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Research Article
Neurotransmitters Modulating Effect of *Andrographis paniculata* Extract and Isolated Pure Andrographolide in Diabetic Rodents

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Abstract

Background and Objective: *Andrographis paniculata* (Burm. F.) Wall. Ex Nees is an Ayurvedic medicinal plant and it has been traditional used for treatment liver disease, diabetes, cold, fever and diarrhoea. Present study investigated the mechanistic activity of standardized extract of *Andrographis paniculata* (AP) and its isolated pure andrographolide in diabetic rodents for the potential neurotransmitters modulating effect. **Materials and Methods:** Diabetes was induced by single dose of streptozotocin (65 mg kg^{-1}, i.p.) and nicotinamide (120 mg kg^{-1}, i.p.). Rodents with blood glucose levels higher than 250 mg dL^{-1} were used as diabetic rodents. In this study, AP (50, 100 and 200 mg kg^{-1}/day, p.o.) or andrographolide (15, 30 and 60 mg kg^{-1}/day, p.o.) was administered for ten consecutive days. Neurotransmitters modulating effects of AP and andrographolide were evaluated in 5-Hydroxytryptophan (5-HTP) head twitches test in mice, spontaneous locomotor activity in rats and L-dopa potentiation test in mice. **Results:** Diabetic rodents demonstrated significant ($p<0.05$) decrease in head twitches, increase in locomotor activity and decrease in behaviour score compared to nondiabetic rodents during respective behavioural tests. However, AP and andrographolide treated rodents demonstrated significant ($p<0.05$) increase in head twitches, decrease in locomotor activity and increase in behaviour score compared to diabetic control during respective tests. **Conclusion:** The potential anti-depressant activity of AP and andrographolide in diabetic rodents might be due to modulation of central serotonergic and dopaminergic transmission.

Key words: *Andrographis paniculata* andrographolide, diabetes, depression, neurotransmitter

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Andrographis paniculata (Burm. F.) Wall. Ex Nees is an Ayurvedic medicinal plant, belonging to Acanthaceae family. Andrographolide is quantitatively the principal bioactive secondary metabolite of *Andrographis paniculata.* Andrographolide diterpenoids and 2'-oxygenated flavonoids are common chemotaxonomic markers of the Andrographis genus to which *Andrographis paniculata* belongs[1-3]. Extracts of this plant and isolated andrographolide have been used to pharmacologically and experimentally verify its traditional usage for liver disease, diabetes, rheumatoid arthritis, inflammation, cold, fever and diarrhoea[3-6]. In the recent years, many studies on neuropharmacological screening, neuroprotective effect and beneficial effects on cognitive function associated with diabetes have been reported[7-12]. Preclinical information on medicinal phytochemistry and pharmacology of *Andrographis paniculata* extracts and isolated and rographolide strongly suggest that they are promising therapeutic leads, which are potentially useful for treatments of diverse spectrums of psychopathologies commonly encountered in almost all lifestyle associated chronic diseases[9,13-18]. Moreover, the possibility that *Andrographis paniculata* extract and andrographolide possess anti-stress or adaptogenic properties have often been pointed out[18,19]. In addition, during last 3 years, also evaluated *Andrographis paniculata* extract and its isolated pure andrographolide for beneficial effects on comorbid brain disorders generally associated with diabetic rodents[12,15,16,20].

Beside other various psychological and physiological comorbidities in diabetic patients, depression has been found to be more associated with diabetes and it has been reported that prevalence of depression in diabetics is higher than prevalence of depression in normal population[21-23]. Patients with diabetes and depression have been shown to have greater number of risk factors, poor quality of life and increased disability burden[24]. Unfortunately, currently available antidepressant and other psychoactive drugs do not meet the therapeutic demands of diabetic patients and many of them are even contraindicated for patients with diabetes[25-28]. Based on earlier antidepressant activity of selected *Andrographis paniculata* extract in nondiabetic and diabetic rodents[15,16], present study was designed to explore mechanistic activity of standardized extract of *Andrographis paniculata* and its isolated pure andrographolide in diabetic rodents for the potential neurotransmitters modulating effect. Results of these experimentally verified possibilities are described and discussed in this article.

MATERIALS AND METHODS

Animals: Adult Swiss Albino mice (20±5 g) and Adult Charles Foster albino rats (150±10 g), of both sexes were obtained from Central Animal House of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (Registration Number: 542/AB/CPCSEA, dated 22-01-2002). They were housed in groups of six in polypropylene cages (260×190×135 mm for mice and 400×250×155 mm for rats) and maintained in ambient temperature of 25±1°C and 45-55% relative humidity, with a 12:12 h light/dark cycle. They were supplied with commercial food pellets (Pranav Agro Industries Ltd., Sangali, India) and water *ad libitum* unless otherwise stated and were acclimatized to laboratory conditions for 1 week before subjecting them to experimental conditions. Behavioural experiments were conducted between 09.00-14.00 h and 'Principles of laboratory animal care' (NIH publication number 85-23, revised in 1985) guidelines were always followed. Prior approval from the Central Animal Ethical Committee of Banaras Hindu University, Varanasi, India, was obtained for the study protocols (Letter No. Dean/11-12/CAEC/325, dated 30-11-2011).

Plant extract and andrographolide: Standardised hydro-methanolic *Andrographis paniculata* leaves extract (AP; KalmCold™, 32.20%, w/w andrographolide) and andrographolide (99.0% pure by HPLC) were generously supplied by Natural Remedies Pvt. Ltd., Bangalore, India. The plant leaves were collected in the month of March and identified as *Andrographis paniculata* (Burm. F.) Wall. Ex Nees by in-house botanist at R and D Centre of Natural Remedies Pvt. Ltd., Bangalore, India and a voucher herbarium specimen (No. NR582) was kept in the R and D Centre of Natural Remedies Pvt. Ltd., Bangalore, India. Extraction procedure and analytical methods used for standardising AP and isolation of andrographolide were described in details elsewhere[29].

Animal grouping and drug administration: Experimental groups consisting of both sex animals (n = 6) per group were used, the animals were randomly allotted to different experimental groups. *Andrographis paniculata* extract was suspended in 0.3% carboxymethylcellulose (CMC) for once daily per-oral administrations. Andrographolide was macerated with Tween 80 (0.2%) and suspended in 0.2% aqueous agar for daily administrations. Vehicle control groups were similarly treated with vehicle only. All the drug treatments were through per-oral (p.o.) route by using *oral-gavage* (volume up to 10 mL kg^{-1} b.wt.). Standard drug

treated group for respective study was exercised parallel as positive control. The experiment groups are as follow: Non-diabetic (ND) Control, Diabetic (D) Control, D+AP 50 mg kg^{-1}, D+AP 100 mg kg^{-1}, D+AP 200 mg kg^{-1}, D+Andro 15 mg kg^{-1}, D+Andro 30 mg kg^{-1}, D+Andro 60 mg kg^{-1} and D+standard reference drug. Carboxymethylcellulose and agar (Central Drug House, New Delhi, India), Tween 80 (Sisco Research Lab., Mumbai, India) and other chemical and reagents used were from commercial sources.

Diabetes mellitus model: Diabetes mellitus was induced in overnight fasting animals by a single intra-peritoneal (i.p.) injection of 65 mg kg^{-1} streptozotocin (STZ, Sigma, India), followed by i.p. administration of 120 mg kg^{-1} nicotinamide (SD Fine-Chemical Ltd., India) as described elsewhere with some modifications[30]. The STZ treated animals were returned to their cages and provided normal food and 10% sucrose water to minimize hypoglycemic shock. Hyperglycemia was confirmed by elevated glucose level in the blood, determined at 72 h and then on day 7 after STZ injection. Preselected diabetic rodents with blood glucose levels higher than 250 mg dL^{-1} were used in the experiments.

Body weight and blood glucose: Changes in body weight of experimental rodents occurring during the 10-day treatment period were recorded. Blood glucose level was estimated by glucose oxidase/peroxidase method by using commercial available kit (Span Diagnostics Ltd., India). Briefly, glucose is converted to gluconic acid and H_2O_2 in presence of glucose oxidase. Subsequently, in a peroxidase catalysed reaction, the oxygen liberated was accepted by the chromogen system to give a red coloured quinoneimine compound. The absorbance of red colour was measured at 505 nm by using absorbance microplate reader (iMarkTM-Bio-Rad Laboratories, USA) and was found to be directly proportional to glucose concentration.

5-Hydroxytryptophan (5-HTP) head twitches test in mice: The method described elsewhere was followed with some modifications[31]. The last administration of AP (50, 100 and 200 mg kg^{-1}, p.o.) andrographolide (15, 30 and 60 mg kg^{-1}/day, p.o.), or imipramine (15 mg kg^{-1}, p.o., Sun Pharmaceutical Industries Ltd., India) was done on day 10th, 60 min prior to injection of 5-HTP (100 mg kg^{-1}, i.p., Sigma-Aldrich, USA). After 5-HTP injection, number of head twitches displayed by each mouse were observed for 2 min at every 20 min intervals up to 1 h and averaged. The head twitch response was characterized by abrupt lateral movements, which may or may not be accompanied by body twitches and hind limb retractions.

Spontaneous locomotor activity in rats: The spontaneous locomotor activity in photoactometer (Techno Electronics, India) was assessed in rats by method described elsewhere[32]. The last administration of AP (50, 100 and 200 mg kg^{-1}, p.o.) andrographolide (15, 30 and 60 mg kg^{-1}/day, p.o.), or lorazepam (1 mg kg^{-1}, p.o., Cipla Ltd., India) was done on day 10th, 60 min prior to spontaneous locomotor activity test. Each animal was allowed for a period of 5 min in a square closed field arena (30×30×30 cm) equipped with 6 photocells in the outer wall for spontaneous locomotion. Interruptions of photocell beams (locomotor activity) were recorded by means of a 6 digits resettable counter.

L-dopa potentiation test in mice: The technique used was described elsewhere with some modifications[33]. In brief, the last administration of AP (50, 100 and 200 mg kg^{-1}, p.o.) andrographolide (15, 30 and 60 mg kg^{-1}/day, p.o.) or imipramine (15 mg kg^{-1}, p.o., Sun Pharmaceutical Industries Ltd., India) was done on day 10th, 30 min prior to injection of L-Dopa (100 mg kg^{-1}, i.p., Sigma-Aldrich, USA). The behaviour of the animals was rated 30, 45 and 60 min after the administration of L-dopa and averaged. The rating scale was from 0 (no any mice showed hyperactivity) to 6 (all six mice showed hyperactivity and/or jumping).

Statistical analysis: Mean ± standard error of mean (SEM) was calculated for the observed values in each experimental group (n = 6). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test. GraphPad Prism-6 software (GraphPad Software Inc., CA, USA) was used for statistical analysis. P value less than 0.05 was always considered as statistically significant.

RESULTS

Analytical characterization of extract and andrographolide: Standardised *Andrographis paniculata* extract (KalmCold™) contained andrographolide (32.2%, w/w), isoandrographolide (0.5%, w/w), neoandrographolide 2.7%, w/w) andrograpanin (0.9%, w/w), 14-deoxy-11,12 didehydroandrographolide 4.7%, w/w) and skullcap flavone I (0.06%, w/w). The HPLC fingerprints of *Andrographis paniculata* extract and isolated pure andrographolide are shown in Fig. 1.

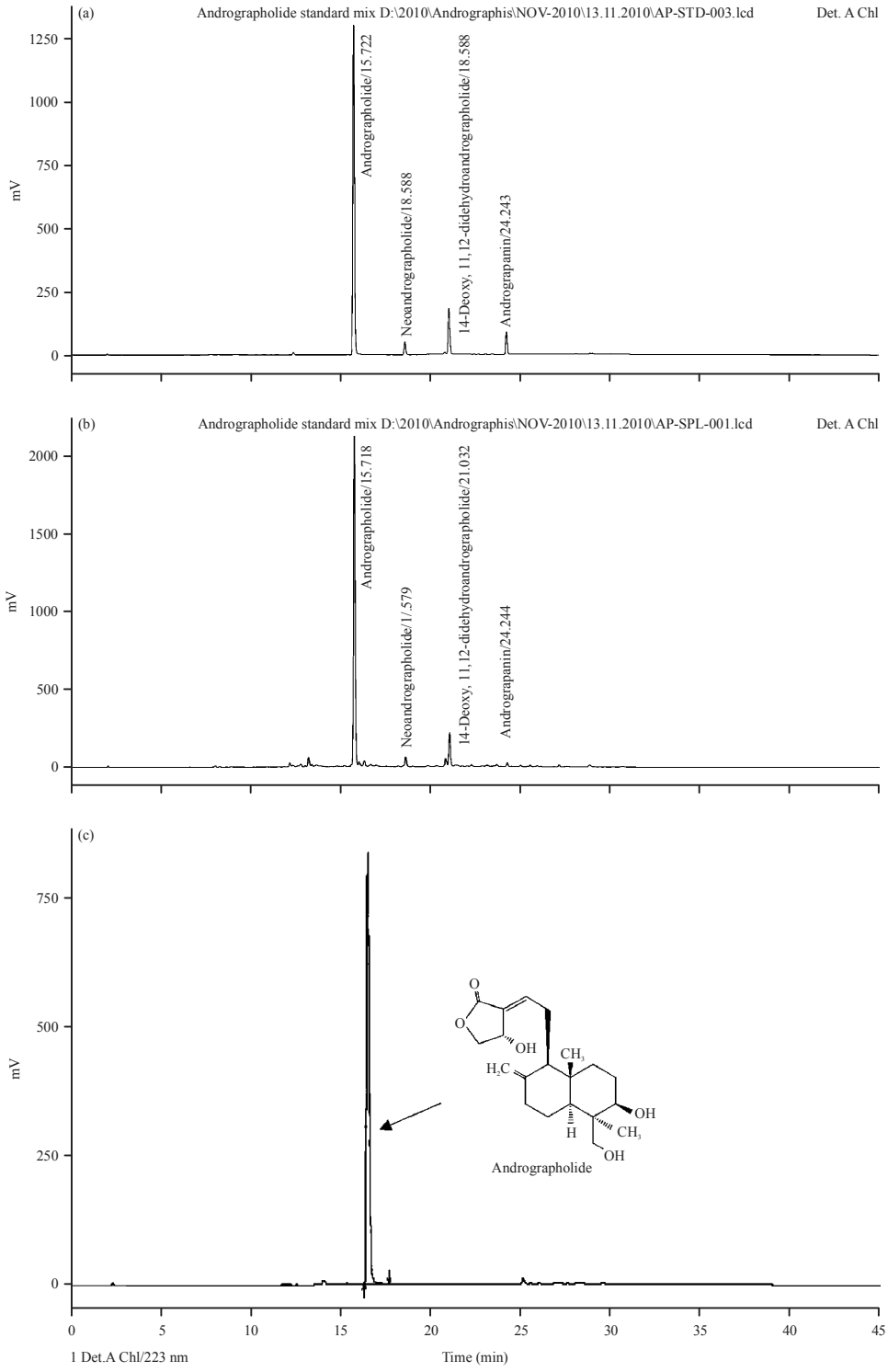

Fig. 1(a-c): HPLC fingerprint of (a) Standard mixtures, (b) *Andrographis paniculata* extract and (c) Isolated pure andrographolide

Body weight and blood glucose: The data for body weight and blood glucose level for each experimental group were recorded and maintained [only data from 5-Hydroxytryptophan (5-HTP) head twitches test is shown in this article]. In this set of experiment, AP or andrographolide treatments significantly ($p<0.05$) reverse the body weight losses and decreased the elevated blood glucose levels in diabetic animals compared to diabetic control mice (Fig. 2).

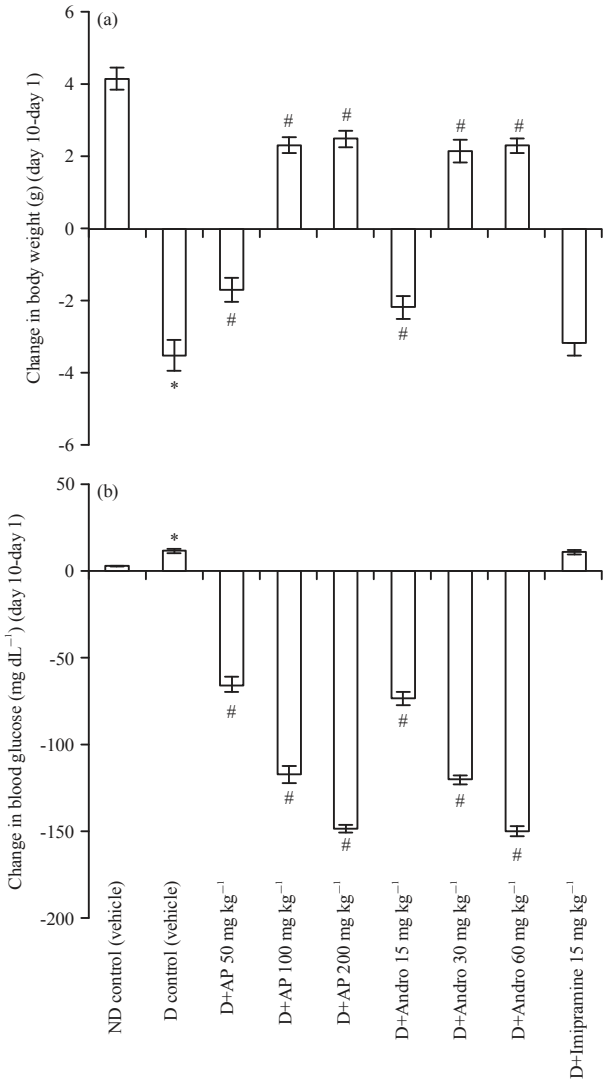

Fig. 2(a-b): Effect of *Andrographis paniculata* extract (AP) and andrographolide on (a) Body weight and (b) Blood glucose level of diabetic mice from 5-Hydroxytryptophan (5-HTP) head twitches test groups

*p<0.05 vs. nondiabetic (ND) control, #p<0.05 vs. diabetic (D) control. Andro: Andrographolide

5-Hydroxytryptophan (5-HTP) head twitches test in mice: The diabetic mice showed significant (p<0.05) decreased head twitches compared to nondiabetic control mice. However, imipramine like significant (p<0.05) increased head twitches were observed in AP (50, 100 and 200 mg kg^{-1}) and andrographolide (15, 30 and 60 mg kg^{-1}) treated diabetic mice (Fig. 3a).

Spontaneous locomotor activity: The diabetic rats showed significant (p<0.05) increase in locomotor activity compared to nondiabetic control rats. However, lorazepam-like significant (p<0.05) decrease in locomotor activity was observed in AP (100 and 200 mg kg^{-1}) and andrographolide (15, 30 and 60 mg kg^{-1}) treated diabetic rats (Fig. 3b).

L-dopa potentiation test in mice: The diabetic mice showed significant (p<0.05) decrease in behaviour score compared to nondiabetic control mice. However, imipramine-like significant (p<0.05) increased behaviour score (potentiation) were observed in AP (100 and 200 mg kg^{-1}) and andrographolide (30 and 60 mg kg^{-1}) treated diabetic mice (Fig. 3c).

DISCUSSION

Although depressive symptoms, anxiety and cognitive dysfunctions are now well-established comorbidities in diabetic patients, but until now more consideration had been paid to depression only[34]. This might be due to the fact that available evidences from extensive population studies suggested that the relationship between diabetes and depression is bi-directional and that such was not necessarily the case for diabetes and anxiety or diabetes and memory impairment[35,36]. However, despite numerous efforts, no definitive statements can yet be made on the complexity of the cause effect relationship between diabetes (and other metabolic disorders) and diverse types of psychosomatic and/or mood related disorders. Recent observations made in laboratory were in agreement with this suggestion and have revealed that an analytically well standardized *Andrographis paniculata* extract rich in andrographolide (>30%) could be a therapeutic option for treatments of comorbid psychopathologies with exaggerated anxiety and depression[15,16]. The observed bio- and neuro-chemical alterations reported earlier revealed that even the lowest tested oral AP dose (50 mg kg^{-1}/day for ten days) was effective in reversing the altered enzymatic activities of both oxidative (MAO-A and MAO-B) as well as anti-oxidative (SOD and catalase) enzymes and lipid peroxide levels in the brain samples of diabetic animals. This dose of AP was also effective in partially reversing the lower hippocampal levels of the three quantified monoamines (NE, DA and 5-HT) in diabetic rats[16]. In view of the observed antihyperglycemic activity in type-2 diabetic animals and antidepressants-like efficacies of AP in diabetic animals[15,37], it was of interest to experimentally verify the possibility of potential neurotransmitters modulating effects of AP and andrographolide in diabetic rodents.

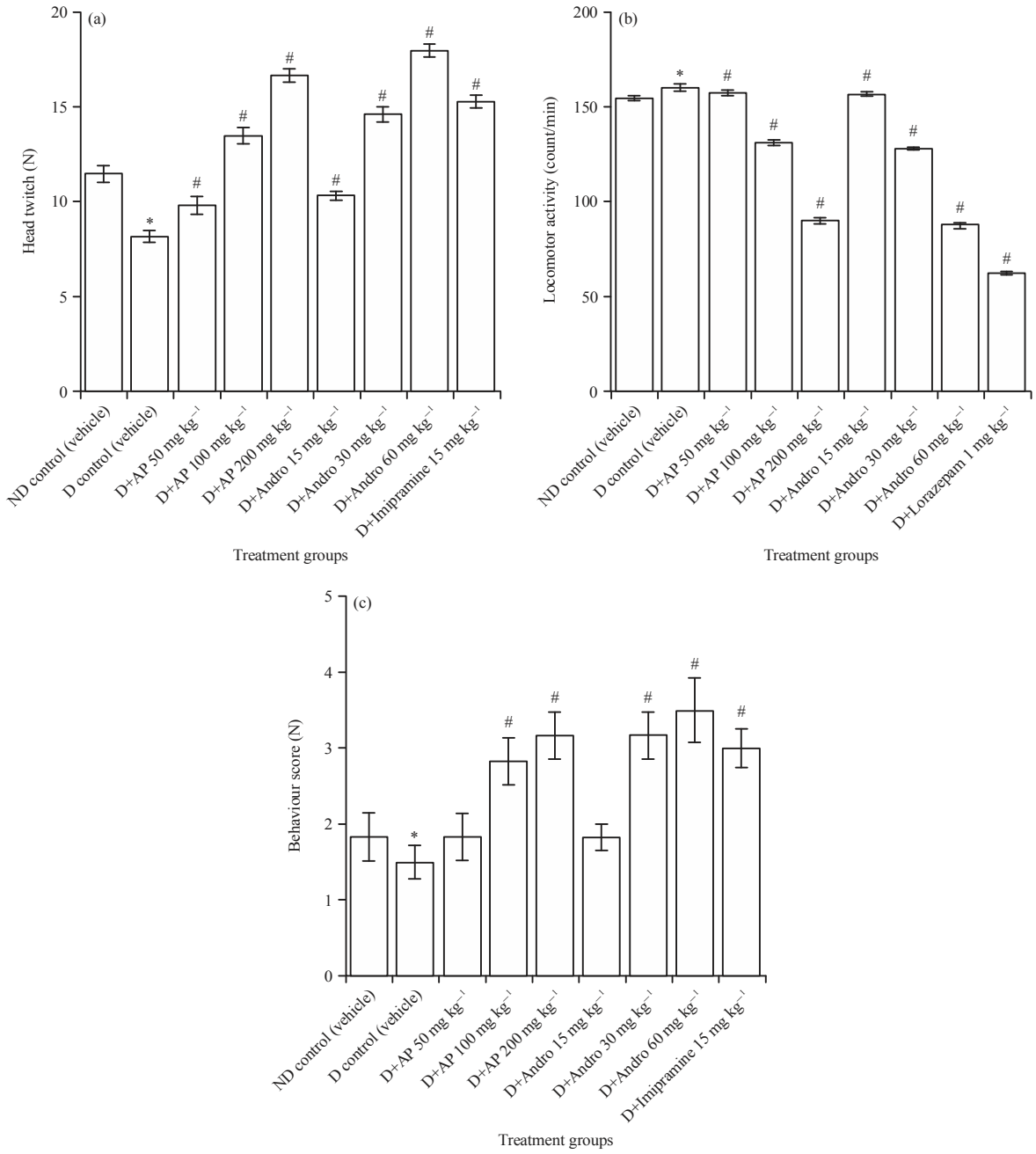

Fig. 3(a-c): Effect of *Andrographis paniculata* extract (AP) and andrographolide on (a) HTP-induced head twitches and (b) Locomotor activity and (c) L-DOPA-induced hyperactivity in diabetic rodents

*p<0.05 vs. nondiabetic (ND) control, #p<0.05 vs. diabetic

Observation reported in this article revealed that neurotransmitters modulating effect of AP and andrographolide is consonant with the antidepressant activity of AP in diabetic rats[16]. 5-Hydroxytryptophan (5-HTP) head twitches test in rodents is based on the monoamine hypothesis of depression. Therefore, antidepressant activity of AP as well as andrographolide might be due to capability of enhancing central serotoninergic functions probably by inhibition of the re-uptake of serotonin, increase in 5-HT level and/or altered MAO enzymatic activities[16]. Moreover, AP and andrographolide treatment in diabetic rats showed significant increase in head twitches compared to diabetic control rats. These results suggest that 5-HTP induced head twitches might be induced by an increase of 5-HT concentration and reduced by MAO enzymatic activities. Locomotor activity is considered as an index of wakefulness or alertness of mental activity and

a decrease in locomotor activity in photoactometer may lead to calming and sedation as a result of reduced excitability of the CNS and CNS acting drugs have been identified to influence the locomotor activity in animals[15,38]. In the case of sedative drugs, they attenuate the motor activity and many authors have used interruption of the light beams as lateral movements of rats or mice in a cage as a tool to investigate CNS active drugs[39,40]. In the present experiment, treatment of AP and andrographolide attenuated motor activity in diabetic rats possibly by its central sedative action and hence, quite possible that earlier observed antidepressant and anxiolytic-like activity of AP is responsible[15,16]. Furthermore, hyperactivity in L-dopa pretreatment in tested extract or andrographolide treated diabetic mice indicating putative effect of AP and andrographolide on dopaminergic system as well. Therefore, central potentiating effects L-dopa pretreatment might be also due to inhibitory effect of MAO and increased DA level in the hippocampus of diabetic rats by AP treatment[16]. These observations were concomitant to the observed monoaminergic activity of AP on diabetic brain by increasing monoamine (5-HT and DA) levels and decreased MAO-A and MAO-B activity.

CONCLUSION

These observations reported in this article justify that AP and andrographolide are involved in modulation of central serotonergic and dopaminergic transmission and responsible for observed antidepressant activity in diabetic rodents. In addition *andrographis paniculata* is another adaptogenic Rasayana herb with an exceptionally broad psychopharmacological activity profile and could be herbal lead for prevention and treatments of diverse spectrums of psychopathologies and other comorbidities commonly associated with diabetes.

SIGNIFICANCE STATEMENT

Andrographis paniculata (Burm. F.) Wall. Ex Nees is an Ayurvedic medicinal plant and it has been traditional used for treatment liver disease, diabetes, cold, fever and diarrhoea. In addition, extracts of this medicinal plant and isolated secondary metabolites have been experimentally verified for neuropharmacological activities, neuroprotective effect and management of depression and cognitive functions associated with diabetes. This study was designed to explore mechanistic activity of standardized extract of *Andrographis paniculata* (AP) and its isolated pure andrographolide in diabetic rodents for the potential neurotransmitters modulating effect. The results suggested that the potential anti-depressant activity of AP and andrographolide in diabetic rodents might be due to modulation of central serotonergic and dopaminergic transmission.

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