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AMELIORATIVE EFFECT OF *ACONITUM HETEROPHYLLUM (WALL EX ROYLE)* ON, ERYTHROCYTE ANTIOXIDANTS AND LIPID PEROXIDATION IN STZ- INDUCED DIABETIC RATS

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ABSTRACT

The present study was carried out to evaluate the beneficial effects of ***Aconitum heterophyllum*** (AH) on altered erythrocyte lipid peroxides and antioxidants in streptozotocin induced diabetic rats. Diabetes mellitus was induced in adult male albino wistar rats by a single intraperitoneal injection of streptozotocin (40 mg/kg of body weight). The streptozotocin induced diabetic rats were treated with *Aconitum heterophyllum* extract 200 mg/kg b.w for 28 days. The enzymatic superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (Gpx) and non-enzymatic antioxidants Vitamin C, Vitamin E and Reduced Glutathione antioxidant activity significantly decreased and simultaneously lipid peroxidation markers like thiobarbituric acid reactive substances (TBARS) and hydroperoxides (LOOH) significantly was increased in erythrocytes of diabetic rats. Administration of Aconitum *heterophyllum* (200mg/kg bw) and glibenclamide reverted these parameters towards normalcy when compared with untreated diabetic rats. From the present study, it is evident that, *Aconitum heterophyllum* extract possesses significant, antioxidant and antilipidperoxidation properties without any toxic effects.

KEY WORDS: Erythrocytes, *Aconitum heterophyllum*, antioxidants.

INTRODUCTION

Diabetes mellitus (DM) is a chief cause of morbidity and mortality in the world's growing population. The prevalence of DM in adults worldwide is estimated to rise from 382 million in the year 2013 to 592 million in the year 2035[1]. DM is a chronic disease caused by the inability of the pancreas to produce insulin or the inability of our body to use the insulin produced appropriately. Oxidative stress is believed to play an important role in the pathogenesis of chronic complications in DM[2]. A relationship between diabetic nephropathy and neuropathy and oxidative stress has been reported, suggesting that oxidative stress affects development of diabetic complications[3].

Oxidative stress induces the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides. However, endogenous antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) are responsible for the detoxification of the deleterious oxygen species[4]. Lipid peroxidation is a key marker of oxidative stress. It is a free radical induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction[5]. In experimental diabetic rats increased lipid peroxidation has also found to be associated with hyperlipidemia[6].

The diabetes mellitus is becoming more and more prevalent in Indian society. In India it is estimated that approximately 2% of the population have diabetes[7]. The number of cases is said to be rising by 5%-6% each year and an estimated 300,000 people die from diabetes and its complication[8]. There are about 3.5 crore diabetics in India and the figure will rise to about 5.2 crores by 2025. Every 5th patient visiting a consulting physician is a diabetic and every 7th patient visiting a family physician is a diabetic. Prevalence of diabetes is higher in Indian subcontinent & it is estimated that 20% of global burden resides in South East Asia Region (SEAR) area which will be tripled to 228 million by the year 2025 from the current 84 million[9]. Keeping in view the alarming increase in the incidence and prevalence of diabetics in India, WHO has declared India as the Diabetic Capital of the World[10].

India has one of the oldest, richest and diverse cultural traditions associated with the use of the plants and herbs for human, livestock and plant health. Vast ethenobotinical knowledge exists in India from ancient

times. However, very few plants used by locals for medicines are subjected to scientific investigation. The need for conservation of medicinal plants and traditional knowledge particularly in developing countries like India, taking into account the socio cultural and economic conditions is urgent[11]. Modern medicines, biguanides, sulfonylureas and thiozolidinediones are available for the treatment of diabetes along with undesired side-effects. Alternative diabetics have been treated with medicinal plants based on traditional medicine information[12]. Hundreds of herbs and traditional Chinese herbal formulas reported to have been used to treat diabetes mellitus[13].

Aconitum heterophyllum wall commonly known as Atis or Patris belonging to family renunculace is a perennial herb distributed over temperate parts of western Himalaya extending from Kashmir to Kumaonh[14]. The plant has shown to contain alkaloids viz heteratisine, heterophyllisine, heterophyllidine, atidine, isoatisine, hetidine, benzonyheteroatisine[15]. Three widely occurring alkaloids in the aconite roots mesaconitine, aconitine, and hypaconitine showed analgesic activities[16]. Recent studies have shown that its roots are used for curing arthritis[17] as well as in the preparation of Caspa drops a polyherbal formulation for improving digestion and preventing abdominal distension[18]. Aconitum has also shown to exhibit antipyretic,analgesic, anti-bacterial,insecticidal,brime shrimp cytotoxicity, and immunostimulant properties [19]. *A. heterophyllum* is traditionally used to control obesity and included in "lekhaneyagana," a pharmacological classification mentioned in Charaka samhita[20]. The development of anti-diabetic agents that are devoid of adverse effects is still a challenge to the health care systems globally .Thus medicinal plants are constantly being explored with the hope of developing a relatively safe antidiabetic plant based product alone or in combination with other agents[21]. Medicinal plants are widely used in the management of diseases all over the world[22, 23]. Mechanism behind the hypolipidemic, antioxidant and antilipidimic effect of *A. heterophyllum* is unknown as so far in diabetic rats. Thus the present study was under taken to explore antioxidant and lipidperoxidation potential of *Aconitum heterophyllium* on STZ induced diabetic rats.

MATERIAL AND METHODS

Collection of plant material

Aconitum heterophyllum plants were taken from Jammu and Kashmir near the areas of Gurez and Uri in the districts of Bandipora and Baramulla respectively and were authenticated by Prof. Dr.D.Kumarasamy, Department of Botany, Annamalai University. A voucher specimen (AU4381C) of the plant has been deposited in the above mentioned department's herbarium for future reference.

Preparation of plant extracts

The roots of *Aconitum heterophyllum* were collected from the sub-alpine regions of Gurez (4000 mls) and Uri located in the upper parts of Western Himalayan region of India. Roots were air dried at room temperature (25 ˚C; 60% relative humidity) until a moisture content of 10–12% (fresh weight basis) was attained. The dried roots were crushed to powder and subsequently extracted using methanol (1:10, w/v) at ambient temperatures between 35 to 60˚C.The extraction was repeated until the solvent becomes yellowish in colour, the extract was filtered through Whatman No 1 filter paper in rotavoparator followed by lyophilisation. The lyophilized extract was stored at 20 ˚C until further use for biochemical parameters.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich (St. Louis, MO, USA).All other chemicals used in this study were of analytical grade obtained from E.Merck and Hi-media, (Mumbai, India.).

Experimental Animals

Adult male albino wistar rats (9 weeks old;180-200 g wt) were obtained from central Animal House, Department of Experimental Medicine, Raja Muthiah Medical College and Hospital, Annamalai University and maintained at constant temperature (25± 1˚C) on a 12 h light / 12 h dark cycle with feeds (Pranav agro industries Ltd, Pune, Maharastra, India) and water were provided *ad libitum*.The experiments were conducted according to the ethical norms approved by the Ministry of Social justices and Empowerment, Government of India and institutional Animals Ethics Committee Guidelines.

Induction of diabetes mellitus

The animals were rendered diabetic by a single intraperitoneal injection of 40 mg/kg b.w, streptozotocin (STZ) diluted in 0.1 M sodium citrate buffer (pH 4.5) solution. STZ-injected animals were given 20% glucose solution for 24h to prevent initial drug induced hypoglycemia. STZ-injected animals exhibited hyperglycemia within a few days. Diabetic condition rats were confirmed by measuring the elevated plasma glucose (by gluose oxidase method) 72 h after injection of STZ. The rats with blood glucose above 235 mg/dl were considered to the diabetic and used for experiments.

Experimental Design

The experimental animals were divided into five groups; each group consists of six animals. *Aconitum heterophyllum (AH)* was dissolved in 2% Gum acaia and administered orally at different doses using an intragastric tube daily for a period of 28 days. Glibenclamide was dissolved in 2% gum acacia and was used as standard drug.

Group I: Normal control rats.
Group II: Normal + AHE (200 mg/kg body weight)
Group III: Diabetic control rats
Group IV: Diabetic + AHE (200 mg/kg body weight)

Group V: Diabetic + glibenclamide (600µg/kg body weight)

After 28 days of treatment the animals were sacrificed by cervical decapitation. The blood was collected in heparinized centrifuge tubes and the plasma was collected.

Preparation of haemolysate

From 2 ml of blood, erythrocytes were separated by centrifugation at 1000 × *g* for 10 min at 4◦C.The erythrocyte layer was washed three times with 10 volumes of 10 mmol / lPBS. The washed erythrocytes were suspended in phosphate buffer saline (PBS) and adjusted to a hematocrit (HCT) of 5 or 10%.An aliquot of 0.5 ml washed RBC was lysed with 4.5 ml of ice cooled distilled water to prepare haemolysate.

Biochemical Analysis

The estimation of TBARS and LOOH in erythrocytes were done by using the methods of Niehaus and Jiang[24, 25] respectively. Gpx activity was measured by the method described by Rotruck[26]. SOD in the erythrocytes was assayed by the method Kakkar[27]. CAT was estimated by the method of Sinha[28] Vitamin C by Kuether[29], Vitamin E by baker[30] and GSH was determined by the method of Ellman[31] respectively.

Statistical analysis

Values were represented as mean± S.D for each group. Data were analyzed by one way Analysis of Variance (ANOVA) and compared with Duncan's multiple range test (DMRT) using SPSS 16 version. The limit of significance was set $p<0.05$.

RESULTS

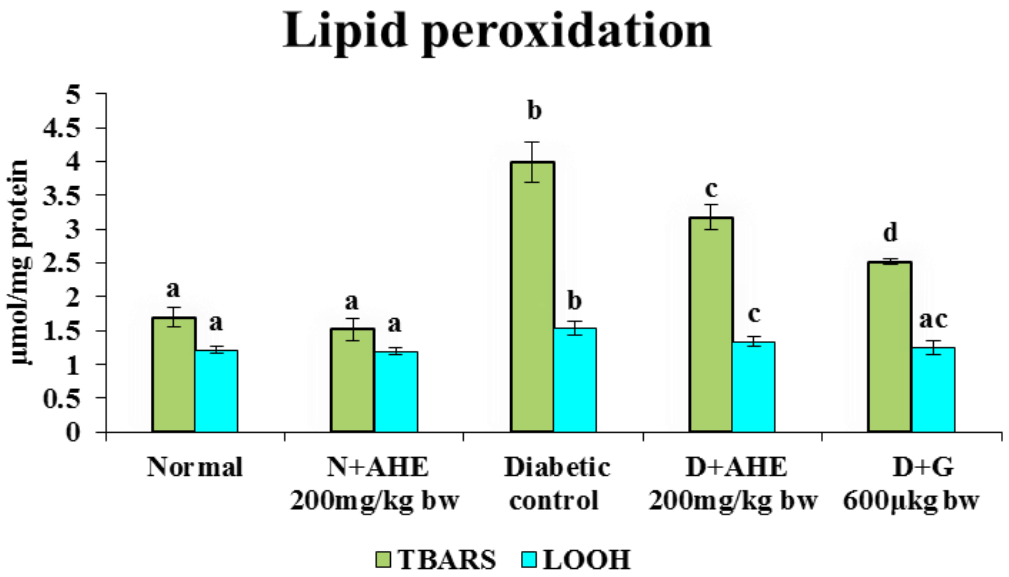

Figure 1.Shows the effect of *Aconitum heterophyllum*on erythrocytelipid peroxidation markers in STZdiabetic rats.

Values are means ± S.D for six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

Table 1 Effect of *Aconitum heterophyllum* on enzymic antioxidants activity in the erythrocytes of STZ-diabetic rats.

Groups	SOD (U^{*}/mg Hb)	CAT ($U^{\$}$/mg Hb)	GPx ($U^{@}$/mg Hb)
Normal	8.38 ± 0.54^{a}	173.01 ± 10.40^{a}	15.97 ± 1.76^{a}
Normal +AHE (200 mg/kg BW)	8.23 ± 1.52^{a}	169.08 ± 10.20^{a}	15.34 ± 0.01^{a}
Diabetic control	3.65 ± 0.99^{b}	102.79 ± 5.43^{b}	8.41 ± 0.65^{b}
Diabetic + AHE (200mg/kg BW)	6.48 ± 0.43^{c}	140.06 ± 11.32^{c}	10.99 ± 0.76^{c}
Diabetic + Glibenclamide (600 µg/kg BW)	6.80 ± 0.65^{c}	157.76 ± 12.28^{ad}	13.26 ± 1.33^{d}

Values are means ± S.D for six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).
U*- Enzyme concentration required for 50% inhibition of NBT reduction/minute.
$U^{\$}$ - µmol of hydrogen peroxide consumed/minute.
$U^{@}$ - µmol of GSH utilized/minute.

Table 2 Effect of *Aconitum heterophyllum* on nonenzymic antioxidants activity in the erythrocytes of STZ-diabetic rats.

Groups	Vitamin C (µg/mg of Hb)	VitaminE (µg/mg of Hb)	Reduced glutathione (mg/g of Hb)
Control	1.92±0.07[a]	1.29± 0.80[a]	77.37±4.73[a]
Control + AHE (200 mg/kg BW)	1.94±0.06[a]	1.31±0.75[a]	78.45±3.56[a]
Diabetic control	0.92±0.09[b]	0.65±0.22[b]	44.67±4.67[b]
Diabetic + AHE (200mg/kg BW)	1.60±0.13[c]	1.83±0.14[c]	58.76±3.53[c]
Diabetic + Glibenclamide (600 µg/kg BW)	1.75±0.86[d]	1.72±0.11[c]	70.27± 1.43[d]

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT).

Figure 1 presents the levels of TBARS and LOOH in the erythrocytes of normal and diabetic rats. Diabetic rats had significantly elevated levels of TBARS and LOOH and treatment with *Aconitum heterohyllum* at the dose of (200mg/kg/bw) and glibenclamide showed reversal of these parameters towards normalcy.

The activities of SOD, CAT and GPx in the erythrocyte of normal and diabetic rats are shown in table 1. Diabetic rats showed decreased activities of SOD, CAT and GPX and treatment with *Aconitum heterohyllum* (200mg/kg/bw) and glibenclamide reversed these changes towards normal.

Table 2 shows the levels of Vitamin C, Vitamin E and GSH in the erythrocytes of normal and diabetic rats. Diabetic rats showed significantly decreased levels of Vitamin C, Vitamin E and GSH and treatment with *Aconitum heterophyllum* at the dose of (200 mg/kg/bw) for 28 days significantly increased the non-enzymatic antioxidants levels.

DISCUSSION

STZ induced hyperglycemia in experimental animals is considered to be an ideal model for the preliminary screening of bioactive constituent's proficient against diabetes[32]. The present study examines the effects of AH on oxidative damage in STZ induced diabetic rats. SOD, an antioxidant enzyme, reduces superoxide radicals to H_2O_2, which in turn is excreted as H_2O based on the activity of GSH-px and catalase, thereby protecting the body from oxygen toxicity. STZ causes irreversible damages to pancreatic β-cells and is mostly used to induce diabetes mellitus in experimental animals through its toxic effects[33, 34]. The diabetogenic effect could be due to the destructive effect of streptozotocin on pancreatic islets and its cytotoxic action is associated with the generation of ROS causing oxidative damage that culminates in β-cell destruction. Thus insulin biosynthesis is suppressed which ultimately results in a clinical condition known as hyperglycemia. Diabetes does not only lead to hyperglycemia but also causes hyperlipidaemia, hyperinsulinemia, hypertension, and athelerosaclerosis[35].

Oxidative stress altered lipid peroxidation and disturbances in glucose metabolism are important risk factors for diabetes, cardiovascular, oncologic and many other diseases. Several antioxidants of plant origin are experimentally proved and widely used as effective agents against oxidative stress[36]. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which upon auto-oxidation generate free radicals[37]. The present study (Fig 1) revealed revealed a significant increase of plasma TBARS and LOOH levels in diabetic rats, which may be due to oxidative stress. The increased levels of erythrocyte lipid peroxidation products observed in STZ-induced diabetes is generally thought to be due to pathological changes in tissues that increase the production and liberation of lipid peroxides into the circulation[38]. The decreased levels of plasma TBARS and LOOH observed in AH and glibenclamide treated STZ-induced diabetic rats are due to its anti-lipidperoxidation effect.

Endogenous antioxidant enzymes (SOD, CAT and Gpx) are responsible for the detoxification of deleterious oxygen radicals and plays an important role in protecting cell from oxidative damage.SOD is capable of reducing the superoxide radical into hydrogen peroxide (H_2O_2).The other enzymatic antioxidant CAT catalyzes the reduction of hydrogen peroxides and protects the tissue against reactive hydroxyl radicals[39]. When the cells have increased SOD without proportional increase in

peroxidase (Gpx), cell faces a peroxide overload challenge. Peroxide can react with transitional metals and generate the harmful hydroxyl radicals. In the present study (Table 1) we observed decrease in the activities of (SOD, CAT and Gpx) in erythrocytes of STZ induced diabetic rats associated with concomitant increase in the lipid peroxidation in blood cells. Corroborating with these results numerous studies have demonstrated that the activates of antioxidant enzymes such as SOD,CAT and Gpx are reduced in blood and tissues of STZ induced diabetic rats[40,41,42]. Treatment with AH and glibenclamide increased the levels these enzymes in erythrocytes are evidenced by decreased lipid peroxidation markers and improved glycemic control. These results are congruent with the report of[43].

The non-enzymatic antioxidants such as GSH, Vitamin C, and Vitamin E are interrelated by recycling process. Glutathione is the most important non-protein compound containing thiol group which acts as a substrate for glutathione transferase and glutathione peroxidase involved in preventing the deleterious effect of oxygen radicals[44]. In our study, diabetic rats showed a significant decrease in the level of GSH (Table 2) which may be due to increased utilization for scavenging free radicals and increased consumption by Gpx and GST. Administration of *Aconitum heterophyllum* extract increases the content of GSH in the erythrocytes of STZ induced diabetic rats. This could be due to the decreased utilization of GSH. In this context a number of other plants have also been reported to have similar effects[45].

Vitamin C is a potent antioxidant widely acts on oxygen free radicals as well as interacts with vitamin E.[46]. Vitamin E is a lipophillic lipid peroxyl radical to yield lipid hydro peroxidase and α-tocopherol radicals[47]. Both Vitamin C and Vitamin E significantly decrease in the erythrocytes of diabetic rats (Table 2). The decrease level of α-tocopherol found in the diabetic rats as compared to of control rats could be due to the increased oxidative stress which accompanies the decrease in the level of antioxidants and may be related to the causation of diabetes mellitus. Decreased level of vitamin C and vitamin E in the tissue of diabetic rats is in the line with the[48]. Administration of *AH* extracts for 28 days increase the Vitamin C and Vitamin E levels in erythrocytes of STZ induced diabetic rats. This signifies that vitamin E is used in combating free radicals and if vitamin C is present, vitamin E level is preserved. Also vitamin C regenerates vitamin E from its oxidized form.

CONCLUSION

In conclusion we put forward that AH treatment restored altered levels of lipid peroxidation,enzymatic and non-enzymatic antioxidants in STZ-induced diabetic rats. Our study also revealed that AH ameliorated altered lipid peroxidation and non-enzymatic antioxidants in the STZ induced diabetic rats from oxidative stress associated complications, by virtue of its antihyperglycaemic and antioxidant effects. On the basis of the results presented in the presented study, it could be concluded that the bioactive phytochemical constituents in Aconitum heterophyllium crude methanolic extract exhibits significant antidiabetic, antioxidant and antilipidperoxidation potential.

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