Therapeutic effects of aqueous, methanol and ethanol extracts of Egyptian *Artemisia herba-alba* in STZ-induced diabetic neuropathy in rats

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ABSTRACT

Diabetes mellitus is a common cause of peripheral neuropathy and patients suffer from chronic peripheral pain. *Artemisia herba-alba* (Ah) is a prevalent plant with extensive traditional uses. This study aimed to investigate the therapeutic effects of Ah in STZ-model of diabetic peripheral neuropathy. After induction of diabetes, rats were treated with aqueous, 100% methanol and 70% ethanol extracts of Ah at doses of 50 and 100mg/kg for each extract; respectively. Behavioral tests (Tail flick, hot plate and locomotor activity) were performed on animals. Blood glucose was measured in serum and oxidative stress biomarkers (GSH, MDA, CAT and GST) were assessed in isolated sciatic nerve. Treatment with aqueous, methanol and ethanol extracts of Ah decreased blood glucose level increased tail withdrawal latency and retention time (in tail flick and hot plate tests; respectively) and increased diabetic rats movements in the activity cage. The extracts also inhibited oxidative stress status by decreasing lipid peroxidation and enhancing GSH and antioxidant enzymes; CAT and GST. Therefore, treatment with Ah ameliorated painful diabetic neuropathy in STZ rats which was evident by improved nociceptive latency and locomotor activity. The therapeutic effect of the plant could be mediated by antioxidant mechanisms.

INTRODUCTION

Neuropathic pain arises due to many reasons, namely injurious CNS diseases e.g. multiple sclerosis, viral infections and postsurgical pain. Neuropathic differs from algesic pain in that it is not protective, but rather pathological. The classical type of painful neuropathy occurs during diabetes mellitus (Boulton, 2004). There are three major complications of diabetes mellitus "DM"; namely diabetic nephropathy, neuropathy and retinopathy. These conditions cause further complications e.g. renal failure, sensory/motor loss and blindness (Fowler, 2008). Diabetic neuropathy (DN) is a devastating problem and leads to foot amputation. Diabetes induces inappropriate expression of sensory mediators resulting in electrophysiological disturbances due to a complex network of correlated vascular, metabolic and

sensory mediators resulting in electrophysiological disturbances due to a complex network of correlated vascular, metabolic and neurotropic defects (Edwards *et al*., 2008). Essentially, chronic pain resulted from peripheral nerve damage has a negative impact on diabetic patients' activity. It affects bothtype-1 and type-2 diabetic patients, resulting in disabilities besides a high mortality rate (Callaghan *et al*., 2012). Furthermore, it is severe and chronic clinical issue that presents resistance to some classical analgesics (Gilron *et al*., 2006). Neuropathic pain is also presented with positive symptoms like pricking and pain. Negative symptoms also occur including loss of sensation, hyperalgesia, paresthesias, allodynia and diminished strength (Melton and Dyck, 1999). Reduced glycemic control leads to activation of several biochemical pathways that ends in nerve damage. When glucose concentration is high, glucose uptake by cells is shifted toward insulin-independent mechanisms. Increased intracellular glucose level activate polyol pathway, which is regulated by aldose

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reductase (AR) and sorbitol dehydrogenase activities. Glucose and sorbitol are converted to sorbitol and fructose by the effect of AR and sorbitol dehydrogenase; respectively (Brownlee, 2005). When AR activity predominates, sorbitol accumulates inside cells, raises osmotic pressure and thus producing oxidative cell damage (Chung *et al*., 2003). Therapies for DN are restricted due to lack of established mechanisms for the neuropathic pain. Interventions targeted to halt sensory pathways without treating the pathological basis of pain are often ineffective (Vincent *et al*., 2011). However, there are different options for treatment of the disease. Fundamental treatments are currently directed to prevent DN through adequate glycemic control to prevent further nerve damage (Tesfaye *et al*., 2007). Additionally, drug treatments aimed to minimize pain and its associated symptoms have been produced such as tricyclic antidepressants, anticonvulsants and serotonin reuptake inhibitors (Tesfaye *et al*., 2010). Oxidative stress is a mutual pathway of cell injury as a result of hyperglycemia. Using antioxidant approaches have been widely investigated but only α-lipoic acid has shown promise in clinical trials (Ziegler *et al*., 2006). Due to various side effects of drug therapy, other measures like physical treatment could alleviate symptoms. These include electrical stimulation of nerves, posture training, manual therapy, foot care, hot wax and phototherapy (Pieber *et al*., 2010; Balbinot *et al*., 2012). However, seeking efficient treatment for this condition remains a great challenge.

Artemisia herba-alba (Ah) is a known herb that has beneficial properties as a herbal Further studies are needed to integrate this popular plant in human health care system (Moufid and Eddouks, 2012). In our previous studies, Ah was shown to be effective as anti-inflammatory, antinociceptive, antipyretic and gastro-protective agent (Abdel Jaleel *et al*., 2016). In addition, it was concluded that the total alcoholic extract of Ah could be used as antidiabetic agent. It was found to exhibit antihyperglycemic, antihyperlipidemic and protective against hepatic and renal toxicities in a rat model of induced type1 diabetes (Abdallah *et al*., 2015). The current work was conducted to extend the use of the Egyptian Ah as a potential treatment of diabetic complications. The study aims to examine the therapeutic potential of this herb on the progress of behavioral as well as biochemical deficits in diabetic neuropathy.

MATERIAL AND METHODS

Plant material

The dried aerial parts of the Ah were purchased from the Egyptian markets and were grinded by electric grinder.

Preparation of plant extract

The plant powder was soaked in 70% ethyl alcohol for about 3 days, filtered using filter paper and the filtrate was concentrated under vacuum using the rotating evaporator (Rotavap), then percolated several times till exhaustion. The total alcoholic (70% ethanol) extract was obtained. Then the residual plant was further extracted with methanol. The obtained 100%

methanol extract was filtered, dried and stored in the refrigerator at 4 °C till further use. For preparation of aqueous extract, the above-ground parts of Ah were also powdered and dissolved in distilled water for 16 h with occasionally shaking each 2 h. The extract was filtered. The filtrate was concentrated under vacuum using the rotating evaporator (Rotavap), and then percolated several times till exhaustion. The obtained aqueous extract of Ah was also used for pharmacological screening. The yield of each extract was also recorded; ethanol (27.5%), methanol (19%) and aqueous (34.5%)

Phytochemical study

Chemical analysis for the presence of potential compounds as well as antioxidant activity was performed for the three extracts of Ah; ethanol, methanol and aqueous extracts (the extraction procedures mentioned above). Values were expressed and calculated based on weight (g) of dry tissue (the main powder weight before extraction).

Determination of total phenolic content

Total concentration of phenolic compounds in the extracts was determined using a series of gallic acid standard solutions (2.5-20μg/ml) as described by Singleton and Rossi (1965) but with some modifications. Each extract solution (0.1ml) was mixed with 2ml of a 2% (w/v) sodium carbonate solution and vortexed vigorously. The same procedure was also applied to the standard solutions of gallic acid. After 3 min, 0.1 ml of Folin Ciocalteau's phenol reagent was added and each mixture was vortexed again. The absorbance at 750 nm of each mixture was measured, after incubation for 30 min at room temperature. Total phenolic content was expressed as mg gallic acid/ g dry tissue.

Determination of total flavonoid content

Total concentration of flavonoid compounds in extracts was determined using a series of standard rutin solutions (2.5-50 μg / ml) as described in the aluminum chloride colorimetric method. A known volume of each extract solution was mixed with 5% sodium nitrite solution, vortexed vigorously, then 10% aluminum chloride solution was added and vortexed again. After 6 min, 4.3% of sodium hydroxide solution was added, followed by addition of water, shaken, and left to stand for 15 min before determination. The sample solution without coloration was used as reference solution and the color was read at 510 nm wavelength (Dae-Ok *et al*., 2003). Total flavonoid content was expressed as mg rutin/g dry tissue.

Free radical scavenging activity by DPPH method

The free radical scavenging activities were determined by DPPH method with some modifications of the method proposed by Blois (1958). Briefly, a 0.1 mM solution of DPPH in absolute ethanol was prepared. The initial absorbance of the DPPH in ethanol was measured at 517 nm (absorbance 1.3) and did not change throughout the period of assay. A series of extract solutions or ascorbic acid (standard antioxidant) with varying concentrations were prepared by dissolving the dried extracts in

ethanol and 0.1 ml of solutions from each extract was added to 1.4 ml of DPPH solution. The absorbance at 517 nm was recorded after 30 min of incubation at room temperature. Radical scavenging capacity of each extract has been calculated as the percent inhibition of DPPH radical (scavenging effect)as follows:

$$\% \text{ of inhibition} = [(A1-A0/A0)] \times 100$$

Where, A0 is the absorbance of the control with ethanol and A1 is the absorbance of the sample in the presence of the extract samples. IC50 value was determined from the graph of percentage of inhibition plotted against the log concentration of the extract using GraphPadPrism Software 6.0. IC50 is defined as the concentration of extract needed to inhibit 50% of DPPH radicals.

Animals

Healthy male Wister rats, weighing 220–250 g, were obtained from the animal house of the National Research Centre (Giza, Egypt). Before initiating the experiments, the rats were allowed to acclimatize for few days under standard environmental conditions (12 h dark/12 h light cycle; temperature 20–22°C; relative humidity 40%–60%). The study was conducted according to regulations of the ethics committee of the National Research Centre which gave its consent in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and complies with the guidelines from the Canadian Council on Animal Care. To minimize animal suffering, number of animals were kept as minimum as possible to perform suitable statistical analysis. After finishing the experiment and all measurements, animals were sacrificed by decapitation using sharp scissors which is rapid and painless.

Induction of Diabetes

Type 1 DM was induced by a single i.p. injection of a freshly prepared solution of streptozotocin (STZ) (52.5 mg/kg body weight) in 0.1 M citrate buffer (pH 4.3) after a fasting period of 24 h (Barrière *et al.*, 2012; Mohan *et al.*, 2013). On the third day of STZ injection, diabetes in surviving rats was confirmed by measuring the glucose level of blood obtained from the tail vein. Rats with a plasma glucose level of 180 mg/dl or greater were accepted as diabetic and included in this study.

Experimental design

After induction of diabetes, rats were divided into seven equal groups (eight rats per group). Group 2 (control positive) comprises rats that received STZ as mentioned above. Groups 3,4 comprises rats that received STZ and oral administration of the Ah aqueous extract at 50 & 100mg/kg; respectively. Doses were selected based on previous studies (Abdallah *et al.*, 2015). Groups 5,6 comprises rats that received STZ and oral administration of the Ah methanolic extract at 50 & 100mg/kg; respectively. Groups 7, 8 comprises rats that received STZ and oral administration of the Ah ethanolic extract at 50 & 100mg/kg; respectively. The first group (control negative group) comprises normal rats that received only 1 ml saline. Treatment with the Ah extract was started three days after STZ injection. Each plant extract was given p.o. at the

selected doses based on toxicity study (data not shown) daily to diabetic rats for 28 days. Thermal algesia was assessed using Tail flick and hot plate tests at the end of the experiment, whereas, locomotor activity was assessed before and after treatment. Blood samples were collected from retro-orbital plexus of only 6 animals in each group under light ether anaesthesia and centrifuged by cooling centrifuge (Sigma and laborzentrifugen, 2k15, Germany) at 3000 rpm . The obtained serum samples were immediately analyzed for glucose concentration using available kits (Stanbio Laboratory, USA). Then behavioral tests were performed on 8 animals from each group in order to achieve adequate statistical analysis as follows:

Thermal nociceptive response
Tail flick test

The method described by Sugimoto *et al.* (2008) used with slight modifications. Acute nociception was induced by using a tail flick apparatus (Tail Flick model DS 20 Sorrel Apelex, France). Briefly, each rat placed in a restrainer and the tail flick latency was determined by focusing the intensity controlled beam of light on the distal last 2 cm of the animal's tail and recording the time in seconds taken to remove the tail from the noxious thermal stimulus. For each animal, 2 to 3 recordings were made at an interval of 15 min; the mean value was used for statistical analysis.

Hot plate test

The protocol of determination of analgesic activity using hot plate method was published by Woolfe and Mc Donald (1962). This test measures the time that elapses before the rat demonstrates hind paw licking/shaking and jumping, which indicate pain in response to the applied heat. Each animal was placed onto a Perspex cylinder on the heated stage maintained at 52 + 0.5°C hot plate to perform the test. Latency to exhibit nociceptive responses was determined in seconds. To avoid tissue damage of the rat paws, cutoff time for the response to thermal stimulus was set at 60 seconds.

Locomotor activity test

Spontaneous locomotor activity of the rats was measured by using a grid floor activity cage (Model No. 7430, Ugo-Basile, Comerio, Italy) to detect the rat's movements. Movements by the rat that interrupted infrared beams were automatically detected, and the beam-interruption information was processed by the activity cage software to provide counts of horizontal movements. Before STZ injection, rats were acclimated to the test room for 1 h. Then, each rat was placed individually into the activity cage for a 5 min session and the basal activity counts were recorded. At the end of the session, each rat was gently removed from the activity cage, and then returned to their home cage. The arena was wiped out with a 70% (v/v) alcohol solution in distilled water between sessions to prevent olfactory cues. Twenty-four hours after the last administration of the test drugs, each rat was then re-exposed to the activity apparatus. The final activity counts were recorded per

5 min, with each count indicating one beam break by the animal (Thome *et al.*, 2001).

Isolation of sciatic nerve

Sciatic nerves were rapidly removed as per the procedure described by Mizisin (2004) from the spin to the peroneal bifurcation and rinsed in ice cold saline solution and frozen at -80 °C. On the day of homogenate preparation sciatic nerve segments were measured and weighed. Sciatic nerves were cut into small pieces and then homogenized at 4°C in adequate volume of of 0.025 mM Tris-HCl buffer, pH 8, with glass homogenizer, resulting homogenate fitted with a Teflon pestle. The homogenates were centrifuged at 3400 xg for 15 min using Beckman L5-50B ultracentrifuge with 220.78VD2 rotor at 4°C.The supernatants were filtered through a plug of glass wool to remove floating lipids, the cytosolic fractions were termed as crude homogenates of sciatic nerve and stored at −20°C   for further analyses. Oxidative stress biomarkers were estimated in sciatic nerve homogenate:

Reduced glutathione content (GSH) and Lipid peroxides content

Aliquots of the sciatic nerve homogenate were immediately used for estimation of Lipid peroxides (reflected by MDA concentration) & GSH contents that were assayed colorimetrically according to the methods of Ohkawa *et al.* (1979) and Ellman (1959); respectively as previously described.

Catalase (CAT) activity

CAT activity is determined spectrophotometrically at 37°C. The decomposition of H_2O_2 was followed as a decline in absorbance at 240 nm for 5 minutes (Aebi, 1984). The assay reaction mixture contained in a total volume of 3 ml, the substrate buffer and a suitable volume of the enzyme solution. The substrate buffer contains 30% H_2O_2 in 100 ml of 50 mM potassium phosphate buffer, pH 7. The final concentration of H_2O_2 is 0.042 M. The activity of enzyme was expressed as K units of decomposed/min/mg proteins by the decomposition of H_2O_2 of the assay were followed at 240 nm. The molar absorptivity of H_2O_2= 43.6 L mol^{-1}cm^{-1}.

(Glutathione-s-transferase GST) activity

GST activity was determined according to the method described by Habig *et al.* (1974) by measuring the increase in the concentration of the conjugation product of GSH and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm over 3 min at 25°C. Unless otherwise stated, the assay mixture contained in a total volume of 1 ml, 0.1 M potassium phosphate buffer, pH 6.5, 1mM CDNB in ethanol (final concentration of ethanol less than 4%), 1mM GSH, and the enzyme solution. One unit is equivalent to the amount of enzyme conjugating 1 μmole of CDNB in 1min at 25°C.

The extinction coefficient of the product was taken to be 9.6 mM-1cm-1. Protein was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

Statistical analysis for was carried out using One-way ANOVA followed by Tukey's post hoc test using SPSS software, version 14.0 (SPSS Inc., Chicago, Illinois, USA). For locomotor activity test, statistical significance was determined by two-way ANOVA was used as a statistical test followed by LSD multiple comparison post hoc test. Data were represented as mean ± SEM. The P values less than 0.05 were considered to be significant.

RESULTS

Phytochemical analysis/ antioxidant activity

Phytochemical investigation showed that 70% ethanolic extract of Ah contains higher content of total phenolics (28.6 ± 0.204 mg gallic acid/g dry tissue and 6.215 ±0. 58mg rutin/g dry tissue) than aqueous and methanol extracts and showed high antioxidant activities (IC50= 0.65±0.08 *vs* 0.63±0.03mg /mL for ascorbic acid).

Table1: Phytochemical analysis/activity of Ah different extracts.

Plant sample	Total phenolics (mg gallic acid/ g dry tissue)	Total flavonoids (mg rutin/ g dry tissue)	DPPH * IC_{50} (mg dry tissue /mL)
Ah aqueous extract	6.31±0.5	0.87±0.1	3.9±0.2
Ah methanol extract	6.30±0.3	0.96±0.03	2.25±0.1
Ah ethanol extract	28. 5±0.2	6.22±0.6	0.65±0.01

Ah: Artemisia herba-alba. Results are represented as the mean values of three replicates of the same sample ±SE. Statistical analysis was performed using one way ANOVA followed by Tukey as post hoc test. DPPH IC_{50} of ascorbic acid as stranded as scavenger for free radical (0.63±0.03dry tissue /mL). * IC_{50} (amount of extract which cause 50% inhibition of DPPH free radical).

Effect on Fasting Blood glucose:

Table 2 reveals that STZ injection induced hyperglycemia successfully. Treatment of animals with different extracts of Ah significantly decreased blood glucose as compared to STZ-diabetic group. Aqueous extract at 50 & 100 mg/kg, methanol extract at 50 & 100 mg/kg and ethanol extract at 50&100mg/kg showed a decrease in blood glucose of 71%, 68%, 75%, 79%, 74% & 80%; respectively.

Table 2: Effect of Ah different extracts on fasting blood glucose level after 3 days of STZ injection and after 28 days in rats:

Treatment	Blood glucose level (mg/dl)	
	After 3 days	After 28 days
Control negative (normal)	109.5 ±5.7[a]	106.2 ± 5.2 [a]
Control positive (STZ)	434.7 ±27.8*	438.0 ± 30.1*
Ah aqueous ext (50mg/kg)	449.0 ± 15.5*	128.6 ± 3.1[a]
Ah aqueous ext (100mg/kg)	429.5 ± 34.8*	141.8 ± 2.6[a]
Ah methanolic ext (50mg/kg)	420.5 ± 24.0*	106.6 ±5.4[a]
Ah methanolic ext (100mg/kg)	358.5 ± 22.4*	91.6 ± 5.9[a]
Ah ethanolic ext (50mg/kg)	411 ± 37.5*	113.8 ± 3.9[a]
Ah ethanolic ext (100mg/kg)	362.5 ± 21.0*	89.0 ± 5.8[a]

Ah: Artemisia herba-alba. All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment started 3 days after STZ injection. Blood glucose was measured twice; after 3days of STZ injection and at the end of the experiment (after 28 days). Each value represents the mean glucose level (mg/dl) ± SEM (n=6). *Significantly different from control negative (saline) at P<0.05, [a] significantly different from control positive (STZ) at P<0.05. One-way ANOVA was used as a statistical test followed by Tukey's post hoc test.

Effect on behavioral changes
Tail Flick test

A significant decrease (P<0.05) in tail flick latency was observed in diabetic rats (about 2 folds) compared to normal group and this decrease was markedly eliminated by Ah administration (Figure. 1). Aqueous extract at 50 &100mg/g, methanolic extract at 50& 100mg, and ethanolic extract at 50&100mg increased tail withdrawal latency by 74%, 107%, 82%, 46%, 54%, 100%; respectively as compared to STZ-diabetic group.
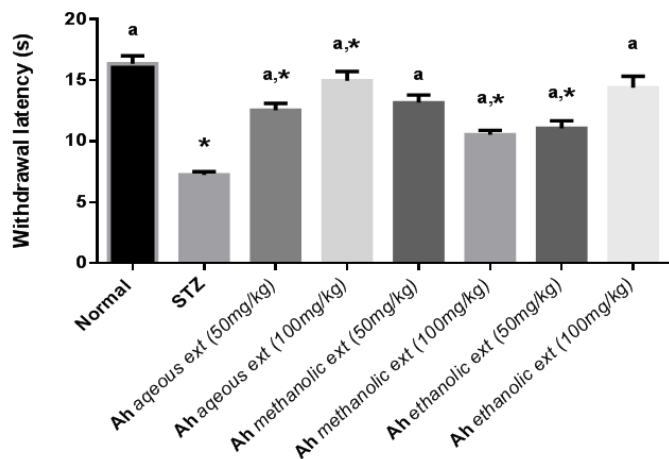

Fig. 1: Effect of Ah different extracts on thermally induced algesia using tail flick test in rats. One-way ANOVA with Tukey as post hoc test were applied. * Significantly different from normal group (p<0.05) and [a] significantly different from STZ group (p<0.05). Values are expressed as Mean ± SE (n = 8). *Ah: Artemisia herba-alba*
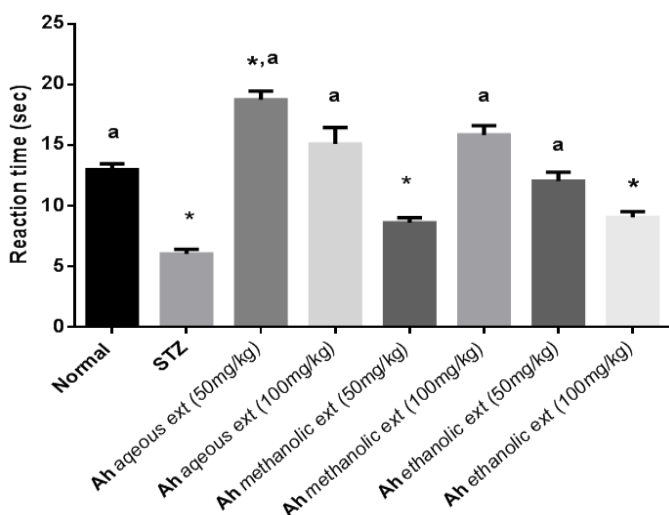

Fig. 2: Effect of Ah different extracts on thermally induced algesia using hot-plate model in rats. One-way ANOVA with Tukey as post hoc test were applied. *Significantly different from normal group (p<0.05) and [a]Significantly different from STZ group (p<0.05). Values are expressed as Mean ± SE (n = 8). *Ah: Artemisia herba-alba.*

Hot plate test

Figure.2 shows that STZ significantly (P<0.05) decreased the retention time in hot plate as compared to the normal group. Administration of Ah aqueous ext. at 50&100mg/kg, methanolic ext. at 100mg/kg, and ethanolic extract at 50mg/kg increased the reaction time by 211%, 150%, 162%, and 100% as compared to STZ-diabetic group.

Locomotor activity test

Normal, diabetic, and other-treated rats showed a significant reduction in their final locomotor activity with respect to their correspondent basal activity. A significant increase in the final locomotor activity of rats treated with aqueous (50mg/kg), methanolic and ethanolic extracts of Ah was noticed as compared to the STZ-diabetic group (table 3).

Table 3: Effect of Ah different extracts on locomotor activity in activity cage test.

Treatments	Locomotor activity (counts/5 min)	
	Basal	Final
Control negative (normal)	169.63±17.43	92.75*[†]±12.06
Control positive (STZ)	133.67±2.88	35.75*[#] ±4.64
Ah aqueous ext (50mg/kg)	182.00±23.42	100.67*[†]±11.90
Ah aqueous ext (100mg/kg)	170.00±16.88	53.67*[#] ±6.17
Ah methanolicext (50mg/kg)	211.50±15.70	102.00*[†]±20.45
Ah methanolicext (100mg/kg)	171.88±15.11	92.50*[†]±17.48
Ah ethanolicext (50mg/kg)	150.64±13.47	80.43*[†]±7.54
Ah ethanolicext (100mg/kg)	207.83±8.37	100.17*[†]±9.80

Ah: Artemisia herba-alba. All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment started 3 days after STZ injection. Results are expressed as mean ± SEM (n=8). *Significant difference from correspondent group at P< 0.05.[#] Significant difference from final Normal group at P< 0.05.[†] Significant difference from final STZ group at P< 0.05. Two-way ANOVA was used as a statistical test followed by LSD multiple comparison post hoc test.

Effect on oxidative stress biomarkers in sciatic nerve
GSH and lipid peroxides contents

Table 4 shows that STZ injection significantly (p<0.05) decreased GSH and increased lipid peroxides contents in sciatic nerve as compared with normal group. Treatment with aqueous, methanol and ethanol extracts at higher doses significantly increased GSH and all treatments significantly decreased lipid peroxide contents compared with STZ-diabetic control group. All extracts could bring lipid peroxide contents back to normal levels.

CAT and GST activities

In sciatic nerve, CAT & GST activities were markedly inhibited in diabetic rats compared to control animals. These activities were significantly (p<0.05) enhanced in diabetic rats treated with different extracts when compared to untreated diabetic rats (table 4).

Table 4: Effect of Ah different extracts on sciatic nerve oxidative stress biomarkers in rats.

Treatment	GSH (µM/mg protein)	MDA (µM/mg protein)	CAT activity (unit/mg protein)	GST activity (unit/mg protein)
Control negative (normal)	2.63±0.24 [a]	0.22±0.02 [a]	25.68 ± 1.19[a]	0.299± 0.023[a]
Control positive (STZ)	1.13±0.08[*]	0.47±0.03[*]	7.26± 0.62[*]	0.170±0.013[*]
Ah aqueous ext (50mg/kg)	1.14±0.03[*]	0.37±0.02[*a]	17.85 ± 1.30[*a]	0.297±0.018[a]
Ah aqueous ext (100mg/kg)	2.39±0.22 [a]	0.26±0.01 [a]	16.82 ± 1.51[*a]	0.276±0.015[a]
Ah methanolic ext (50mg/kg)	1.84±0.19 [*]	0.29± 0.01 [a]	23.49 ± 1.21 [a]	0.241±0.021[a]
Ah methanolic ext (100mg/kg)	2.26±0.20 [a]	0.22±0.02 [a]	14.91± 0.64[*a]	0.301± 0.022[a]
Ah ethanolic ext (50mg/kg)	1.91±0.15[*]	0.19±0.01 [a]	15.06 ± 0.13 [*a]	0.218±0.011[a]
Ah ethanolic ext (100mg/kg)	2.67±0.33 [a]	0.15± 0.01[a]	16.74 ± 1.67[*a]	0.270±0.017[a]

Ah: Artemisia herba-alba. All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment started 3 days after STZ injection. Each value represents the mean ± SEM (n=6). *****Significantly different from control negative (saline) at p<0.05, [a] significantly different from control positive (STZ) at P<0.05. One-way ANOVA was used as a statistical test followed by Tukey's post hoc test.

DISCUSSION

Neuropathic pain is amongst the basic complications of diabetes mellitus. About half of the diabetic patients develop neuropathy with manifestations such as spontaneous pain, allodynia and hyperalgesia (Apfel *et al.*, 2001). Evaluation of behavioral reactions to foreign stimuli in animals developing diabetes gives profitable data regarding mechanisms mediating associated pain (Calcutt *et al.*, 2004).

In the current work, tail flick and hot plate tests were used to estimate sensory responses as a result of thermal stimuli. STZ-diabetic rats exhibited significantly shorter tail withdrawal latency than that observed in normal animals. In addition, STZ injection showed significant hyperalgesia appeared in the hot-plate test. Hyperalgesia induced by STZ was tested in different experimental models and was found to produce various pathophysiological symptoms (Courteix *et al.*, 1994; Hounsom and Tomlinson, 1997; Kamei *et al.*, 2001). This also is in parallel with other previous studies that described thermal hyperalgesia resulted when the tail of STZ-diabetic animals was exposed to noxious stimuli (Ohsawa and Kamei, 1999; Kamboj *et al.*, 2010). This induced nociception in the current study was accompanied with increased blood glucose in STZ group. Treatment with aqueous, methanolic and ethanolic extracts of Ah decreased blood glucose level and increased tail withdrawal latency and retention time. Mechanisms of induced diabetic neuropathy are complicated and overlapped. However, hyperglycemia is considered a primary factor in pain hypersensitivity accompanies diabetes as it causes direct toxicity in the peripheral nervous system. It enhances the activity of primary afferent fibers, potentiates glutamate release and diminishes the opioidergic and GABAergic inhibitory systems activities (Nourooz-Zadeh *et al.*, 1997). Chronic hyperglycemia also causes alteration in sensitivity of the dopaminergic receptors as well as responsiveness of the dopaminergic system due to inclusion of the enkephalinergic system (Wohaieb and Godin, 1987). It affects L-type Ca2+ channels that are directly responsible for modulation of nociception in diabetic rats (Feillet-Coudray *et al.*, 1999). In parallel, Ah extracts enhanced locomotor activity indicating that it was protective against neuronal damage. It was noticed that all groups showed reduction in their final locomotor activity with regard to their corresponding basal activity. This may be a result of rats' habituation to the activity cage. From above results, it is clear that proper adjustment of blood glucose could mitigate peripheral pain in long-lasting diabetes via direct mechanisms as established previously (Courteix *et al.*, 1996).

It is well known that oxidative stress mediates sciatic nerve dysfunction and decreased blood flow in diabetic rats (Figueroa –Romero *et al.*, 2008; Zherebitskaya *et al.*, 2009; Aziza *et al.*, 2014). Oxidative stress is induced by chronic hyperglycemia which induces auto-oxidation of monosaccharides. This causes superoxide and hydroxyl radicals to be evolved. Superoxide anions mediate a lot of the oxidative changes in hyperglycemic conditions, such as potentiating aldose reductase and protein kinase C activities which also have an impact on pain sensation (Kamei *et al.*, 2001). The present results show that STZ injection for 28 days decrease GSH, increased lipid peroxidation and inhibited the activity of antioxidant enzymes; CAT and GST in sciatic nerve. CAT is responsible for the catalytic decomposition of H_2O_2 to oxygen and water. The decreased CAT activity during diabetes reduces protection against free radicals. Simultaneous reduction in SOD and CAT activities makes sciatic nerve more vulnerable to oxidative stress induced by chronic hyperglycemia. Similarly, GST and GSH-peroxidase work together with GSH in the decomposition of H_2O_2 or other organic hydroperoxides. A reduction in GST activity in diabetic rats might reflect decreased protein thiols as -SH groups play a critical role in enzyme catalysis (Mak *et al.*, 1996). Treatment with Ah extracts enhanced GSH, decreased lipid peroxidation and retained the activity of CAT and GST in sciatic nerve indicating that this herb may exert the antinociceptive effect, in part, via its antioxidant properties. In a previous work of us, 70% ethanol extract of Ah was reported to possess antioxidant properties through scavenging DPPH free radical in-vitro (Abdel Jaleel *et al.*, 2016). This antioxidant effect was ascribed to presence of high phenolic compounds including flavonoids and polyphenols (Abdallah *et al.*, 2015). This challenged us to continue phytochemical and pharmacological investigation on other types of extracts for Ah; aqueous and 100% methanol extracts. These extracts also produced neuroprotective effects comparable to the 70% ethanol one.

CONCLUSION

Treatment with the three extracts of *Artemisia herba-alba* ameliorated peripheral pain which was evident by nociceptive latency in hot plate and tail flick tests as well as improved locomotor activity. It is proposed that the extracts act, in part, by anti-hyperglycemic and antioxidant mechanisms. Investigation of more mechanistic pathways is warranted in order to achieve efficient targeting of diabetic neuropathy as a major complication of D.M.

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