Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

EFFECT OF *AEGLE MARMELOS* LEAF EXTRACT TREATMENT ON DIABETIC NEUROPATHY IN RATS: A POSSIBLE INVOLVEMENT OF A_2 ADRENOCEPTORS

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Received: 16 Mar 2012, Revised and Accepted: 26 Apr 2012

ABSTRACT

The aim of present study was to investigate the effect of *Aegle marmelos* leaf extract (AME) on hyperalgesia in alloxan-diabetic rats. Alloxan (150 mg/kg; i.p.) was injected to induce diabetes in wistar rats (150-200 g). The diabetic animals were treated with vehicle (diabetic control), varying doses of AME (25, 50, 100, 200 and 400 mg kg^{-1}), fluoxetine (20 mg kg^{-1}), propanolol (30 mg kg^{-1}) followed by AME (100 mg kg^{-1}) and yohimbine (2 mg kg^{-1}) followed by AME (100 mg kg^{-1}) from 3rd to 14th days of induction of diabetes. AME was found to increase the paw licking and tail flicking latency ($p < 0.05$) as compared to the vehicle treated diabetic controls. The effect of AME was found to be dose dependent with maximum dose dependent increase observed at a dose of 100mg kg^{-1}. The pretreatment with propanolol did not alter the *perse* effect of AME. On the other hand administration of yohimbine prior to AME was found to attenuate the protective effect of AME. From the above findings, it may tentatively be concluded that AME provides protection against alloxan induced diabetic neuropathy in rats and this effect might be mediated via the autonomic nervous.

Keywords: Nutraceuticals; Hyperalgesia; Natural products; Alloxan; Diabetic Neuropathy, Antioxidant.

INTRODUCTION

Diabetic neuropathy (DN) is one of the most common complications of diabetes which has equal incidence in both type I and type II diabetes[1]. It is characterized by hyper responsiveness to pain typically originating in the extremities, progressive loss of neuronal function in a distal to proximal gradient[2]. The exact etiopathogenesis of DN is multifactorial and involves various factors such as hyperglycemia, neuronal loss, alterations in neurotransmitters and growth factors etc[3]. Other mechanisms include insulin deficiency, oxidative stress, nitrosative stress, ischaemia, osmolyte accumulation, neurotropic factor deficiency, autoimmune nerve destruction, alterations in cellular signaling pathways and gene expression of proteins[1].

Because of the limited therapeutic options available to improve the morbidity and treat the syndrome, plants and natural products are increasingly being explored for their effectiveness. Consumption of *Arachis hypogaea* is documented to improve the glycaemic control and oxidant stress in rats[4]. Studies have shown beneficial effects of *Cannabis sativa*[5], resveratrol[6] on hyperalgesia in diabetic rats. Pycnogenol, a herbal formulation[7] and sylimarin[8] have been evidenced to improve hyperalgesia and motor nerve conduction velocity in diabetic rats. Recently, extract of *Embellica officinalis* has been reported to correct the functional, biochemical and molecular deficits in diabetic neuropathy in rats [9]. Circulat, a standardized plant extract combination has been shown to clinically cure diabetic foot and prevent amputations in diabetic subjects[10].

Aegle marmelos is well known for its antihyperglycemic[11-12], analgesic, anti-inflammatory[13] and anti-oxidant properties[14]. In this study, it has been evaluated for its protective effect in diabetic neuropathy. Therefore the present study is designed to investigate the effect of different doses of the ethanol leaf extracts of *Aegle marmelos* on hyperalgesia and nerve conduction parameters in diabetic rats and also to see the involvement of adrenergic receptors in the anti-nociceptive effect of *Aegle marmelos* leaf extract in diabetic rats.

MATERIALS AND METHODS

Plant Material: The leaves of *Aegle marmelos* were collected from the botanical garden of Guru Nanak Dev University, Amritsar. The plant species was identified and authenticated by Dr. Amarjit Singh Soodan, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar and the voucher specimen (SR./Bot.Sci./0350) has been deposited in the herbarium of the said department.

Preparation of *Aegle marmelos* leaf extract (AME): *Aegle marmelos* leaves (100 g) were cleaned, shade dried and crushed in a grinder to give a coarse powder. The extract was prepared with ethanol using soxhlet extraction and the solvent was evaporated under reduced pressure in rotatory vaporiser (Laborota 4001, Heidolph). The yield of the extract was 9.6% w/w. The extract was stored in screw tight bottle at -10 ºC in incubator (Thermotech TIC-4000, Metrex Scientific Instrument, India). The extract was suspended in 0.5% sodium carboxy methyl cellulose (CMC) solution before administration. Freshly prepared suspension of extract was used for the study of each group.

Experimental Design: Wistar rats (150-200 g) of either sex were used in the present study. The animals were kept on straw bedding in cages under natural light and dark cycle in central animal house facility of Guru Nanak Dev University and were acclimatized to the laboratory conditions (room temperature) for 7 days before the start of the experiments. Animals were fed with standard rodent diet and water *ad libitum.* The experimental protocol was duly approved by institutional ethics committee and care of animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Drugs and Chemicals: Alloxan was procured from Sigma, St. Louis, Germany. Fluoxetine was obtained as a gift sample from Q.P. Pharmachem, Derabassi, India, yohimbine as a gift sample from Chemical Resources, Panchkula, India and propanolol was purchased from Namco Chemicals, Amritsar, India. All other chemicals were purchased from local suppliers and were of analytical grade.

Experimental Protocols: The animals were divided into 10 groups of six each, for a protocol of 14 days. Group I: Non-diabetic Group: the animals were injected with vehicle 0.5% carboxymethyl cellulose (1 $mlkg^{-1}$) daily for a period from 3rd to 14th day. In the rest of the groups, diabetes was induced by a single intraperitoneal injection of alloxan 150 $mgkg^{-1}$ on 0th day. The animals having blood glucose range of 150-200 $mgdl^{-1}$ on 3rd day were considered diabetic and included in the study and animals having hyperalgesic response on 0th day were discarded from the study. Group II, diabetic control: the animals were administered vehicle (carboxy methyl cellulose 0.5%) at a dose of (1 $mlkg^{-1}$). Group III, fluoxetine treated group: the animals were treated with fluoxetine at a dose of 20mg/kg from 7th day of inducing diabetes. Group IV, ethanol extract (25 $mgkg^{-1}$) treated group: the animals received a daily dose (25 $mgkg^{-1}$) of ethanol extract suspended in 0.5% CMC. Group V, ethanol extract (50 $mgkg^{-1}$) treated group: the animals received a daily dose (50 $mgkg^{-1}$) of ethanol extract suspended in 0.5%

CMC. Group VI, ethanol extract (100 mg/kg) treated group: the animals received a daily dose (100 mg kg^{-1}) of ethanol extract suspended in 0.5% CMC. Group VII, ethanol extract (200 $mgkg^{-1}$) treated group: the animals received a daily dose (200 $mgkg^{-1}$) of ethanol extract suspended in 0.5% CMC. Group VIII, ethanolic extract (400 mg kg^{-1}) treated group: the animals received a daily dose (400 mg kg^{-1}) of ethanol extract suspended in 0.5% CMC. Group IX, propanolol treated group: the animals received a daily dose of propanolol (30 $mgkg^{-1}$) 30 minutes before AME (100 $mgkg^{-1}$). Group X, yohimbine treated group: the animals received a daily dose of yohimbine (2 $mgkg^{-1}$) 30 minutes before AME (100 $mgkg^{-1}$).

The anti-hyperalgesic effect was examined in the diabetic rats using tail immersion method and hot plate method. The tail of the animal was dipped in hot water bath maintained at 52.5 ± 0.5ºC [6] and the flicking response of rat tail was observed. The cut off period of 12 s was observed. The animals were individually placed on Eddy's Hot Plate maintained at 55 ±1ºC and paw licking or the jump response was taken as the end point. The cut off period was 10 s. The assessment of hyperalgesic activity was done by measuring the nociceptive latency at 15, 30, 60, 90 and 180 min after each drug injection on 14th day.

All data is expressed as mean ± S.E.M. One way analysis of variance with Tukey test was used for evaluating statistical significance between different groups and values with $p<0.05$ were considered to be statistically significant using Instat software (Graph Pad Software Inc., San Deigo).

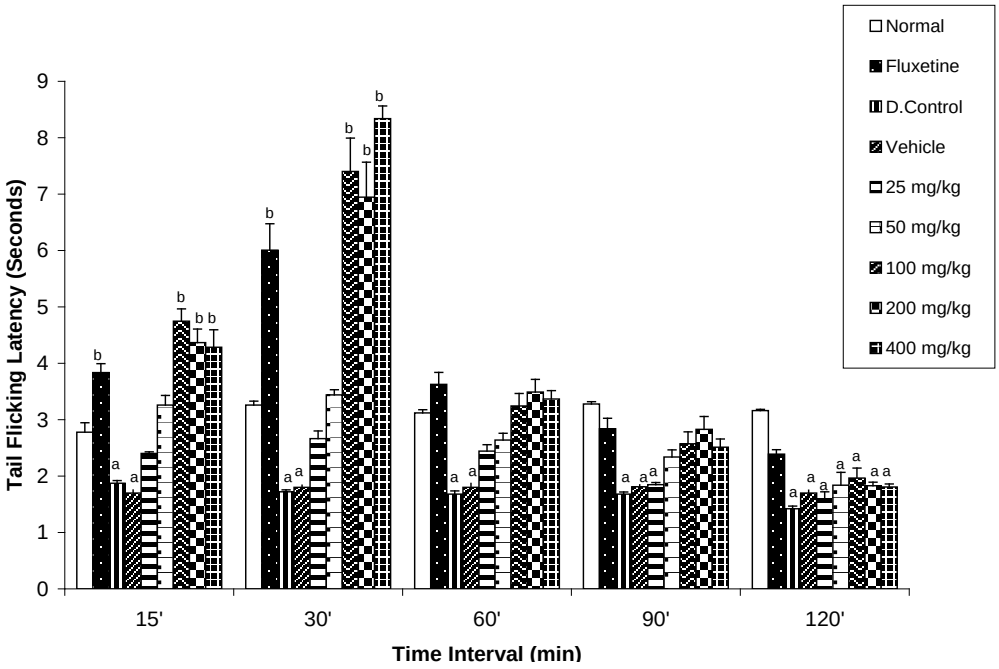

Fig. 1: Effect of various pharmacological interventions on tail flicking latency (seconds) in control and diabetic rats. All values are expressed as mean ± SEM. a *vs.* non-diabetic (p< 0.05), b *vs.* diabetic control (p< 0.05)

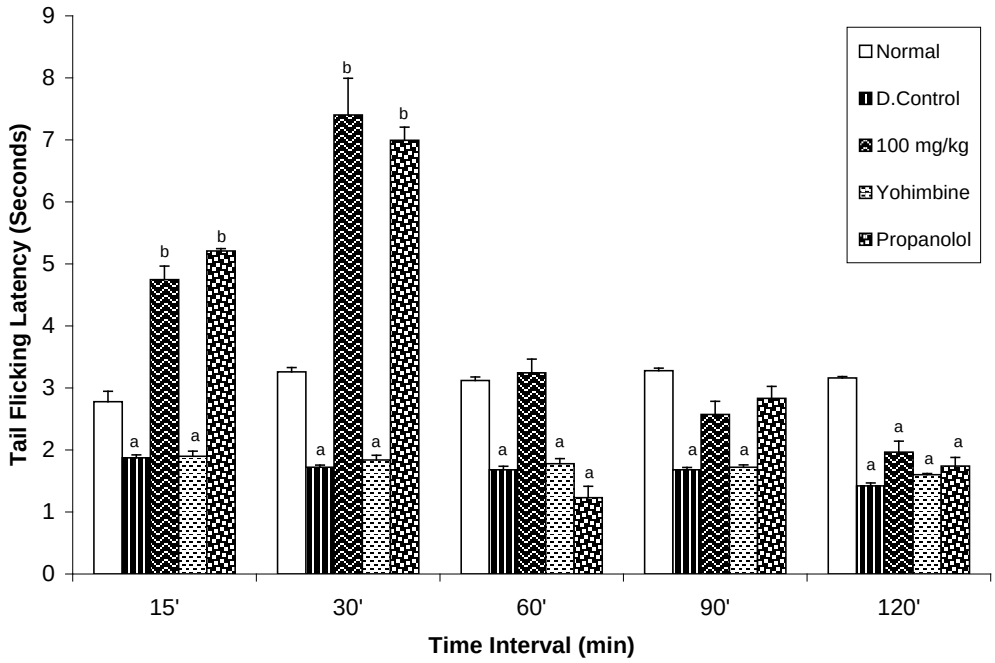

Fig. 2 Effect of yohimbine and propanolol on tail flicking latency in control and diabetic rats. All values are expressed as mean ± SEM. a *vs.* non-diabetic (p< 0.05), b *vs.* diabetic control (p< 0.05)

RESULTS

Effect of different pharmacological interventions on tail flicking latency of diabetic rats in tail immersion method

The nociceptive threshold was found to decrease from 7th day onwards in the diabetic animals and maximum hyperalgesia was observed between 12th to 14th days. On 14th day, the maximum effect was observed at 30 min after administration of each dose. The nociceptive threshold was significantly lower in diabetic control group as compared to non-diabetic and standard (fluoxetine) treated group as observed in tail immersion method. A significant anti-hyperalgesic response was observed in fluoxetine (used as standard in studying hyperalgesia) treated group as compared to diabetic control group. The administration of the ethanolic extract at different doses (25, 50, 100, 200 & 400 mgkg^{-1}) showed an increase in nociceptive threshold dose dependently.

The treatment with propanolol did not alter the increase in nociceptive threshold due to AME whereas the treatment with yohimbine attenuated the AME induced increase in nociceptive threshold (Fig.1, 2).

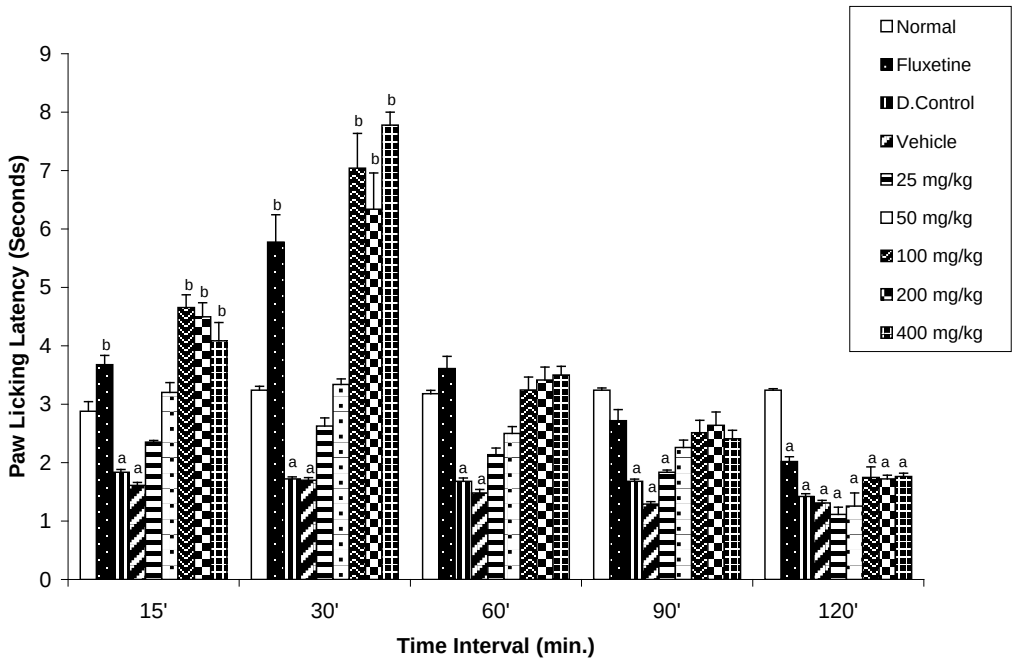

Fig. 3 Effect of various pharmacological interventions on paw licking latency in control and diabetic rats. All values are expressed as mean ± SEM. a *vs.* non-diabetic (p< 0.05), b *vs.* diabetic control (p< 0.05)

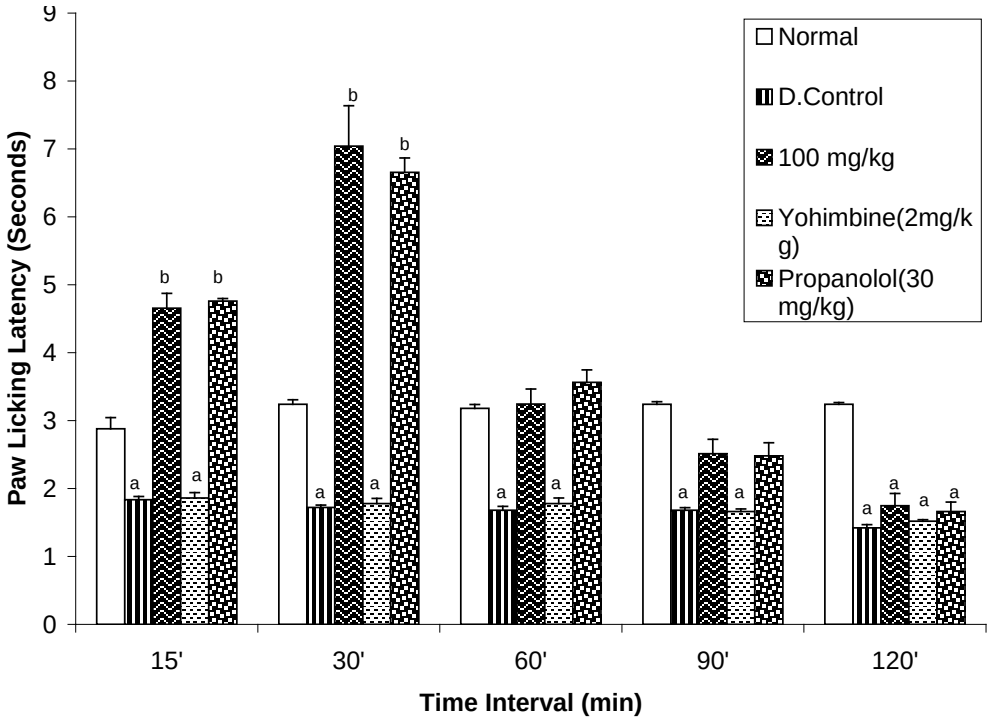

Fig.4 Effect of yohimbine and propanolol on paw licking latency in control and diabetic rats. All values are expressed as mean ± SEM. a *vs.* non-diabetic (p< 0.05), b *vs.* diabetic control (p< 0.05)

Effect of different pharmacological interventions on paw licking latency of diabetic rats in hot plate method

The nociceptive threshold was found to decrease from 7th day onwards in the diabetic animals and maximum hyperalgesia was observed between 12th to 14th days. On 14th day, the maximum effect was at 30 minutes after administration of each treatment. The nociceptive threshold was significantly lower in diabetic control group as compared to non-diabetic and standard treated group as observed in hot plate method. A significant anti-hyperalgesic response was observed in fluoxetine treated group as compared to diabetic control group. The administration of various doses of ethanol extracts (25, 50, 100, 200 & 400 mg kg^{-1}) in diabetic rats showed significant increase in paw licking latency as compared to diabetic control in a dose dependent pattern. The treatment with propanolol did not alter the increase in nociceptive threshold due to AME whereas the treatment with yohimbine attenuated the AME induced increase in nociceptive threshold (Fig.3, 4).

DISCUSSION

Peripheral neuropathy is one of the most common early complications of diabetes. The hyperalgesia due to diabetic neuropathy is evident after 7 days and reaches its peak after 14 days [15-16]. A similar course of hyperalgesia in diabetic rats was observed in the present study. The diabetic animals exhibited first symptoms of hyperalgesia from 7th day of alloxan injection and maximal hyperalgesia was observed between 12th to 14th days of inducing diabetes. The pathophysiology of diabetic neuropathy is complex and the exact contributing factors are poorly understood.

Diabetic neuropathy is associated with axonal demyelination [17] which manifests itself by the decrease in the amplitude of compound muscle action potential (CMAP). This decrease is directly proportional to the decrease in the number of myelinated axons. The choice of drugs available for diabetic neuropathy is limited and the treatment is largely symptomatic. Moreover, the drugs used have numerous side effects that further limit their usefulness. Due to these factors, a lot of emphasis is being laid on the factors affecting the quality of life[18] and plant drugs for the treatment of diabetic syndrome [19].

Aegle marmelos is well known for its antidiabetic and anti-oxidant properties [14]. Studies have reported for the leaf extract of the plant to possess analgesic and anti-inflammatory activity [13]. In the present study, extract of leaves of *Aegle marmelos* was found to increase the paw licking latency in the Eddy's hot plate and tail withdrawal latency in the tail immersion model. In the different doses of alcoholic extract, the optimum effect was found at a dose of 100 mg kg^{-1}. Aegeline found in the alcohol extract of *A. marmelos* has been proposed to have a structural similarity to adrenergic receptor ligands[20]. It has been generally accepted that both α and β adrenergic receptors allocated on the membrane surface of beta cells of pancreas regulate the insulin release. The α_2 adrenergic receptors are proposed to be the major adrenergic receptor involved in the modulation of insulin release in pancreatic beta cells[21]. Clonidine, a α_2 receptor agonist has been reported to inhibit mechanical allodynia and hyperalgesia in streptozotocin-induced diabetes[22]. Systemic administration of clonidine and yohimbine has been reported to produce dose-dependent analgesic and hyperalgesic effects in diabetic animals[23]. Yohimbine, an α_2 receptor blocker was found to attenuate the antihyperalgesic effect of *Aegle marmelos* in both hot plate and tail immersion models. However, administration of propranolol prior to *Aegle marmelos* ethanolic extract (100mg/kg) did not attenuate the antihyperalgesic effect. This suggests that β-adrenoceptors may not be involved in the analgesic effect of *Aegle marmelos* whereas α_2 adrenergic receptors may be mediating the effect of *Aegle marmelos* in diabetic neuropathy. Also, the antinociceptive effect of *Aegle marmelos* may involve interplay between adrenergic neurons and other neurotransmitters. There are reports demonstrating interaction with noradrenaline in spinal antinociception mediated by 5-HT[24]. α_2-adrenergic receptor agonists have been reported to increase the release of acetylcholine release while yohimbine blocks the release of acetylcholine. The increase in acetylcholine is proposed to mediate the anti-allodynic effect of clonidine in diabetic rats. The noradrenergic and serotonergic activities are altered in the brain stem of diabetic rats. The insulin treatment is reported to reverse these changes[25]. *Aegle marmelos* leaf extract treatment is reported to regenerate the pancreas in diabetic rats thereby restoring the insulin secretion[26]. From the above studies, it may tentatively be concluded that the antihyperalgesic effect of *Aegle marmelos* may be mediated through the activation of α_2 adrenoceptors.

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