IJBPAS, February, 2021, 10(2): 748-758

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PROTECTIVE ROLE OF COMBINATION OF HERBAL EXTRACT AGAINST STREPTOZOTOCIN INDUCED *IN VIVO* ANTIOXIDANT ACTIVITY OF MALE WISTAR RATS

RAGHAVENDRA PAI[*1], SANIL KUMAR R[2] AND SANIL KUMAR[3]

1, 2: Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, India

3: Accenture, Bangalore, India

***Corresponding Author: Raghavendra Pai: E Mail: paiannu143@gmail.com**

Received 29th July 2020; Revised 18th Aug. 2020; Accepted 16th Sept. 2020; Available online 1st Feb. 2021

https://doi.org/10.31032/IJBPAS/2021/10.2.5365

ABSTRACT

Background: To investigate the effect of various extracts of combination of herbal drug on the occurrence of oxidative stress in the organ of rats during diabetes by measuring the extent of oxidative damage as well as the status of the antioxidant defense system.

Methods: Various extracts of combination of herbal drug was administered orally and different doses of the extract on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidaase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH) were estimated in streptozotocin-induced diabetic rats. Glibenclamide was used as standard reference drug.

Results: Significant increase in the activities of plasma insulin, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and vitamin C and E were observed in brain on treatment with 200 mg/kg body weight of combination of herbal extract and glibenclamide for 3 weeks.

Conclusions: Since the study of induction of the antioxidant enzymes is considered to be a reliable marker for evaluating the antiperoxidative efficacy of the medicinal plant, these findings are suggestions of possible antiperoxidative role played by combination of herbal extract.

Keywords: Streptozotocin, *in vivo* antioxidant activity, male wistar rats

INTRODUCTION

Background

The neurological significances of diabetes mellitus in the Central Nervous System (CNS) are now receiving larger consideration. Cognitive deficits, along with morphological and neurochemical modifications demonstrate that the neurological difficulties of diabetes are not limited to peripheral neuropathies **[1]**. The central difficulties of antihypoglycaemia also include the potentiation of neuronal damage detected following hypoxic/ischemic events, as well as stroke **[2]**. Glucose consumption is diminished in the brain during diabetes **[2]**, provided that a potential mechanism for increased susceptibility to acute pathological measures.

Oxidative stress, leading to an increased production of reactive oxygen species (ROS), as well as lipid peroxidation, is increased in diabetes **[3]** and also by stress in euglycemic animals **[4]**. Similarly, oxidative damage in rat brain is increased by experimentally induced hyperglycemia **[5]**. Under experimental conditions, hyperglycaemia dramatically increases neuronal alterations and glial cell damage caused by temporary ischaemia **[6]**. Several lines of evidence indicate that the modified oxidative state induced by chronic hyperglycaemia **[7]** may contribute to nervous tissue damage: free radical species impair both the central nervous system, attacking neurons and Schwann cells **[8]** and the peripheral nerves **[9]** because of their high polyunsaturated lipid content, Schwann cells and axons are particularly sensitive to oxygen free radical damage: lipid peroxidation may increase cell membrane rigidity, damaging cell function.

Intensifications in superoxide production are observed in the serum of Type 1 diabetic patients, increases that are reduced with enhanced glycemic control **[10]** lipid peroxidation products are also increased in the organs of Type 1 diabetic rats **[11]** and Type 2 diabetic mice **[8]**. Diabetes and stress mediated increases in oxidative stress, as well as decreases in antioxidant activity, may make the brain more susceptible to subsequent pathological events. Nowadays, the use of complementary/alternative medicine and especially the consumption of botanicals have been increasing rapidly worldwide, mostly because of the supposedly less frequent side effects when compared to modern western medicine **[12]**.

The herbal seed of *Trigonella foenum-graecum* L. (fenugreek) is widely used for its medicinal properties all over the

world and it is a very important spice in Indian culture. Around 260 species of *Trigonella* are diffused worldwide **[13-17]**. The genus name *Trigonella* means 'tri-angled', may be because of triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay'. It is an annual crop and dicotyledonous plant belonging to the subfamily *Papilionaceae,* family *Fabaceae.* Another herbal root of *Withania somnifera* Linn commonly known as Ashwagandha, Indian ginseng, winter cherry is an important medicinal plant in the *solanaceae* **[18-22]** family that has been used in ayurvedic and indigenous medicine for more than 3,000 years. Ashwagandha in Sanskrit means "horse's smell" probably originated from the odour of its root, which resembles that of sweaty horse. The species name *somnifera* means "sleep-making" in Latin, attributed to sedating properties.

MATERIALS AND METHODS

Animals

Adult male albino Wistar rats (8 weeks), weighing 180-200 g bred in the AKCP, Krishnankoil, were used. All animal experiments were approved by the ethical committee (AKCP/IAEC/09/2019-2020), AKCP and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were fed *ad libitum* with normal laboratory pellet diet (Hindustan Lever Ltd., India) and water**.** Animals were maintained under a constant 12 h light and dark cycle and at an environmental temperature of 21-23°C.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade.

Collection and authentication of plant material

The herbal drug was collected from Madurai, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu, India. The herbal drug powdered materials were put away in a hermetically sealed holder. The herbal drug was shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Preparation of plant extract

The equal amount of seed of *Trigonella foenum graecum* and root of *Withania somnifera* herbal drug were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was

evaporated by rotavapor to obtained viscous semi solid masses.

Experimental design

Study protocol for dose fixation

The animals were randomly divided into 7 groups of six animals each. Feeding was started by 9.30 a.m. and various extracts and glibenclamide (suspended in 0.5% CMC) were administered post orally using intragastric tube at 10.30 a.m. The duration of treatment was 21 days. After 21 days of treatment, the animals were fasted for 12 h, sacrificed by cervical dislocation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of plasma glucose and ethylenediamine tetra acetic acid (EDTA) for the estimation of various biochemical parameters. Tissue (liver, kidney, heart and brain) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers. Erythrocytes were also prepared for the estimation of various biochemical preparations.

Group I :	**Normal control**
Group II :	**Diabetic control**
Group III :	**Diabetic + Ethyl acetate extract 200 mg**
Group IV :	**Diabetic + Ethyl acetate extract 200 mg+Glibenclamide (600µg/kg. b.wt.)**
Group V :	**Diabetic + Ethanolic extract 200 mg**
Group VI :	**Diabetic + Ethanolic extract 200 mg+Glibenclamide (600 µg/kg. b.wt.)**
Group VII:	**Diabetic + Glibenclamide (600 µg/kg b.wt.)**

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (40 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg **[23]**. 48 h after streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e with blood glucose of 200-300 mg/dl) were taken for the experiment.

Biochemical Analysis

Catalase (CAT) was assayed by the method of Sinha **[23]**. Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar *et al*., **[24]**. Glutathione peroxidase (GPx) activity was measured by the method described by Rotruck *et al.,* **[25]**. Reduced glutathione (GSH) and glutathione-S-transferase (GST) activity were determined spectrophotometrically by the method of Ellman **[26]** and Habig *et al.*, **[27]** respectively.

Statistical analysis

All data were expressed as mean ± S.D of number of experiments (n = 6). The statistical significance was evaluated by one-

way analysis of variance (ANOVA) using SPSS version 7.5 (SPSS, Cary, NC, USA) and the individual comparison were obtained by Duncan's Multiple Range Test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups **[32]**.

RESULTS

Table 1-4 shows the effect of combination of herbal drug on free radical production, the activities of SOD, CAT, GPx,, GSH and Vitamin C & E were measured **(Table 5 and 6)**. They presented significant increases in high dose of ethyl acetate and ethanolic extract treatment when compared with diabetic control rats. The extent of increase was higher in groups treated with ethanolic extract than ethyl acetate extracts treated groups.

Table 1: Effect of combination of herbal extracts on SOD activity in the tissues of normal and STZ-diabetic rats

Name of the group	Superoxide dismutase (U^*/mg protein)		
	Brain	Kidney	Liver
Group I	10.14 ± 0.65^a	15.81 ± 0.95^a	9.75 ± 0.66^a
Group II	3.22 ± 0.26^b	9.32 ± 0.74^b	4.08 ± 0.35^b
Group III	8.45 ± 0.74^a	13.02 ± 1.18^a	7.76 ± 0.65^a
Group IV	9.38 ± 0.58^c	13.69 ± 0.85^c	8.30 ± 0.50^c
Group V	8.82 ± 0.86^a	13.52 ± 1.22^a	7.98 ± 0.72^a
Group VI	9.68 ± 0.64^c	14.12 ± 1.10^c	8.45 ± 0.68^c
Group VII	9.34 ± 0.56^d	13.76 ± 1.24^c	8.26 ± 0.56^d

Table 2: Effect of combination of herbal extracts on CAT activity in the tissues of normal and STZ-diabetic rats

Name of the group	Catalase (U^*/mg protein)		
	Brain	Kidney	Liver
Group I	2.75 ± 0.18^a	40.22 ± 2.86^a	84.12 ± 5.28^a
Group II	0.90 ± 0.05^b	23.46 ± 1.82^b	40.94 ± 3.34^b
Group III	1.88 ± 0.25^a	30.68 ± 2.60^a	60.40 ± 4.93^a
Group IV	2.36 ± 0.18^c	34.90 ± 2.56^c	75.78 ± 4.66^c
Group V	2.15 ± 0.24^a	32.28 ± 2.43^a	69.36 ± 5.58^a
Group VI	2.48 ± 0.16^c	36.35 ± 2.14^c	78.10 ± 4.45^c
Group VII	2.39 ± 0.08^d	35.24 ± 2.38^d	76.15 ± 5.41^d

Table 3: Effect of combination of herbal extracts on GPx activity in the tissues of normal and STZ-diabetic rats

Name of the group	GPx (U^*/mg protein)		
	Brain	Kidney	Liver
Group I	6.41 ± 0.52^a	8.20 ± 0.56^a	9.95 ± 0.68^a
Group II	3.10 ± 0.28^b	4.32 ± 0.35^b	4.70 ± 0.32^b
Group III	4.56 ± 0.62^a	6.86 ± 0.52^a	8.24 ± 0.74^a
Group IV	5.28 ± 0.38^c	7.37 ± 0.38^c	9.02 ± 0.58^c
Group V	5.12 ± 0.56^a	7.22 ± 0.46^a	8.45 ± 0.86^a
Group VI	5.65 ± 0.48^c	7.97 ± 0.48^c	9.26 ± 0.66^c
Group VII	5.32 ± 0.44^d	7.40 ± 0.58^c	8.92 ± 0.58^c

Table 4: Effect of combination of herbal extracts on GSH activity in the tissues of normal and STZ-diabetic rats

Name of the group	Plasma GSH (mg/dL)	GSH (mg/100 g wet tissue)		
		Brain	Kidney	Liver
Group I	27.23 ± 2.08^{a}	33.98 ± 2.18^{a}	33.14 ± 2.23^{a}	42.06 ± 3.45^{ab}
Group II	12.46 ± 0.88^{b}	16.45 ± 2.14^{c}	18.46 ± 1.28^{b}	23.98 ± 2.12^{c}
Group III	22.08 ± 2.30^{a}	22.68 ± 2.42^{b}	22.38 ± 2.32^{a}	30.84 ± 3.35^{a}
Group IV	23.96 ± 1.52^{c}	28.86 ± 1.78^{d}	30.36 ± 1.46^{c}	38.45 ± 2.56^{d}
Group V	23.33 ± 2.20^{a}	24.16 ± 2.24^{b}	26.64 ± 2.15^{a}	32.78 ± 2.90^{a}
Group VI	25.18 ± 1.36^{c}	30.26 ± 1.55^{d}	31.06 ± 1.85^{c}	39.56 ± 2.78^{d}
Group VII	24.10 ± 1.54^{d}	29.18±2.33^{e}	30.42 ± 2.04^{d}	38.95±2.28^{e}

Table 5: Effect of combination of herbal extracts on vitamin C in the plasma and tissues of normal and STZ-diabetic rats

Name of the group	Plasma vitamin C (mg/dL)	Vitamin C (µg/mg protein)		
		Brain	Kidney	Liver
Group I	2.13 ± 0.18^{a}	1.69 ±0.14^{a}	0.85 ± 0.09^{a}	0.96 ± 0.08^{a}
Group II	0.76 ± 0.06^{b}	0.75 ± 0.07^{b}	0.53 ± 0.05^{c}	0.49 ± 0.05^{b}
Group III	1.54 ± 0.18^{a}	1.04± 0.18^{a}	0.58 ± 0.09^{b}	0.62 ± 0.012^{a}
Group IV	1.94 ± 0.10^{c}	1.52 ± 0.07^{c}	0.74 ± 0.06^{d}	0.80 ± 0.06^{c}
Group V	1.75 ± 0.16^{a}	1.18± 0.14^{a}	0.62 ± 0.10^{b}	0.77 ± 0.08^{a}
Group VI	2.04 ± 0.14^{c}	1.61 ± 0.08^{c}	0.82 ± 0.07^{d}	0.90 ± 0.06^{c}
Group VII	1.98 ± 0.16^{d}	1.57 ± 0.12^{c}	0.78 ± 0.06^{d}	0.84 ± 0.08^{d}

Table 6: Effect of combination of herbal extracts on vitamin E in the plasma and tissues of normal and STZ-diabetic rats

Name of the group	Plasma vitamin E (mg/dL)	Vitamin E (µ mol/mg protein)		
		Brain	Kidney	Liver
Normal	1.48 ±0.12^{a}	0.69 ± 0.08^{a}	0.63 ± 0.23^{a}	0.78 ± 0.14^{a}
Group I	0.64 ±0.40^{b}	0.26 ± 0.05^{b}	0.22 ± 0.04^{b}	0.29 ± 0.04^{b}
Group II	0.98 ±0.84^{a}	0.35 ± 0.05^{a}	0.30 ± 0.05^{a}	0.36 ± 0.06^{a}
Group III	1.29 ±0.86^{c}	0.59 ± 0.06^{c}	0.43± 0.04^{c}	0.58 ± 0.05^{c}
Group IV	1.14 ±0.80^{a}	0.43 ± 0.04^{a}	0.38 ± 0.06^{a}	0.45 ± 0.07^{a}
Group V	1.40 ±0.92^{c}	0.62 ± 0.08^{c}	0.52± 0.05^{c}	0.67 ± 0.05^{c}
Group VI	1.32 ±0.14^{c}	0.60 ± 0.05^{d}	0.45 ± 0.04^{c}	0.60 ± 0.06^{d}

DISCUSSION

The streptozotocin diabetic rat serves as an excellent model to study the molecular, cellular and morphological changes in organs induced by stress during diabetes **[7]**. Under normal conditions, the generation of free radicals or of active species in the brain, as in other tissues, is maintained at extremely low levels **[4]**. Diabetes also contributes to cerebrovascular difficulties, decreases in cerebral blood flow, interruption of the blood brain barrier and cerebral edema **[5]**. All of these neurochemical and neurophysiological changes ultimately contribute to the long-term complications associated with diabetes, including morphological abnormalities, cognitive impairments and increased vulnerability to pathophysiological event **[6]**.

In the present study, treatment with various extract of combination of herbal drug showed significant antihyperglycaemic activity. The antihyperglycaemic activity of this plant may be, at least in part, through release of insulin from the pancreas in view

of the measured rise in the plasma insulin concentrations.

The reformed balance of the antioxidant enzymes instigated by decrease in CAT, SOD, GPx, GST, GSH and Vitamins C & E activities may be responsible for the insufficiency of the antioxidant defenses in combating ROS-mediated damage. The decreased activities of CAT, SOD may be a retort to increased production of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation **[5]**. Treatment with various extract of combination of herbal drug increased the activity of enzymes and may help to control free radicals, as herbal drugs has been reported to be rich in alkaloids, flavonoids and terpenoids **[16, 17]**, well-known antioxidants, which scavenge the free radicals generated during diabetes. The increase in SOD activity may defend CAT and GPx against inactivation by $O_2^{\cdot-}$ anions as these anions have been shown to deactivate CAT and GPx **[31]**.

Under *in vivo* experiments, GSH acts as an antioxidant and its reduction was reported in diabetes mellitus **[32]**. We have observed significant decrease in GSH levels in organ during diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress **[33]**. The diminution of GSH content may also lower the GST activity **[34]**. Depression in GPx activity was also observed in tissues of diabetic rats. GPx has been shown to be an important adaptive response to condition of increased peroxidative stress **[33]**. The significant increase in GSH content and GSH dependent enzymes GPx and GST in diabetic rats treated with various extract of combination of herbal drug indicates an adaptive mechanism in response to oxidative stress. The increased vitamin C and E content in the organs of the rats treated with various extract of combination of herbal drug and glibenclamide may be a factor responsible for inhibition of lipid peroxidation. The elevated level of vitamin C and E protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to streptozotocin **[34]**.

It may be concluded that in diabetes, organ tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The above observation shows that the various extract of combination of herbal drug possesses antioxidant activity, which could exert a beneficial action against pathological alterations caused by the presence of free radicals in streptozotocin induced diabetes.

CONCLUSION

The organs exhibit numerous morphological and functional alterations during diabetes. Oxidative stress, a factor implicated in the pathogenesis of diabetic complications may contribute towards some of these alterations. Treatment of diabetic rats with combination of herbal extract significantly increased the antioxidant status. Since the study of induction of the antioxidant enzymes is considered to be a reliable marker for evaluating the antiperoxidative efficacy of the medicinal plant, these findings are suggestions of possible antiperoxidative role played by combination of herbal extract in addition to its antidiabetic effect.

REFERENCES

[1] Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH: Cerebral function in diabetes mellitus. *Diabetologia* 1994, 37: 643-650.

[2] McCall AL: The impact of diabetes on the CNS. *Diabetes* 1992, 41: 557-570.

[3] Wolff SP: Diabetes mellitus and free radicals. *Bri Med Bull* 1993, 49: 642-652.

[4] Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN: Immobilization stress causes oxidative damage to lipid, protein and DNA in the brain of rats. *FASEB J* 1996, 10: 1532-1538.

[5] Aragno M, Brignardello E, Tamagno O, Boccuzzi G: Dehydro-eppiandrosterone administration prevents the oxidative damage induced by acute hyperglycaemia in rats. *J Endocrinol* 1997, 155: 233-240.

[6] Li PA, Gisselsson J, Keuker J, Vogel ML, Kuschinsky SW, Siesjo K: Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cerebral artery occlusion is not due to capillary obstruction. *Brain Res* 1998, 804: 36–44.

[7] Aragno M, Parola S, Tamagno E, Brignardello E, Manti R, Danni O Boccuzzi G: Oxidative derangement in rat synaptosomes induced by hyperglycaemia: restorative effect of dehydro-epiandrosterone treatment. *Biochem Pharmacol* 2000, 3: 389-395.

[8] Kumar JS, Menon VP: Effect of diabetes on levels of lipid peroxides and glycolipids in rat brain. *Metabolism* 1993, 42: 1435-1439.

[9] Kawai N, Keep RP, Betz AL, Nagao S: Hyperglycaemia induces progressive changes in the cerebral microvasculature and blood-brain barrier transport during focal cerebral ischemia. *Acta Neurochir* 1998, 71: 219-221.

[10] Ceriello A, Giugliano D, Quatraro P, Russo D, Lefebvre PJ: Metabolic control may influence the increased superoxide generation in diabetic serum. *Diab Med* 1991, 8: 540-542.

[11] Makar TK, Hungund BL, Cook GA, Kashfi K, Cooper AJL: Lipid metabolism and membrane composition are altered in the brains of Type II diabetic mice. *J Neurochem* 1995, 64: 2159-2168.

[12] Hu X, Sato J, Oshida Y, Yu M, Bajotto G, Sato Y: Effect of Gosha-jinki-gan (Chinese herbal medicine): Niu-che-sen-qi-wan) on insulin resistance in streptozotocin induced diabetic rats. *Diab Res Clin Pract* 2003, 59: 103 – 111.

[13] Abozeid, Zaki Turki, Fathi El-Shayeb and Zhonghua Tang. *2017*. Embryo and seedling morphology of some *Trigonella* L. species (Fabaceae) and their taxonomic importance. *ELSEVIER,* 2(30): 57-65.

[14] Ahmad Sulaeman and Mahani Mahani. (2019). Trigona Propolis and Its Potency for Health and Healing Process. *Science Direct journal and books*, 5(23): 1-8.

[15] Fohad Mabood Husain, Iqbal Ahmad, Mohd Shahnawaz Khan, and Nasser Abdulatif Al-Shabib. 2015. Trigonella foenum-graceum (Seed) Extract Interferes with Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of *Pseudomonas aeruginosa* and Aeromonas hydrophila. *Journals/ ecam*, 5(3): 25-30.

[16] Gausiya Bashri Sheo and Mohan Prasad. 2016. Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in Cd stressed *Trigonella foenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate - glutathione cycle. *Ecotoxicology and Environmental Safety*, 13(2): 329-338.

[17] Gausiya Bashri Sheo and Mohan Prasad. 2016. Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in Cd stressed *Trigonella foenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate-glutathione cycle. *Ecotoxicology and Environmental Safety*, 13(2): 329-338.

[18] Akash Saggam, Girish Tillu, Santosh Dixit, Preeti Chavan-Gautam, Swapnil Borse, Kalpana Joshi and

Bhushan Patwardhan. 2020. *Withania somnifera* (L.) Dunal: A potential therapeutic adjuvant in cancer. *Journal of Ethnopharmacology*, 25(5): 11-27.

[19] Anju Thakur & Himangini. 2015. Revival of germination and vigour of aged seeds of *Withania somnifera* by seed invigoration treatments. *Indian Journal of Plant Physiology*, 20(5): 391-395.

[20] Aradhana Mishra, Satyendra Pratap Singh, Sahil Mahfooz, Arpita Bhattacharya, Nishtha Mishra, Pramod Arvind Shirke and Nautiyal CS. 2018. Bacterial endophytes modulates the withanolide biosynthetic pathway and physiological performance in *Withania somnifera* under biotic stress. *Microbiological Research*, 12(5): 17-28.

[21] Bakhtiar Choudhary, Shetty A, and Deepak G Langade. 2015. Efficacy of *Ashwagandha* (*Withania somnifera* [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. *Ayu.,* 36(1): 63–68.

[22] Bipradut Sil, Chiranjit Mukherjee, Sumita Jha and Adinpunya Mitra. 2015. Metabolic shift from with a steroid formation to phenylpropanoid accum-ulation in cryptogeinco transformed hairy roots of *Withania somnifera* (L.) Dunal. *Protoplasma*, 25(2): 1097–1110.

[23] Sinha KA: Colorimetric assay of catalase. *Anal Biochem* 1972, 47: 389-94.

[24] Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys* 1984, 21: 130-132.

[25] Rotruck JT, Pope AL, Ganther HE, Swanson AB: Selenium: Biochemical roles as a component of glutathione peroxidase. *Science* 1984, 179: 588-590.

[26] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959, 82: 70-77.

[27] Habig WR, Pbst MJ, Jakpoly WB: Glutathione transferase. A first enzymatic step in mercaturic acid formation. *J Biol Chem* 1974, 249: 7130-7139.

[28] Nichans WG, Samuelson D: Formation of Malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1968, 6:126-130.

[29] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959, 82: 70-77.

[30] Habig WR, Pbst MJ, Jakpoly WB: Glutathione transferase. A first enzymatic step in mercaturic acid formation. *J Biol Chem* 1974, 249: 7130-7139.

[31] Lowry OH, Roesborough MJ, Farr AL, Randall RJ: Protein measurement with Folin-Phenol reagent. *J Biol Chem* 1951, 193: 265-275.

[32] Duncan BD: Multiple range tests for correlated and heteroscedastic means. *Biometrics* 1957, 13: 359-364.

[33] Prince PSM, Menon VP, Pari L: Hypoglycaemic activity of *Syzigium cumini* seeds: Effect on Lipid Peroxidation in Alloxan Diabetic Rats *J Ethnopharmacol* 1998; 61: 1-7.

[34] Carney JM, Strake-Reed PE, Oliver CN, Landum RW, Chang MS, Wu JF, Floyd RA: Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity and loss in temporal and spatial memory by chronic administration of the spin trapping compound N-tert-buty-alfa-pheynitrone. *PNAS* 1991, 88: 3633-3636.