Glucose (IFG) and/or Impaired Glucose Tolerance (IGT). Experimental studies on pre-diabetic dogs showed high subcutaneous fat deposits, diminished insulin sensitivity and impaired fasting glucose (Ionut *et al.,* 2010).

Insulin therapy is often essential for diabetic dogs since virtually all dogs have Insulin Dependent Diabetes Mellitus (IDDM). Also, oral hypoglycemic drugs such as

Division of Medicine, ICAR- Indian Veterinary Research Institute, Izatnagar, BareillY-243 122 Uttar Pradesh, India.
[1]ICAR-Indian Veterinary Research Institute, Regional Station, Palampur-176 061, Himachal Pradesh, India.
[2]ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122 Uttar Pradesh, India.
[3]Banaras Hindu University, Mirzapur-231001, Uttar Pradesh, India.
[4]Collge of Veterinary animals Sciences, Pookot, Wayanad-673 576 Kerala, India.
[5]FVS cand AH, SKUAST-K, Shuhama Alusteng Srinagar-191 202, Jammu and Kashmir, India.
[6]COVAS, Chennai, Tamil Nadu, India.
[7]Precise Pet Clinic and Diagnostic, Bengaluru-560 075, Karnataka, India.

**Corresponding Author:** Devi Gopinath, ICAR-Indian Veterinary Research Institute, Regional Station, Palampur-176061, Himachal Pradesh, India. Email: drdevigopinath@gmail.com

**How to cite this article:** Gopinath, D., Dimri, U., Ajith, Y., Deepa, P.M., Yatoo, M.I.,Gopalakrishnan, A. and Madhesh, E. (2021). The Anti-oxidant and the Anti-diabetic Effects of *Terminalia chebula* and *Withania somnifera* in Subclinically Diabetic Dogs . Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4355.

**Submitted:** 03-11-2020 **Accepted:** 25-03-2021 **Online:** 30-04-2021

sulphonylurea, metformin etc. were not effective in dogs suffering from IDDM (Nelson, 2000).

In diabetes mellitus, chronic hyperglycemia lead to multiple biochemical damages. Oxidative stress may be the basis of pathogenesis and clinical manifestations of this multi-systemic syndrome (Giugliano *et al.,* 1996). Free radicals were synthesized in disproportionately higher rate and quantities in DM patients, by means of elevated glucose oxidation, non-enzymatic glycation of proteins followed by their oxidative degradation. Hence, the vital role of antioxidant therapy is significant. Antioxidants cannot be considered as a complete curative for DM. But intake of antioxidants can reduce the oxidative stress by interfering with the synthesis of free radicals or by inactivating them and thereby ameliorate the progression of pathological damages related to the disease (Cheng *et al.,* 2003). Earlier, the role and usage of natural antioxidants in prevention of oxidative damage associated with experimental diabetes in rats has been studied (Coldiron *et al.,* 2002; Vessal, *et al*. 2003). Strong natural antioxidants of plant origin included carotenoids, limonoids, tocopherols, ascorbates, and polyphenols (Bajpai e*t al.,* 2005). Also, some plants showed hypoglycemic activity by interfering with insulin release/ stimulation (Pari and Latha, 2002).

The development of new drugs and therapeutic strategies with least side effects were of greater interest to the scientific community at every point of time. Scientists were striving to evaluate antidiabetic activities of such natural products and its scientific validation (Florence *et al.,* 2014). The ethanolic extract of *T.chebula* fruit showed potent hypoglycemic activity in streptozotocin-induced diabetic rats and was found more efficacious than glibenclamide, a standard hypoglycemic drug in commercial use (Kumar *et al.,* 2006). Ethanolic extract of *W. somnifera* root have potent anti-inflammatory as well as anti-diabetic activity (Udayakumar *et al.,* 2009). Keeping this as background data, the present study was carried out to evaluate the ameliorative role of *T. chebula* and *W. somnifera* via redox modulation in subclinically diabetic dogs.

## MATERIALS AND METHODS

### Animal selection

The work was carried out at Division of Medicine, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh during 2015-2016. Subclinically diabetic dogs were identified based on preliminary screening of random blood glucose, fasting blood glucose and Benedict's test for the presence of sugar in urine (Kaneko *et al.* 2008). Animals selected via preliminary tests were further confirmed through glycated haemoglobin estimation by ion exchange chromatography using commercial kit (Coral clinical systems, India) as per Trivelli *et al.* (1971), serum fructosamine estimation by nitrobluetetrazolium reduction method (Sahu and Sarkar, 2008) and serum insulin estimation by ELISA (Bioassay technology laboratory, China).

### Plant extract preparation

*T. chebula* fruits and *W. somnifera* roots were procured and later identified by Dr. R.K. Tiwari, Reader and Head, Department of Dravyaguna, SRM Govt. Ayurvedic College, Bareilly, UttarPradesh. *T. chebula* dried fruit was crushed to separate outer seed husk and the seed was ground into powder. Dried *W. somnifera* roots were also ground into powder form. Extraction of the coarse powder in 70% ethanol was done using Soxhlet extraction apparatus by running 20 cycles at 60°C. After filtration through Whatman No.1 filterpaper, the extract was then dried using evaporator. The dried extract was then crushed into fine powder and was filled in commercially available gelatin capsule covers at the rate of 700 mg powder in each capsule. Capsules were then preserved in a refrigerator at 4°C till usage.

### Experimental study design

The study is approved by Institutional Animal Ethics Committee (IAEC) under institute project IVRI/MED/12-15/ 008. Thirty subclinically diabetic dogs were selected based on the diabetic biomarkers (Kaneko *et al.* 2008) and were divided into five groups of six animals each; Group I was positive control without any treatment, Group II fed with *T. chebula* extract @ 100 mg/kg body weight, Group III fed with *W. somnifera* extract @ 100 mg/kg body weight, Group IV fed with both *T. chebula* extract, and *W. somnifera* extract @ 100mg/kg body weight each and Group V fed with standard antioxidant N-acetyl cysteine @ 10 mg/kg body weight. The antioxidants were administered for 30 days at the above mentioned daily dosages. Six apparently healthy dogs were kept without any treatment as negative control (Group VI). The animals were evaluated based on various diabetic biomarkers and oxidative stress markers on days 0, 15 and 30 of the study.

### Blood sample processing

Blood samples were collected from the cephalic vein of the dogs in sterile vials with sodium fluoride (2 mg/ml) and heparin (0.2 mg/ml) as anticoagulants for the assessment of blood glucose and oxidative stress indices, respectively. About 2ml of blood was collected into another clot activator vial and the serum was garnered for the evaluation of other diabetic biomarkers. Heparinised blood samples were centrifuged at 3000 rpm for 10 min to remove plasma and buffy coat and the red blood cells (RBCs) were harvested. The RBCs were washed thrice using normal saline solution and 10% hemolysate was prepared in chilled distilled water. This hemolysate was used for the estimation of oxidative stress indices namely LPO, SOD and Catalase activities. Reduced glutathione (GSH) level was estimated in 50% RBC suspension prepared by mixing with chilled normal saline (1:1).

### Estimation of diabetic biomarkers

Random blood glucose level and fasting glucose level were estimated using portable glucometer (Accu-Chek, Roche

Diagnostics) and Glucose oxidase and peroxidase (GOD-POD) method, respectively (Trinder 1969). Glycated haemoglobin (GHb), serum fructosamine, and serum insulin concentration were estimated as per the method mentioned above.

**Estimation of oxidative stress indices**

Reduced glutathione (GSH) concentration in RBC suspension was estimated by 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) method as per Prins and Loos (1969). LPO level in the RBC hemolysate was estimated by the method of Placer (1967) based on the formation of a colored complex between the lipid peroxidation products and thiobarbituric acid (TBA). Superoxide dismutase (SOD) activity was estimated by the method described by Madesh and Balasubramanian (1998). And finally, catalase (CAT) activity in hemolysate was appraised by the method of Bergmayer (1983) using $H_2O_2$ as a substrate.

**Statistical analysis**

The values are expressed as mean ± S.E. Data were analyzed by Analysis of Variance followed by the Post-Hoc Tukey test using statistical software package, SPSS 16.0. A value of $P<0.05$ was considered to be statistically significant.

## RESULTS AND DISCUSSION

Effect of different treatments on diabetic biomarkers and oxidative stress indices of subclinically diabetic dogs are presented on (Table 1). All the treatment groups showed significant ($P<0.01$) reduction in random blood glucose, fasting blood glucose, glycated hemoglobin, serum fructosamine and serum insulin concentration than the positive control group after treatment. Among treatment groups, group V (N-acetylcysteine) showed better improvement in random blood glucose (15.19%), fasting blood glucose (15.23%), glycated haemoglobin (15.19%), serum fructosamine (33.5%) and Insulin (20%) levels followed by group IV (15.14%, 14.67%, 13%, 31.03%, 19.74%) treated with combination of *T. chebula* and *W. somnifera*, group II (14.09%, 13.66%, 6.6%, 26.22%, 16.13%) treated with *T. chebula* and group III (8.2%, 8.1%, 3.58%, 25.56%, 6.7%) treated with *W. somnifera*, respectively, on day 30 of the therapy (Fig 1-5). Significant improvement in oxidative biomarkers was noticed in all the four treatment groups on day 30. N-acetylcysteine treated group (group V) showed highest increment in antioxidant systems such as GSH (33.33%), SOD (59%) and CAT (69.57%), followed by group IV (20.45%, 45%, 50%) treated with combination of herbs, group II (8.5%, 36%, 31.96%) treated with *T. chebula* and group III (6.5%, 29.16%, 27.8%) treated with *W. somnifera,* respectively, after therapy (Fig. 6-8). However, better reduction of LPO was exhibited by Group V (32.38%) followed by Group IV (29.94%), Group II (22.86%) and Group III (21.91%), respectively (Fig 9). No significant ($P>0.01$) difference in diabetic as well as oxidative biomarkers were observed between Group IV and Group V animals after treatment, unfolding the possibility of *W. somnifera* and *T. chebula* combination as an alternative to the standard antioxidant drug in management of subclinical diabetes mellitus in dogs.

Varied biological properties of *T. chebula* is attributed by its tannins, vitamin C, gallic acid, anthraquinones, triterpenoids and other miscellaneous compounds (Juang *et al.* 2004). The antioxidant constituents are flavonol glycosides, triterpenoids and chebulin. *T. chebula* extract showed significant antioxidant and antidiabetic effect in alloxan-induced diabetic rats when administered at the dose rate 100mg/kg body weight (Sabu and Kuttan, 2002). About thirty five pharmacologically important chemical constituents were reported from the root of *W. somnifera*. It includes various alkaloids, steroidal lactones, withanolides, withaferins, saponins *etc*. Among them, withaferin A and withanolide D were responsible for most of the pharmacological activity of *W. somnifera* (Mishra *et al.* 2000). It is reported to reduce hepatic lipoperoxidation and stimulate thyroidal activity (Verma, 2010). Immunostimulant property of *Withania somnifera* has also been detected in poultry vaccinated against NewCastle's Disease (Mohanambal *et al*., 2017). Udayakumar et al. (2009) demonstrated the hypoglycaemic effect of *W. somnifera* root ethanolic extract on experimentally induced diabetic rats at 100mg/kg body weight.

N-acetyl cysteine has significant therapeutic potential in ameliorating oxidative stress by means of its GSH precursor effect as well as free radical scavenging ability (Gillissen, *et al.* 1997; Messonier, 2001). This antioxidant effect of N-acetylcysteine would have contributed in improving the diabetic biomarkers of the subclinically diabetic dogs by modulating redox mechanisms and preventing further progression of disease. Dried ripe fruit of *T. chebula* Retz (Combretaceae) is a vital constituent of Triphala, a traditional medicine in Asia (Perry and Metzger1980). Antidiabetic effect of chebulic acid derived from *T. chebula* has been reported by Lee *et al.* (2010). *W. somnifera* (Dunal), a well-known adaptogen, has proven hypocholesterolemic and antioxidant properties (Singh *et al.,* 2011). Also, it possesses anti-inflammatory, anticancer, antistress, immunomodulatory, rejuvenating properties along with endocrine, cardiopulmonary and central nervous system modulatory properties (Mishra *et al.,* 2000). *In vivo* and *In vitro* antioxidant potential of *T. chebula* and *W. somnifera* has already been reported (Singh, 2007; Deepa, 2014). Previous reports also suggest *W. somnifera* as an anti-diabetic agent (Udayakumar, 2009).

Subclinical diabetic/prediabetic animals were apparently healthy with persistently elevated fasting blood glucose concentrations above the reference range, but below the threshold concentration necessary for glycosuria (Rucinsky *et al.* 2010). The present study reports elevated glycated haemoglobin, serum fructosamine, and serum insulin level in prediabetic dogs which is in agreement with

**Table 1:** Effect of different treatments on diabetic biomarkers and oxidative stress indices (Mean±SE).

| Parameters | Day | Group I Positive control (n=6) | Group II *T. chebula* (n=6) | Group III *W. somnifera* (n=6) | Group IV *T. Chebula* & *W. Somnifera* (n=6) | Group V N-acetyl cysteine (n=6) | Group VI Negative control (n=6) |
|---|---|---|---|---|---|---|---|
| Random glucose(mg/dl) | 0 | 162.75±15.0$^{aA}$ | 168.50±6.18$^{aA}$ | 163.50±9.03$^{aA}$ | 158.50±6.99$^{aA}$ | 164.50±11.9$^{aA}$ | 83.75±6.00$^{bA}$ |
| | 15 | 167.00±15.8$^{aA}$ | 151.75±7.28$^{abAB}$ | 155.50±7.27$^{abA}$ | 138.00±1.47$^{bB}$ | 147.00±7.29$^{abA}$ | 82.12±6.00$^{cA}$ |
| | 30 | 157.50±12.8$^{aA}$ | 144.75±6.12$^{abB}$ | 150.00±3.93$^{abA}$ | 134.50±1.75$^{bB}$ | 139.50±4.29$^{abB}$ | 83.99±6.00$^{cA}$ |
| Fasting glucose (mg/dl) | 0 | 148.00±10.1$^{aA}$ | 155.50±6.18$^{aA}$ | 154.25±9.68$^{aA}$ | 146.25±9.83$^{aA}$ | 151.00±8.88$^{aA}$ | 70.5±3.88$^{bA}$ |
| | 15 | 149.00±10.5$^{aA}$ | 138.75±5.34$^{abcB}$ | 145.50±3.96$^{abA}$ | 126.50±3.12$^{cB}$ | 129.75±2.05$^{bcB}$ | 71.5±3.88$^{dA}$ |
| | 30 | 148.50±10.33$^{aA}$ | 134.25±3.75$^{abcB}$ | 141.75±3.25$^{abA}$ | 124.75±0.47$^{cB}$ | 128.00±2.27$^{bcB}$ | 70.8±3.88$^{dA}$ |
| Glycated haemoglobin (%) | 0 | 7.08±0.17$^{aA}$ | 7.02±0.23$^{aA}$ | 7.26±0.08$^{aA}$ | 7.15±0.06$^{aA}$ | 7.24±0.07$^{aA}$ | 5.02±0.05$^{bA}$ |
| | 15 | 7.21±0.09$^{aA}$ | 6.95±0.16$^{aA}$ | 7.09±0.05$^{aAB}$ | 6.92±0.06$^{aA}$ | 7.08±0.08$^{aA}$ | 5.06±0.05$^{bA}$ |
| | 30 | 7.24±0.11$^{aA}$ | 6.55±0.26$^{bB}$ | 7.00±0.05$^{aB}$ | 6.22±0.12$^{bB}$ | 6.14±0.04$^{cB}$ | 4.99±0.04$^{dA}$ |
| Serum fructosamine (mmol/L) | 0 | 3.65±0.16$^{aA}$ | 3.89±0.11$^{aA}$ | 3.95±0.15$^{aA}$ | 3.77±0.06$^{aA}$ | 3.88±0.19$^{aA}$ | 1.92±0.03$^{bA}$ |
| | 15 | 3.69±0.18$^{aA}$ | 3.76±0.12$^{aA}$ | 3.82±0.17$^{aA}$ | 3.61±0.07$^{aA}$ | 3.66±0.20$^{aA}$ | 1.97±0.03$^{bA}$ |
| | 30 | 3.77±0.20$^{aA}$ | 2.87±0.16$^{bB}$ | 2.94±0.06$^{bB}$ | 2.60±0.08$^{bB}$ | 2.58±0.08$^{bB}$ | 1.96±0.03$^{cA}$ |
| Insulin (IU/L) | 0 | 33.18±0.94$^{aA}$ | 32.48±0.57$^{aA}$ | 34.17±0.82$^{aA}$ | 32.37±0.59$^{aA}$ | 33.09±0.97$^{aA}$ | 19.19±0.70$^{bA}$ |
| | 15 | 33.29±0.83$^{aA}$ | 29.53±0.61$^{bcB}$ | 33.10±0.76$^{aA}$ | 28.25±0.42$^{cB}$ | 30.33±0.65$^{bB}$ | 20.13±0.56$^{dA}$ |
| | 30 | 33.37±0.79$^{aA}$ | 27.24±0.20$^{bC}$ | 31.88±0.71$^{aA}$ | 25.98±0.21$^{bC}$ | 26.46±0.42$^{bC}$ | 19.57±0.55$^{cA}$ |
| GSH (µmol/ml of packed RBC) | 0 | 0.45±0.01$^{bA}$ | 0.47±0.02$^{bA}$ | 0.46±0.02$^{bA}$ | 0.44±0.02$^{bB}$ | 0.42±0.01$^{bB}$ | 0.61±0.01$^{aA}$ |
| | 15 | 0.43±0.01$^{cA}$ | 0.49±0.02$^{bcA}$ | 0.47±0.02$^{bcA}$ | 0.50±0.00$^{bA}$ | 0.51±0.02$^{bA}$ | 0.62±0.01$^{aA}$ |
| | 30 | 0.42±0.00$^{dA}$ | 0.51±0.01$^{cdA}$ | 0.49±0.02$^{cdA}$ | 0.53±0.01$^{bcA}$ | 0.56±0.00$^{bA}$ | 0.61±0.01$^{aA}$ |
| LPO (nmol MDA/mg Hb) | 0 | 5.92±0.20$^{aA}$ | 5.73±0.19$^{aA}$ | 5.43±0.43$^{aA}$ | 5.71±0.40$^{aA}$ | 5.99±0.40$^{aA}$ | 3.99±.07$^{bA}$ |
| | 15 | 5.92±0.21$^{aA}$ | 5.22±0.15$^{bA}$ | 5.02±0.14$^{bAB}$ | 4.98±0.15$^{bA}$ | 4.77±0.15$^{bB}$ | 3.97±0.07$^{cA}$ |
| | 30 | 5.87±0.21$^{aA}$ | 4.42±0.16$^{bB}$ | 4.24±0.05$^{bcB}$ | 4.00±0.11$^{cB}$ | 4.05±0.08$^{bcB}$ | 3.95±0.07$^{cA}$ |
| SOD (µmolMTTformazan/ mg Hb) | 0 | 0.23±0.01$^{bA}$ | 0.25±0.01$^{bC}$ | 0.24±0.01$^{bB}$ | 0.20±0.01$^{bC}$ | 0.22±0.02$^{bB}$ | 0.38±0.01$^{aA}$ |
| | 15 | 0.23±0.01$^{dA}$ | 0.30±0.00$^{cB}$ | 0.26±0.00$^{dB}$ | 0.25±0.00$^{dB}$ | 0.34±0.00$^{bA}$ | 0.36±0.01$^{aA}$ |
| | 30 | 0.22±0.00$^{dA}$ | 0.34±0.01$^{abA}$ | 0.31±0.01$^{bcA}$ | 0.29±0.01$^{cA}$ | 0.35±0.01$^{aA}$ | 0.37±0.01$^{aA}$ |
| CAT (K/mg Hb) | 0 | 3.97±0.14$^{bA}$ | 3.41±0.11$^{bC}$ | 3.99±0.32$^{bB}$ | 3.56±.40$^{bC}$ | 3.32±0.28$^{bC}$ | 6.93±0.10$^{aA}$ |
| | 15 | 4.04±0.09$^{dA}$ | 4.04±0.14$^{dB}$ | 4.35±0.21$^{cdB}$ | 4.52±0.12$^{cB}$ | 5.04±0.10$^{bB}$ | 6.92±0.10$^{aA}$ |
| | 30 | 3.88±0.11$^{eA}$ | 4.50±0.14$^{cA}$ | 5.10±0.07$^{dA}$ | 5.34±0.12$^{bcA}$ | 5.63±0.03$^{bA}$ | 6.92±0.10$^{aA}$ |

Values with superscript a,b,c,d differ significantly (P<0.01) in same row; Values with superscript A, B, C differ significantly (P<0.01) in same column

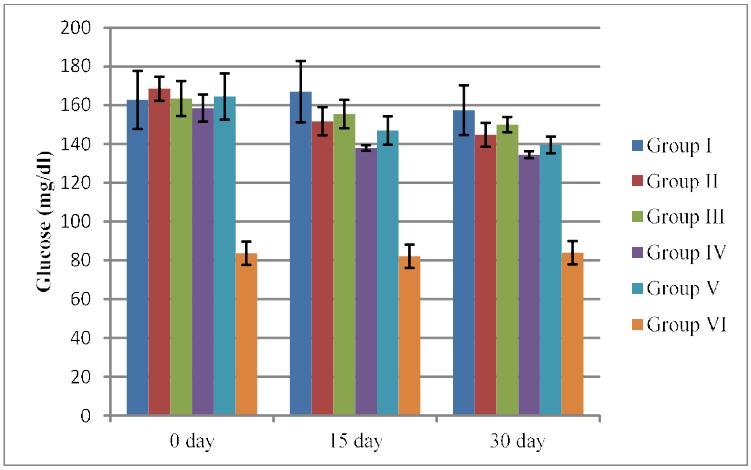


**Fig 1:** Effect of different treatments on random blood glucose (mg/dl).

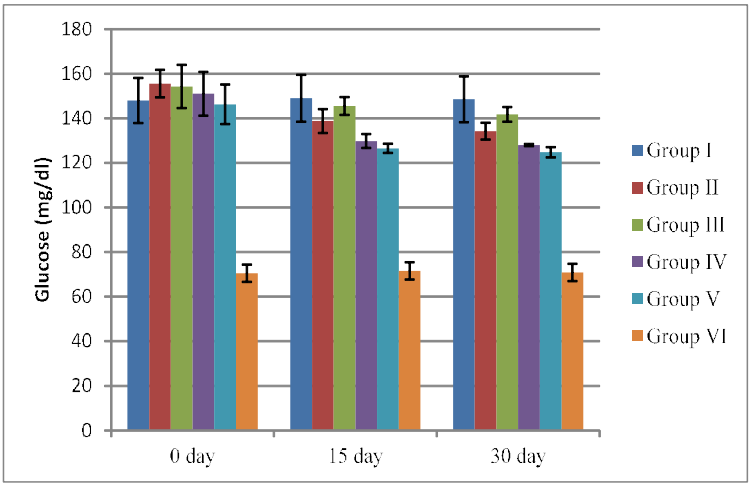


**Fig 2:** Effect of different treatments on Fasting blood glucose (mg/dl).

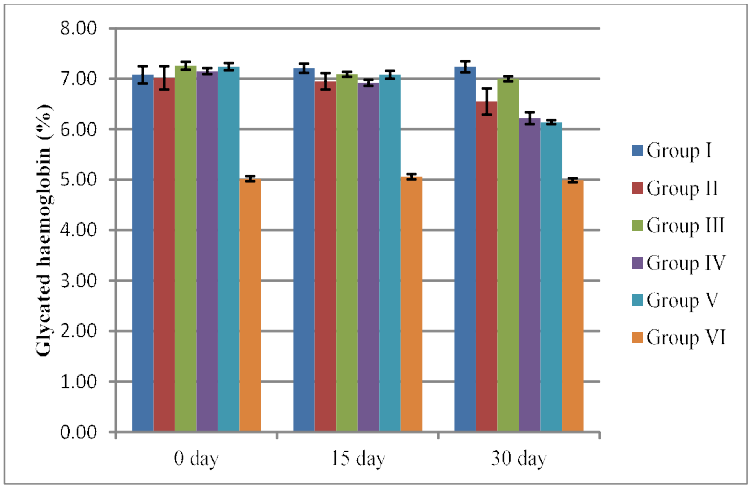


**Fig 3:** Effect of different treatments on glycated haemoglobin (%).

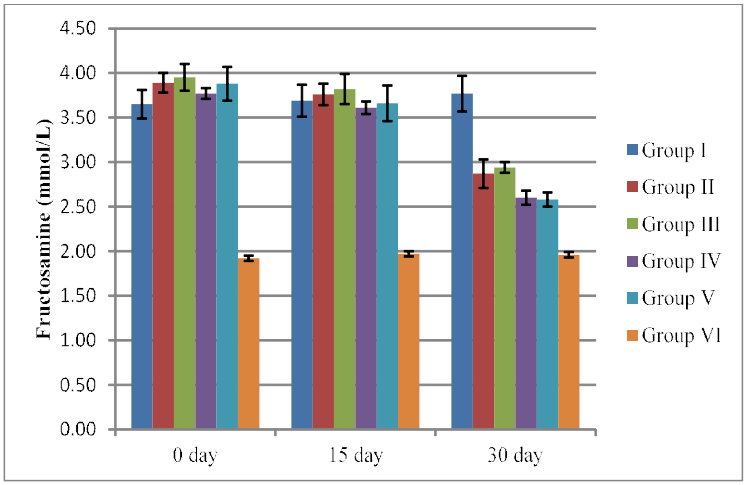


**Fig 4:** Effect of different treatments on serum fructosamine (mmol/L).

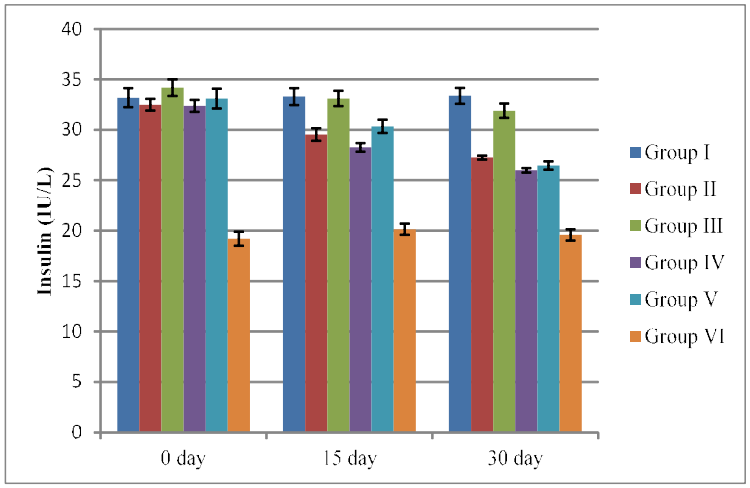


**Fig 5:** Effect of different treatments on serum Insulin (IU/L).

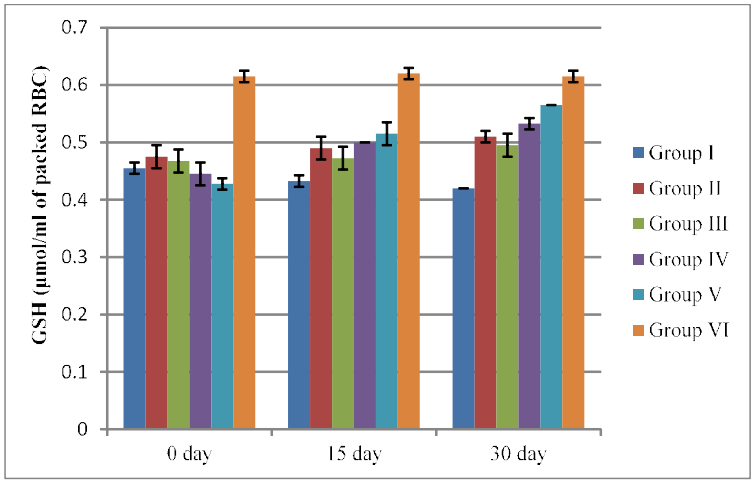


**Fig 6:** Effect of different treatments on GSH (µmol/ml of packed RBC).

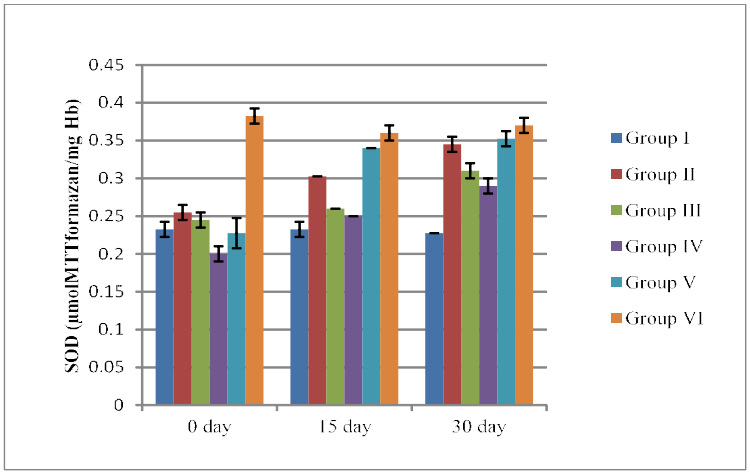


**Fig 7:** Effect of different treatments on SOD (µmol MTT formazan/mg Hb).

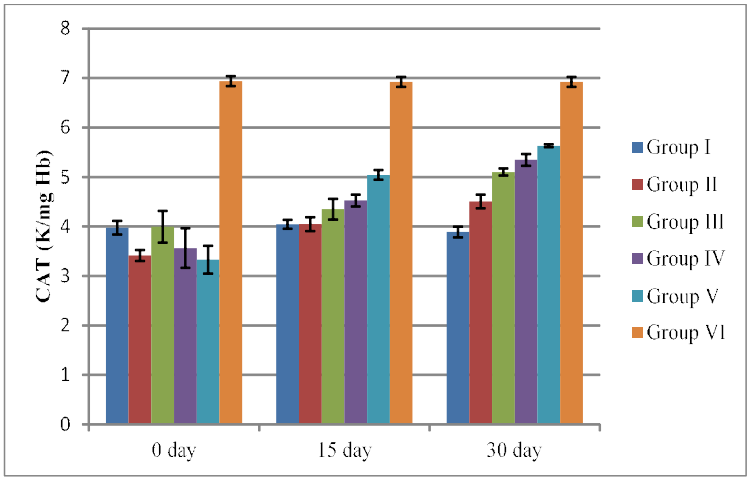


**Fig 8:** Effect of different treatments on CAT (K/mg Hb).

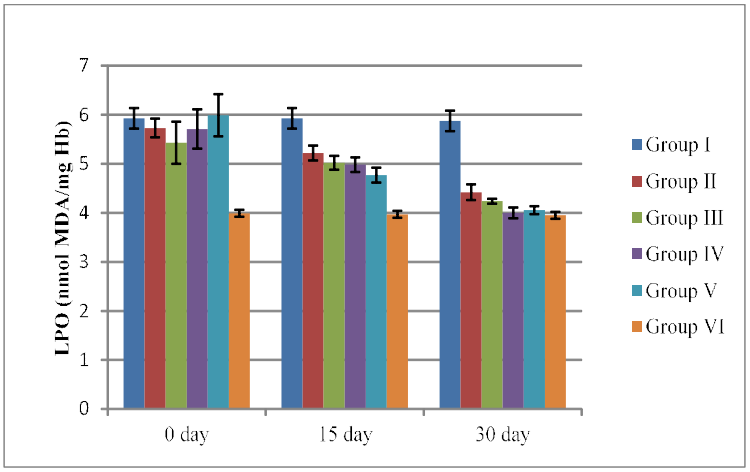


**Fig 9:** Effect of different treatments on LPO (nmol MDA/mg Hb).

Deepa (2014) and Kaneko (2008). Elevated glycated hemoglobin (HbA1c) is important predictive biomarker of chronic hyperglycemia, whereas, the serum fructosamine provide the previous 2-3 weeks' estimate (Mared *et al.* 2012). Recently canine HbA1c has shown least correlation with haematological parameters and therefore it can be suggested as a reliable biomarker of canine diabetes (Neumann, 2020). Canine diabetes mellitus is usually compared to latent autoimmune diabetes mellitus in human beings; initial hyperstimulation of pancreatic â cells followed by insulin deficient phase due to â cell exhaustion (Shanik *et al.* 2008). The present study explains that the antioxidant therapy can curb this pathological mechanism by modulating oxidative metabolisms preventing exhaustive damage to â cells. Similarly, the modulation of host immune mechanisms by the antioxidant N-acetylcysteine in Th1 cytokine dominant disease conditions was previously suggested by Ajith *et al.* (2017). Also, the human form of IDDM is usually considered as a Th1 dominant condition with increased expression of Th1 cytokines (Eizirik *et al.* 2009). Hence, early detection and strategic management of subclinical DM using better antioxidants is necessary for preventing its progression to clinical form, improving lifespan and ensuring better quality life.

## CONCLUSION

Subclinical diabetes mellitus in dogs is a disease condition which needs more attention from clinical veterinary practitioners. Significant oxidative impairments were observed in even subclinical stages of diabetes mellitus and may be elaborated in the pathogenesis of clinical diabetes. N- acetylcysteine, *T. chebula* and *W. somnifera* ameliorate oxidative stress in subclinical diabetes mellitus and prevent further evolution of the disease. Hence, the current study established the antidiabetic potential of *W. somnifera* and *T. chebula* via improved oxidant-antioxidant profile. Further, they prevented damages on the body system resulted from appropriate glucose utilization, which itself is a triggering force for free radical generation and unstable body system. The present study emphasizes the early detection of subclinical form of diabetes and stress the need for antioxidant therapy in preventing progression of the clinical disease.

## REFERENCES


Ajith, Y., Dimri, U., Dixit, S.K., Singh, S.K., Gopalakrishnan, A., Madhesh, E., Rajesh, J.B. and Sangeetha, S.G. (2017). Immunomodulatory basis of antioxidant therapy and its future prospects: An appraisal. Inflammopharmacology. 25: 487-98. DOI: 10.1007/s10787-017-0393-5

Bajpai, M., Pande, A., Tewari, S. K. and Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. International Journal of Food sciences and Nutrition. 56(4): 287-91.

Bergmayer, H.U. (1983). UV method of catalase assay. Methods of enzymatic analysis. 3: 273.

Buysschaert, M. and Bergman, M. (2011). Definition of prediabetes. Medical Clinics. 95(2): 289-97.

Cheng, H.Y., Lin, T.C., Yu, K.H., Yang, C.M. and Lin, C.C. (2003). Antioxidant and free radical scavenging activities of *Terminalia chebula*. Biological and Pharmaceutical Bulletin. 26(9): 1331-5.

Coldiron Jr, A.D., Sanders, R.A. and Watkins III, J.B. (2002). Effects of combined quercetin and coenzyme Q10 treatment on oxidative stress in normal and diabetic rats. Journal of Biochemical and Molecular Toxicology. 16(4): 197-202.

Das, S. and Lodh, C. (2015). Epidemiology of canine diabetes mellitus in West Bengal. Indian Journal of Canine Practice. 7(1): 38-41.

Deepa, P.M. (2014) Exploring subclinical/clinical Diabetes mellitus with respect to Biomarkers and alleviation through antioxidant, immunomodulator and stem cells in animals (Thesis). Uttar Pradesh. Indian Veterinary Research Institute.

Devi, G., Dimri, U., Jatav, R.S., Yatoo, M.I., Deepa, P.M., Gopalakrishnan, A. and Ajith, Y. (2016). Study on prevalence of subclinical/clinical diabetes in dogs of Uttarpradesh, Uttarakhand and Kerala. National Journal of Life Science. 13: 73-75.

Eizirik, D.L., Colli, M.L. and Ortis, F. (2009). The role of inflammation in insulitis and â-cell loss in type 1 diabetes. Nature Reviews Endocrinology. 5(4): 219.

Florence, N.T., Benoit, M.Z., Jonas, K., Alexandra, T., Désiré, D.D.P., Pierre, K. and Théophile, D. (2014). Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 151(2): 784-90.

Gillissen, A., Jaworska, M., Orth, M., Coffiner, M., Maes, P., App, E. M., Cantin, A.M. and Schultze-Werninghaus, G. (1997). Nacystelyn, a novel lysine salt of N-acetylcysteine, to augment cellular antioxidant defence *in vitro*. Respiratory Medicine. 91(3): 159-68.

Giugliano, D., Ceriello, A. and Paolisso, G. (1996) Oxidative stress and diabetic vascular complications. Diabetes Care. 19: 257-67.

Ionut, V., Liu, H., Mooradian, V., Castro, A.V.B., Kabir, M., Stefanovski, D., Zheng, D., Kirkman, E.L. and Bergman, R.N. (2010). Novel canine models of obese prediabetes and mild type 2 diabetes. American Journal of Physiology-Endocrinology and Metabolism. 298(1): E38-E48.

Juang, L. J., Sheu, S. J. and Lin, T. C. (2004). Determination of hydrolyzable tannins in the fruit of *Terminalia chebula* Retz. by high performance liquid chromatography and capillary electrophoresis. Journal of Separation Science. 27(9): 718-24.

Kaneko, J.J., Harvey, J. W. and Bruss, M.L. (Eds.). (2008). *Clinical biochemistry of domestic animals*. Academic press. Kumar, G.P.S., Arulselvan, P., Kumar, D.S. and Subramanian, S.P. (2006). Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. Journal of Health Science. 52(3): 283-91.

Lee, H.S., Koo, Y.C., Suh, H.J., Kim, K.Y. and Lee, K.W. (2010). Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation endproduct-induced endothelial cell dysfunction. Journal of Ethnopharmacology. 131(3): 567-74.

Madesh, M. and Balasubramanian, K.A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics. 35(3): 184-8.

Mared, M., Catchpole, B., Kämpe, O. and Fall, T. (2012). Evaluation of circulating concentrations of glucose homeostasis biomarkers, progesterone, and growth hormone in healthy Elkhounds during anestrus and diestrus. American. Journal of Veterinary Research. 73(2): 242-7.

Marmor, M., Willeberg, P., Glickman, L.T., Priester, W.A., Cypess, R.H. and Hurvitz, A.I. (1982). Epizootiologic patterns of diabetes mellitus in dogs. American Journal of Veterinary Research. 43(3): 465-70.

Mattheeuws, D., Rottiers, R., Kaneko, J.J. and Vermeulen, A. (1984). Diabetes mellitus in dogs: relationship of obesity to glucose tolerance and insulin response. American Journal of Veterinary Research. 45(1): 98-103.

Messonnier, S. (2001). Natural Health Bible for Dogs and Cats: Your AZ Guide to Over 200 Conditions, Herbs, Vitamins, and Supplements. Three Rivers Press.

Mishra, L.C., Singh, B.B. and Dagenais, S. (2000). Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): a review. Alternative medicine review. 5(4): 334-46.

Mohanambal, K., Selvaraju, G., Palanivel, K.M. and Rajeswar, J.J., (2018). Evaluation of immunopotentiating effect of medicinal plant products in commercial layer flock vaccinated against Newcastle disease. Indian Journal of Animal Research. 52(9): 1343-1346.

Nelson, R.W. and Reusch, C.E. (2014). Animal models of disease: classification and etiology of diabetes in dogs and cats. Journal of Endocrinology. 222(3): T1-T9.

Nelson, R.W. (2000). Oral medications for treating diabetes mellitus in dogs and cats. Journalof Small Animal Practice. 41: 486-90.

Neumann, S. (2020). Reference Interval of Hemoglobin A1c and Influence of Hematological Parameters on Its Serum Concentration in Dogs. Veterinary Medicine International.

Pari, L. and Latha, M. (2002). Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Medical Journal. 43(12): 617-21.

Perry, L.M. and Metzger, J. (1980). Medicinal plants of east and southeast Asia: attributed properties and uses. MIT press. Placer, Z. (1967). Lipoperoxydationssysteme von Subzellularpartikeln. Food/Nahrung. 11(7 8): 623-30.

Prins, H.K. and Loos, J.A. (1969). Glutathione. In *Biochemical methods in red cell genetics* (pp. 115-137). Academic Press New York.

Raffan, E., Smith, S.P., O'Rahilly, S. and Wardle, J. (2015). Development, factor structure and application of the Dog Obesity Risk and Appetite (DORA) questionnaire. Peer Journal. 3: 1278.

Rand, J.S. (2020). Diabetes Mellitus in Dogs and Cats. Clinical Small Animal Internal Medicine (pp. 93-102).

Rucinsky, R., Cook, A., Haley, S., Nelson, R., Zoran, D.L. and Poundstone, M. (2010). AAHA diabetes management guidelines for dogs and cats. Journal of the American Animal Hospital Association. 46(3): 215-24.

Sabu, M.C. and Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of Ethnopharmacology. 81(2): 155-60.

Sahu, A. and Sarkar, P.D. (2008). Comparative study of NBT reduction method for estimation of glycated protein (serum fructosamine) with glycated HbA1c estimated on DCA 2000+ analyzer (immunoagglutination inhibition). Indian Journal of Physiology and Pharmacology. 52(4): 408-12.

Shanik, M.H., Xu, Y., Škrha, J., Dankner, R., Zick, Y. and Roth, J. (2008). Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse. Diabetes care. 31(Supplement 2): S262-S268.

Singh, S.K., Dimri, U., Kataria, M. and Kumari, P. (2011). Ameliorative activity of *Withania somnifera* root extract on paraquat-induced oxidative stress in mice. Journal of Pharmacology and Toxicology. 6(4): 433-39.

Singh, S.K. (2007). *In vitro* disease resistance and free radical scavenging potential of certain indigenous bio-organics and *in vivo* corroboration in mice and canine [Thesis]. Uttar Pradesh. Indian Veterinary Research Institute.

Temelkova-Kurktschiev, T. and Stefanov, T. (2012). Lifestyle and genetics in obesity and type 2 diabetes. Experimental and Clinical Endocrinology and Diabetes. 120(1): 1-6.

Trinder, P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of Clinical Pathology, 22(2), 158-61.

Trivelli, L.A., Ranney, H.M. and Lai, H.T. (1971). Hemoglobin components in patients with diabetes mellitus. New England Journal of Medicine. 284(7): 353-357.

Udayakumar, R., Kasthurirengan, S., Mariashibu, T.S., Rajesh, M., Anbazhagan, V.R., Kim, S.C., Ganapathi, A. and Choi, C.W. and Choi, C. W. (2009). Hypoglycaemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan-induced diabetic rats. International Journal of Molecular Sciences. 10(5): 2367-82.

Verma, K.C. (2010). Ashwagandha (*Withania somnifera Dunal*): wonder medicinal plant. Agricultural Reviews. (31): 292-297.

Vessal, M., Hemmati, M. and Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comparative Biochemistry and Physiology Part C: Toxicology amd Pharmacology. 135(3): 357-64.

Wilkinson J.S. (1960). Spontaneous diabetes mellitus. Veterinary Record. 72: 548-5.