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Monoaminergic and L-arginine-no-cGMP pathways mediate the antidepressant–like action of alkaloids from the stem bark of *Trichilia monadelpha*

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ABSTRACT

Background: The role of L-arginine-nitric oxide-cGMP and monoamine pathways in the pathophysiology of depression and as target for antidepressant drugs is well documented. Previously, we reported that *Trichilia monadelpha* possesses antidepressant activity. In that study, total alkaloids (ALK) from *T. monadelpha* showed greatest activity when compared with other phytochemicals. The mechanism of action for ALK in this previous study was, however, not elucidated.
Objective: The current study investigated the involvement of the monoaminergic and L-arginine-NO-cGMP pathways in the antidepressant action of ALK.
Materials and methods: The modified forced swim test (FST) and tail suspension test (TST) in mice were used as models to investigate the involvement of the monoaminergic and L-arginine-NO-cGMP pathways in the antidepressant action of ALK. ALK doses of 30-300 mg/kg, *p.o.* were administered to mice. Experimental process involved pre-treating mice with para-chlorophenylalanine [*p*CPA] (200 mg/kg, *i.p.*); cyproheptadine (80 mg/kg, *i.p.*); reserpine (1 mg/kg, *s.c.*); methyldopa (200 mg/kg, *i.p.*); and reserpine (1 mg/kg, *s.c.*) concomitantly administered with methyldopa (200 mg/kg, *i.p.*), prazosin (3 mg/kg, *p.o.*) and yohimbine (3 mg/kg, *p.o.*). NO pathway was assessed by pre-treating mice with L-arginine (750 mg/kg, *i.p.*), N^G-nitro-L-arginine methyl ester [L-NAME] (30 mg/kg, *i.p.*), methylene blue (10 mg/kg, *i.p.*) and sildenafil (5 mg/kg, *i.p.*).
Results: The antidepressant-like action of ALK was reversed by *p*CPA, methyldopa and/or reserpine. Similarly, cyproheptadine was found to decrease the antidepressant-like action of ALK, while a synergistic effect was observed with yohimbine, but not prazosin. The

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antidepressant-like action of ALK was also decreased by L-arginine and sildenafil. In contrast, a synergistic effect was observed with pre-treatment of L-NAME and methylene blue. **Conclusion**: The monoaminergic systems and L-arginine-NO-cGMP pathways were found to mediate the antidepressant-like action of ALK.

5- HT	5-Hydroxytryptamine
α-MD	alpha methyldopa
ALK	Alkaloids
ATO	Atomoxetine
cGMP	cyclic guanosine 3′5′-monophosphate
CNS	Central nervous system
DA	Dopamine
FLX	Fluoxetine
FST	Forced swimming test
HEE	Hydroethanolic extract
IMI	Imipramine
ICR	Imprinting control region
i.p	Intraperitoneal
L-DOPA	Levodopa
L-NAME	NG-nitro-L-arginine methyl ester
NO	Nitric oxide
NA	Noradrenaline
*p*CPA	Para-chlorophenylalanine
p.o.	*per os*
s.c	subcutaneous
SSRIs	Selective serotonin reuptake inhibitors
TST	Tail suspension test
VMAT	Vesicular monoamine transporter 2

Introduction

According to the World Health Organization (WHO), depression will be the second most prevalent disorder after ischemic heart disease by 2020 [17]. Depression is often linked with suicidal tendencies. Reports suggest that about two-thirds of depressed patients experience suicide thoughts, and about 10-15% attempt suicide [22]. Generally, management of depression is challenging. Several attempts have been made to develop novel drugs (antidepressants) with high efficacy and few adverse effects [13]. In the quest for searching for new antidepressants, the focus has been to develop and/or isolate compounds that have multi-target sites in the brain [14].

Several scientific reports have shown the role of monoamines in the neurobiology of depression. Depressed patients often show symptoms that reflect abnormalities in brain monoamine neurotransmitters, specifically noradrenaline, serotonin, and dopamine [3]. The aforementioned hypothesis of depression has been proven by antidepressants that increase the concentrations and/or activity of these neurotransmitters in the brain.

Over the last decade, the L-arginine–nitric oxide (NO)–cyclic guanosine 3′5′-monophosphate (cGMP) pathway has been found to be a target in the management of depressive disorders [15]. Several studies have associated the action of antidepressants to significant reduction in NO synthesis and with increased monoaminergic neurotransmission [37]. Earlier studies have demonstrated that inhibition of the nitrergic system produces antidepressant-like actions in a variety of animal models [12]. Reports suggest that NO signaling pathway contribute to the antidepressant activities of venlafaxine, bupropion and berberine chloride [16].

Although there are several conventional antidepressants on the market, depression still remains a major cause of morbidity and mortality. The existing conventional antidepressants often have poor remission rates, slow onset of action, persistent side effects, and high refractory rates [2]. These aforementioned challenges with a number of conventional antidepressants are justification for novel agents to be identified.

In recent years, the search for novel therapeutic agents from medicinal plants for brain disorders has increased significantly [16]. A number of medically active phytochemicals have been found in the stem bark of *Trichilia monadelpha* [20]. Secondary metabolites found in plants, for example alkaloids, have been shown to possess antidepressant activities [10]. In our previous study, we reported the antidepressant potential of the total alkaloids from the stem bark of *T. monadelpha*

[18]. However, in that study, we did not show possible mode of action of the total alkaloids. In that study, we reported that the ethanolic extract was safe in the animals after toxicity studies [18]. We also showed that *T. monadelpha* exhibited analgesic effect at low doses [18]. In addition, we hypothesized that the extract might enhance serotoninergic, noradrenergic and opioidergic neurotransmission. The contribution of pain in the pathophysiology of depression is well documented [30]. Moreover, analgesics such as tramadol, that enhance opioidergic activity have shown antidepressant effect [5]. These aforementioned potential beneficial effects of *T. monadelpha* make it a good candidate for antidepressant agent investigations. Since a substantial number of scientific reports have attributed the role of NO pathway in the pathophysiology of depression [11], we investigated the involvement of L-arginine-NO-cGMP as well as the monoaminergic systems in the antidepressant action of *T. monadelpha*.

Materials and methods

Trichilia monadelpha

Botanical name: *Trichilia monadelpha* (Thonn.) J.J. de Wilde
Family: Meliaceae
Local names: Akan (Tanduro), Nzema (Tenuba)
Commercial status: Class IV

T. monadelpha stem bark was harvested from Bomaa, Brong-Ahafo Region, Ghana (7°05'06.60"N,2°10'01.66"W) and authenticated at the herbarium of the Department of Plant and Environmental Biology, University of Ghana (Voucher No. DPT/JM/001).

Preparation of extracts from stem bark of Trichilia monadelpha

The fresh stem bark of *Trichilia monadelpha* was air-dried for 2 weeks, sliced into smithereens and ground into fine powder. The powder was serially extracted with petroleum ether (40-60 °C), ethyl acetate and 70% ethanol over 24 h using the cold maceration technique. The ethanolic fraction, chosen based on earlier study [18] was concentrated to dryness in vacuo using a rotary evaporator at 40-60 °C. This gave a brown syrupy mass, which was further dried using water bath and kept in a desiccator. The yield of the ethanolic extraction was 7.3% w/w.

Preliminary phytochemical screening

The extract was screened for the presence of alkaloids as described by Trease and Evans [33].

Further extraction of alkaloids from the ethanolic fraction [26]

The ethanolic fraction of *Trichilia monadelpha* (50 g) was weighed into a beaker, after which 10% acetic acid in 1 L ethanol was added. This was covered and allowed to stand for 4 h. The solution was filtered and the resultant filtrate was concentrated on a water bath to about one-quarter of the original volume. Drops of concentrated ammonium hydroxide were added until precipitates were formed. The precipitate was decanted, washed with dilute ammonium hydroxide and then filtered. The residue was then collected and dried as total alkaloids (ALK). The final yield was 14.2%.

Experimental animals

Male ICR mice (20±5 g) were acquired from the Noguchi Memorial Institute for Medical Research, University of Ghana. Animals were housed in a well-ventilated, standard clean cages at the animal house of the School of Biomedical and Allied Health Science, University of Ghana. Standard laboratory conditions; temperature at 20–23°C, 12:12 hour light/dark cycle, and relative humidity of 60-70% were maintained. The mice had access to pelletized feed and water *ad libitum*. The animals were made to acclimatize to laboratory conditions for two weeks before start of experiment. All animals used in this study were handled according to the Guide for the Care and Use of Laboratory Animals [25].

Drugs and chemicals

Alpha-methyldopa, reserpine, *para*-chlorophenylalanine, N-nitro-L-arginine methyl ester (L-NAME), L-arginine, noradrenaline, yohimbine, and methylene blue were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Sildenafil and prazosin hydrochloride were obtained from Pfizer, USA. Fluoxetine hydrochloride (Prozac) was from Eli Lilly and Co., Basingstoke, England. Imipramine hydrochloride (Tofranil) was obtained from Mallinckrodt Pharmaceuticals, Ireland.

Site for experiment

All experiments were performed at the Neuropsychopharmacology laboratory of the Department of Pharmacology and Toxicology, School of Pharmacy, University of Ghana.

Animal groupings for tail suspension test

Mice were generally put into groups (n=7) for each tail suspension test. The number of groups varied depending on each experiment.

Tail suspension test (TST)

The tail suspension test (TST) was carried out as initially described by Steru et al. [31]. One hour after oral administration of ALK (30, 100 and 300 mg/kg), fluoxetine (3, 10 and 30 mg/kg), imipramine (30, 100 and 300 mg/kg) and 45 min after intraperitoneal injection of other test agents, mice were individually suspended by the tail from a horizontal bar (distance from floor was 30 cm) using adhesive tape (distance from tip of tail was 1 cm). Test sessions were recorded with a videotape and lasted for 6 min. Behaviour of mice for the last 5 min of the 6-min period were analyzed. Duration of immobility (defined as the absence of all movements except those required for respiration) was scored. Decline in immobility score in the mice was an index of the antidepressant-like effect of *Trichilia monadelpha* or test agent(s).

Animal groupings for forced swimming test

Mice were generally put into groups (n=7) for each tail suspension test. The number of groups varied depending on each experiment using the forced swimming test.

Forced swimming test (FST)

The FST was carried out based on that described by Porsolt et al. [28]. One hour after oral administration of ALK (30, 100 and 300 mg/kg), fluoxetine (3, 10 and 30 mg/kg), imipramine (30, 100 and 300 mg/kg) and 45 min after intraperitoneal injection of other test agents, mice were placed individually in polypropylene cylinders (height 25 cm, diameter 10 cm) filled with water to a height of 15 cm, and maintained at room temperature of 25°C–27°C. With a public domain software JWatcher, version 1.0 behaviour of mice was assessed. For each mouse, the duration of immobility was time of floating upright and mouse making small movements to keep its head above the water. The duration of immobility was scored during the last 5 min of the 6 min test period. A reduction in immobility score was an indication of antidepressant-like effect of *Trichilia monadelpha* or test agent(s).

Assessment of the involvement of catecholaminergic system in the antidepressant-like effect of Trichilia monadelpha

Mice were put into forty (40) groups (n=7). Groups 1 - 10 were pre-treated with α- MD (400 mg/kg, *i.p.*) 3.5 h before administration of ALK (30, 100 and 300 mg/kg, *p.o.*), fluoxetine (3, 10 and 30 mg/kg, *p.o.*), imipramine (3, 10 and 30 mg/kg, *p.o.*) and vehicle (saline). Groups 11 – 20 were also pre-treated with reserpine (1 mg/kg, *s.c.*) 24 hours before administration of ALK (30, 100 and 300 mg/kg, *p.o.*), fluoxetine (3, 10 and 30 mg/kg, *p.o.*) imipramine (3, 10 and 30 mg/kg, *p.o.*) and vehicle (saline). Groups 21-30 were pre-treated with a combination of reserpine (1 mg/kg, *s.c.*) and α-MD (200 mg/kg, *i.p.*) 24 h and 3.5 h respectively before administration of ALK (30, 100 and 300 mg/kg, *p.o.*), fluoxetine (3, 10 and 30 mg/kg, *p.o.*), imipramine (3, 10 and 30 mg/kg, *p.o.*) and vehicle (saline). Group 31 (vehicle) received saline only, groups 32-34 received ALK only (30, 100 and 300 mg/kg *p.o.*), group 35-37 received fluoxetine only (3, 10 and 30 mg/kg *p.o.*) and group 38-40 received imipramine only (3, 10 and 30 mg/kg, *p.o.*) respectively. Mice were pre-treated with reserpine and/or α-methyldopa (α-MD) in order to investigate the possible contribution of catecholaminergic system in the antidepressant activity of the alkaloids of *Trichilia monadelpha*. The doses of α-MD and reserpine were chosen based on previous studies [19]. To deplete newly synthesized pools of noradrenaline (NA) and dopamine (DA), mice were pretreated with a single dose of α- MD prior to behavioural testing. To deplete vesicular pools of NA and DA, mice were pretreated with a single dose of reserpine 24 h before behavioural testing. In an effort to deplete both the vesicular and cytosolic pools of NA and DA, mice were pretreated with a combination of reserpine and α-MD before behavioural testing, respectively**.** TST and FST were conducted again to assess antidepressant action of *Trichilia monadelpha* via the catecholaminergic pathway.

In a separate experiment, mice were put into twelve (12) groups (n=7). Groups 1 - 3 were pre-treated with prazosin (3 mg/kg, *p.o.*) 30 min before administration of ALK (30, 100 and 300 mg/kg, *p.o.*). Groups 4 – 6 were also pre-treated with prazosin (3 mg/kg, *p.o.*) thirty (30) minutes before administration of atomoxetine (1 mg/kg, *p.o.),* imipramine (10 mg/kg, *p.o.*) and saline (vehicle) treatment on the day of experiment. Group 7 received saline only, groups 8 - 10 received ALK only; 30, 100 and 300 mg/kg, *p.o*. respectively, group 11 received atomoxetine only (1 mg/kg, *p.o.)* and group 12 received imipramine only (10 mg/kg, *p.o.*). The effects of a selective α-1 receptor antagonist (prazosin, 3 mg/kg *p.o.*) on the antidepressant-like actions of the ALK (30, 100 and 300 mg/kg, *p.o.*), imipramine (10 mg/kg, *p.o.*) and atomoxetine (1 mg/kg, *p.o.*) were assessed. TST and FST were used to assess period of immobility.

In another experiment, mice were put into twelve (12) groups (n=7). Groups 1 - 3 were pre-treated with yohimbine (3 mg/kg, *p.o.*) 30 min before the ALK (30, 100 and 300 mg/kg, *p.o.*). Groups 4 -6 were also pre-treated with yohimbine 30 min before atomoxetine (1 mg/kg, *p.o.*), imipramine (10 mg/kg, *p.o.*) and saline on the day of experiment. Group 7 received saline only, group 8 - 10 received the ALK (30, 100 and 300 mg/kg *p.o.*) only. Group 11 received atomoxetine (1 mg/kg, *p.o.*)

only and group 12 received imipramine (10 mg/kg, *p.o.*) only. This was to evaluate the effect of a selective α-2 receptor antagonist, yohimbine, on the antidepressant actions of the ALK, imipramine and atomoxetine. TST and FST were used to assess period of immobility.

Assessment of the involvement of serotoninergic systems in the antidepressant effect of Trichila monadelpha

Mice were put into twenty (20) groups made of 7 mice each. *Para*-chlorophenylalanine (*p*CPA; 200 mg/kg, *i.p.*) was administered once daily for 3 consecutive days to 10 groups of animals. On the fourth day, group 1 received saline, groups 2 - 4 received the ALK (30, 100 and 300 mg/kg *p.o.*); groups 5 - 7 received fluoxetine (3, 10 and 30 mg/kg, *p.o.*), groups 8 - 10 received imipramine (3, 10 and 30 mg/kg, *p.o.*). The remaining 10 groups that had no pre-treatment received: ALK 30, 100 and 300 mg/kg, *p.o.*, groups 11 – 13; fluoxetine 3, 10 and 30 mg/kg, *p.o.*, groups 14 – 16; imipramine 3, 10 and 30 mg/kg, *p.o.*, Groups 17 – 19; and saline (group 20). TST and FST were used to assess period of immobility.

In a separate experiment, mice were put into twelve (12) groups (n=7). Groups 1 - 6 were pretreated with cyproheptadine (8 mg/kg, *p.o.*); 30 min before administration of other agents. Mice in groups 1 – 3 received 30, 100 and 300 mg/kg, *p.o.*, and mice in groups 4, 5 and 6 received imipramine (10 mg/kg, *p.o.*), fluoxetine (10 mg/kg, *p.o.*) and saline, respectively. Mice in group 7 received saline only, group 8-10 received only ALK (30, 100 and 300 mg/kg *p.o.*), respectively. Mice in group 11 received fluoxetine (10 mg/kg, *p.o.*) only and group 12 received imipramine (10 mg/kg, *p.o.*) only. The effect of 5-HT2 receptor antagonist cyproheptadine (8 mg/kg, *p.o.*) was assessed on the antidepressant-like actions of the ALK using the TST and modified FST.

Assessment of the involvement of L-arginine-NO-cGMP pathway in the antidepressant-like effect of Trichilia monadelpha

Mice [(n=7) in each group] were pre-treated with a sub-effective dose of L-arginine (750 mg/kg, *i.p.*), a precursor of nitric oxide (NO) or saline 15 min before administration of ALK (30, 100 and 300 mg/kg, *p.o.*). Assessment was conducted 45 min later for period of immobility in the TST and FST [24].

In separate experiments, mice [(n=7) in each group] were pre-treated with L-NAME (30 mg/ kg, *i.p.*), a non-selective nitric oxide synthase (NOS) inhibitor or saline 15 min before administration of ALK (30, 100 and 300 mg/kg, *p.o.*). Assessment was conducted 45 min later to ascertain period of immobility in the TST and FST [1].

In separate experiments, mice [(n=7) in each group] were pretreated with methylene blue (10 mg/ kg, *i.p.*), an inhibitor of NOS and an inhibitor of soluble guanylate cyclase (sGC) or saline 15 min before administration of ALK (30, 100 and 300 mg/kg, *p.o.*). Assessment was conducted 45 min later for immobility time in the TST and FST.

In another experiment, the possible role of cyclic guanosine monophosphate (cGMP) in the antidepressant action of ALK was investigated. Mice received an injection of sildenafil (5 mg/kg, *i.p.*, a phosphodiesterase 5 inhibitor) or saline 15 min before administration of ALK (30, 100 and 300 mg/kg *p.o.*). Assessment was conducted 45 min later for immobility time in the TST and FST.

Statistical analysis

GraphPad Prism for windows version 5.03 (GraphPad Software, San Diego, CA, USA) was used to analyze all data. Differences in means were analyzed by one-way or two-way ANOVA, followed by either Newman Keul's or Bonferroni *post hoc* tests, respectively. A P value < 0.05 was considered statistically significant.

Results

Qualitative phytochemical screening

The presence of alkaloids was determined in the ethanolic extract before proceeding to extract total alkaloids (Refer to supplementary data Table 1).

Involvement of catecholaminergic systems in the antidepressant-like effect of Trichilia monadelpha

Alpha-methyldopa pre–treatment

Pre-treatment with α-methyldopa (400 mg/kg, *p.o.*), diminished the antidepressant-like effect of ALK (30, 100 and 300 mg/kg) and IMI (3, 10 and 300 mg/kg) but not FLX (3, 10 and 300 mg/kg) in both TST (Fig. 1A) and (Fig. 1B) FST ($F19,120 = 80.50$, $P<0.0001$; $F19,120 = 60.83$, $P <0.0001$) respectively. In contrast, α-methyldopa alone did not alter immobility score when compared to the (vehicle) saline treatment

Reserpine pre–treatment

Pre-treatment with reserpine (1 mg/kg, *s.c.*) significantly diminished the antidepressant-like effect of ALK (30, 100 and 300 mg/kg) and IMI (3, 10 and 300 mg/kg) but not FLX (3, 10 and 300 mg/kg) in both TST (Fig. 2A) and FST (Fig. 2B) ($F19,120 = 59.92$, $P<0.0001$, TST; $F19,120 = 64.89$, $P <0.0001$; FST). However, the administration of reserpine alone did not alter immobility score when compared to the (vehicle) saline treated group.

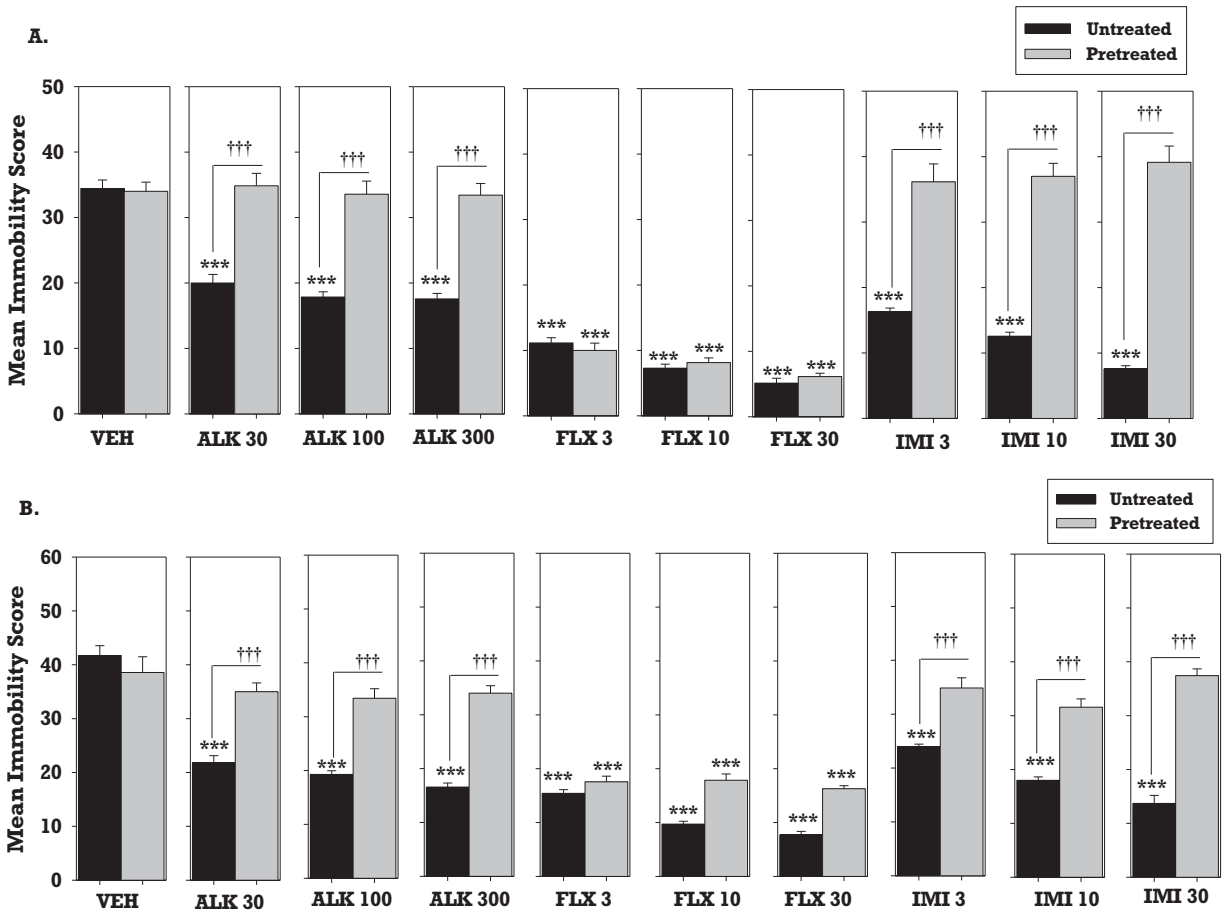

Fig. 1. Effects of α-methyldopa pre-treatment (400 mg/kg, *p.o.*) on murine immobility scores of ALK (30, 100 and 300 mg/kg; *p.o.*) group, FLX (3, 10 and 30 mg/kg, *p.o.*) group and IMI (3, 10 and 30 mg/kg, *p.o.*) group in (A) TST and (B) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle are indicated as: $^{***}P<0.0001$; (One-way ANOVA followed by Newman-Keul's test). †††$P<0.001$; significant difference between drug pretreated and untreated group (Two-way ANOVA with Bonferroni *post hoc* test).

Pre-treatment with both α-methyldopa and reserpine

Pre-treatment with both α-methyldopa (400 mg/kg, *p.o.*) and reserpine (1 mg/kg, *s.c.*) inhibited the antidepressant-like effect of ALK (30, 100 and 300 mg/kg) and IMI (3, 10 and 300 mg/kg) group but not FLX (3, 10 and 300 mg/kg) in both TST (Fig. 3A) and FST (Fig. 3B) ($F19,120 = 62.29$, $P<0.0001$:TST; $F19,120 = 60.89$, $P<0.0001$; FST). See supplementary data.

Pre-treatment with prazosin

Pre-treatment with prazosin (3 mg/kg, *p.o.*), a specific α1-receptor antagonist had no effect on the antidepressant-like effect of ALK (30, 100 and 300 mg/kg, *p.o.*) group and IMI (10 mg/kg, *p.o.*) group but blocked the antidepressant potentials of ATX (1 mg/kg, *p.o.*) group in both TST (Fig. 4A) and FST (Fig. 4B) ($F19,120 = 42.13$, $P<0.0001$:TST; $F19,120 = 46.01$, $P<0.0001$; FST). Mice treated with prazosin alone, however, did not show any significant difference in mean immobility score when compared to mice administered with saline only. See supplementary data.

Pretreatment with yohimbine

Pre-treatment with yohimbine (3 mg/kg, *p.o.*), an α2-receptor antagonist significantly potentiated the antidepressant-like effect of the ALK (30, 100 and 300 mg/kg *p.o.*) and ATX (1 mg/kg *p.o.*), but not IMI (10 mg/kg *p.o.*) in both TST (Fig. 5A) and FST (Fig. 5B) ($F19,120 = 28.18$, $P<0.0001$:TST; $F19,120 = 17.80$, $P<0.0001$: FST). See supplementary data.

Involvement of serotoninergic systems

Pre–treatment with pCPA

Pre–treatment of mice with *p*CPA (200 mg/kg, *p.o.*) diminished the antidepressant effect of ALK (30, 100 and 300 mg/kg, *p.o.*) group, FLX (3, 10 and 30 mg/kg, *p.o.*) group, but partially inhibited the antidepressant-like effect of the IMI (3, 10 and

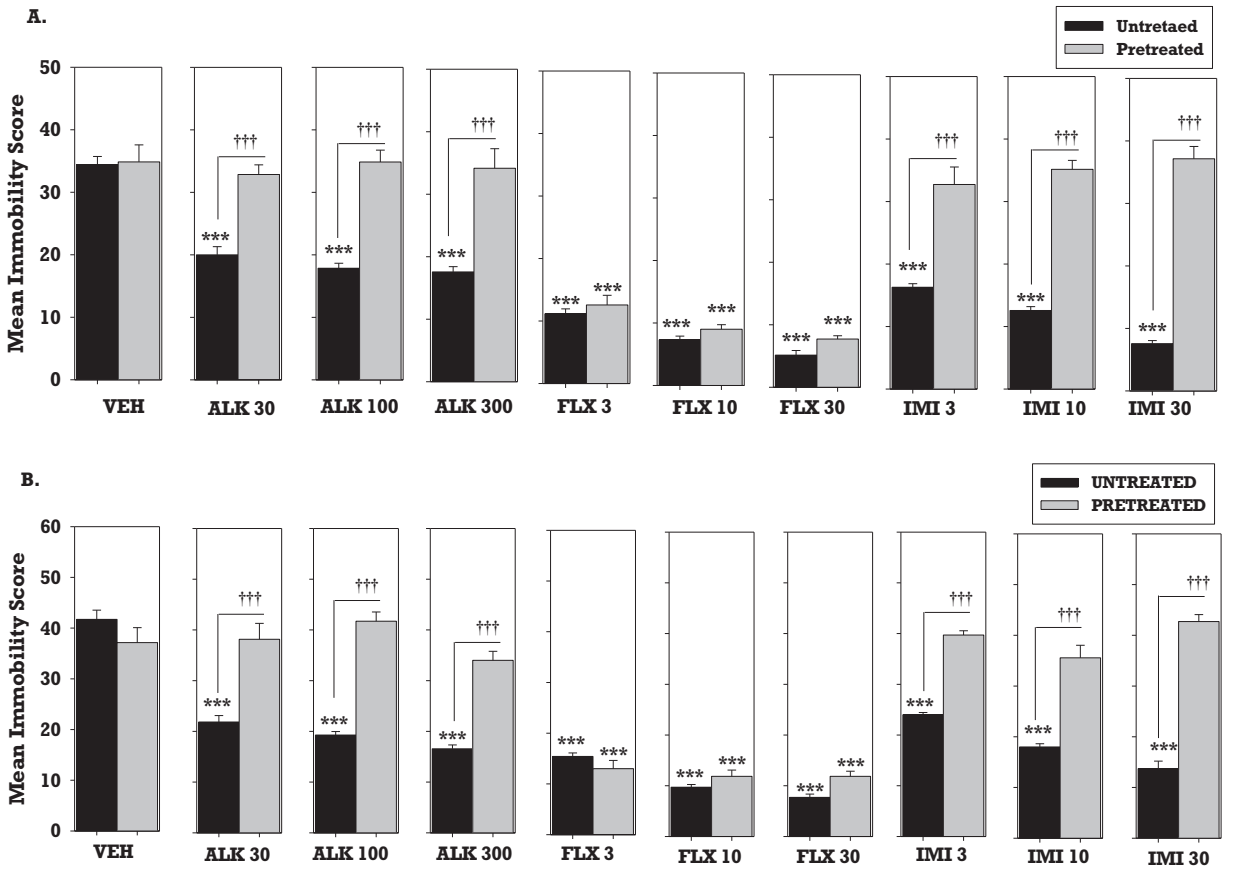

Fig. 2. Effects of reserpine (1 mg/kg, *s.c.*) pre-treatment of mice on immobility scores of ALK (30, 100 and 300 mg/kg, *p.o.*) group, FLX (3, 10 and 30 mg/kg, *p.o.*) group and IMI (3, 10 and 30 mg/kg, *p.o.*) group in (A) TST and (B) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle are indicated as: ***$P<0.0001$; (One-way ANOVA followed by Newman-Keul's test). †††$P<0.001$; significant difference between drug pretreated and untreated group (Two-way ANOVA with Bonferroni *post hoc* test).

30 mg/kg, *p.o.*) group in both the TST and FST. The mean counts for immobility [TST (Fig. 6A); $F19,120 = 118.7$; $P<0.0001$] and [FST (Fig. 6B); $F19,120 = 81.45$; $P<0.0001$] in the ALK and FLX-treated groups after *p*CPA treatment did not show any difference when compared with the control.

Pre-treatment with cyproheptadine

Pre-treatment with cyproheptadine (8 mg/kg, *p.o.*), a 5-HT2 receptor antagonist, diminished the antidepressant-like effect of ALK (30, 100 and 300 mg/kg, *p.o.*), FLX (10 mg/kg, *p.o.*) and IMI (10 mg/kg, *p.o.*) in both the TST (Fig. 7A) and FST (Fig. 7B) ($F11,72 = 92.24$, $P<0.0001$: TST; $F11,72 = 46.18$, $P<0.0001$). Cyproheptadine alone did not show any antidepressant activity.

Involvement of nitric oxide

Pre-treatment with L-arginine

Pretreatment with L-arginine (750 mg/kg, *i.p.*, a precursor of nitric oxide) reversed the reduction in mean immobility score induced by ALK (30, 100 and 300 mg/kg) in both TST (Fig. 8A) ($F7,48 = 17.04$, $P< 0.0001$) and FST (Fig. 8B) ($F_{7,48} = 42.48$ $P< 0.0001$), showing that pre-treatment with L-arginine significantly inhibited the antidepressant effect of ALK. See supplementary data.

Pre-treatment with L-NAME

L-NAME (30 mg/kg, *i.p.*, a nonselective nitric oxide synthase inhibitor) enhanced the antidepressant-like effect of ALK (30, 100 and 300 mg/kg) in both TST (Fig. 9A) ($F7,48 = 42.89$ $P< 0.0001$) and FST (Fig. 9B) ($F7,48 = 59.88$ $P< 0.0001$). There was further reduction in the mean immobility score after L-NAME pretreatment in the ALK-treated mice.

Pre-treatment with methylene blue

Intraperitoneal administration of methylene blue (10 mg/kg, an inhibitor of NO synthase and an inhibitor of soluble guanylate cyclase) did not alter the immobility score of the vehicle-treated mice. In contrast, methylene blue significantly

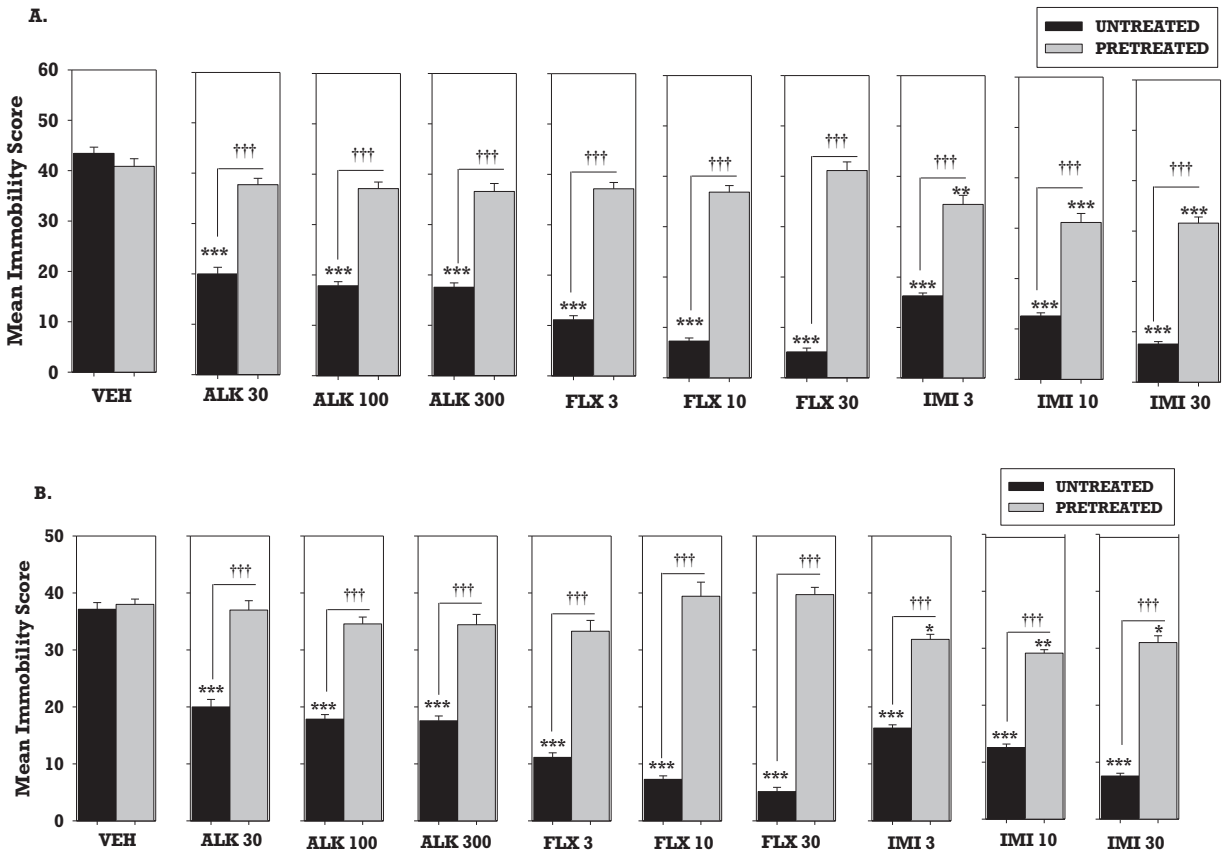

Fig. 6. Effects of pre-treatment of mice with *p*CPA (200 mg/kg, *p.o.*) on immobility scores of ALK (30, 100 and 300 mg/kg, *p.o.*) group, FLX (3, 10 and 30 mg/kg, *p.o.*) group and IMI (3, 10 and 30 mg/kg, *p.o.*) group in (A) TST and (B) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle is indicated as: $^{***}P<0.0001$ (One-way ANOVA followed by Newman-Keul's test). †††$P<0.001$; significant difference between drug pretreated and untreated group (Two-way ANOVA with Bonferroni *post hoc* test).

potentiated the antidepressant-like effect of the ALK (30, 100 and 300 mg/kg) in both TST (Fig. 10A) ($F7,48 = 45.09$ $P< 0.0001$) and FST (Fig. 10B) ($F7,48 = 298.1$, $P< 0.0001$).

Pre-treatment with sildenafil

Pretreatment of animals with sildenafil (5 mg/kg, *i.p.*, a phosphodiesterase 5 inhibitor) significantly increased the mean immobility score elicited by ALK (30, 100 and 300 mg/kg) in both TST (Fig. 11A) and FST (Fig. 11B). See supplementary data.

Discussion

The present study has shown that the total alkaloids from the stem bark of *Trichilia monadelpha* (ALK) has antidepressant-like action mediated *via* the monoaminergic and L-arginine- NO-cGMP systems. In the tail suspension (TST) and forced swimming test (FST), ALK treatment caused significant reduction in immobility, which may be considered as antidepressant effect. Reports suggest that a number of antidepressant agents have the tendency to reduce immobility during TST and FST (Petit-Demouliere et al. [27]).

A number of studies support the role of monoamines in the pathogenesis and management of depression. Reports suggest that drugs that do not have antidepressant actions via the monoaminergic systems may eventually do so after repeated administration [6].

Pre-treatment with α-methyldopa, a competitive inhibitor of the enzyme DOPA decarboxylase (which inhibits the conversion of L-DOPA to dopamine and hence inhibit the biosynthesis of catecholamines) decreased the antidepressant action of ALK and imipramine but failed to decrease the behavioural effects of fluoxetine. This suggests that the antidepressant effect of ALK may depend on the biosynthesis of noradrenaline and/or dopamine. Moreover, α-methyldopa can be converted to α-methylnoradrenaline (a pre-synaptic α_2-adrenergic receptor agonist) by beta-dopamine hydroxylase. Stimulation of α_2-adrenergic receptor in the brain leads to inhibition of vesicular release of noradrenaline (Chhabra [7]). Results from current study also suggest that ALK may also be involved in the vesicular release of noradrenaline; probably via blockade of presynaptic α_2-adrenergic receptors.

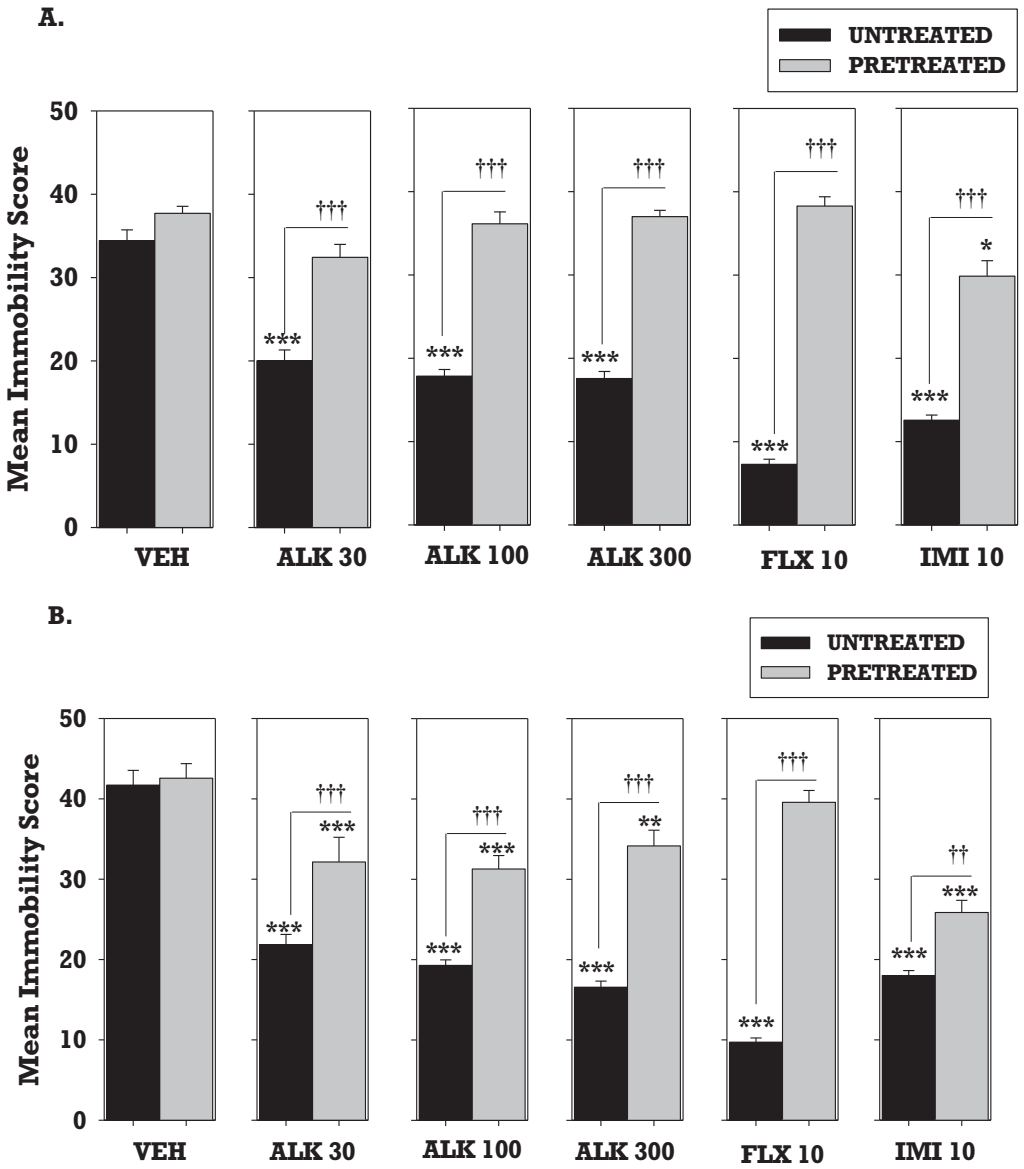

Fig. 7. Effects of pre-treatment of mice with cyproheptadine (8 mg/kg, *p.o.*) on immobility scores of ALK (30, 100 and 300 mg/kg, *p.o.*), FLX (10 mg/kg, *p.o.*) and IMI (10 mg/kg, *p.o.*) in (A) TST and (B) FST. Data are represented as group Means ± SEM of 7 animals. Significantly different from vehicle: $^{***}P<0.0001$; $^{**}P<0.01$ (One-way ANOVA followed by Newman-Keul's test). †††$P<0.001$, ††$P<0.01$; significant difference between drug pretreated and untreated group (Two-way ANOVA with Bonferroni *post hoc* test).

In addition, the ability of reserpine pre-treatment to decrease the antidepressant-like effect of ALK suggests that the depletion of vesicular pools of monoamines may reduce the antidepressant-like action of ALK. Reserpine irreversibly blocks the vesicular monoamine transporter 2 (VMAT-2) which is located primarily within the central nervous system (CNS) and is responsible for transporting free intracellular monoamines into secretory vesicles (Metzger et al. [22]). The ability of reserpine pre-treatment to inhibit the antidepressant-like effect of ALK and imipramine but not fluoxetine seem to suggest that reserpine does not affect vesicular storage of 5-HT to the same extent as that of noradrenaline. Wimalasena [36], reported that reserpine has a low affinity to inhibit VMAT 2–mediated 5HT transport into secretory vesicles. Furthermore, a combination of reserpine and α-methyldopa significantly decreased the antidepressant effect of the ALK, confirming that the antidepressant-like effect of ALK may be dependent on the presence of synaptic catecholamines which are either biosynthesized and/or released in the process.

Results of the present study shows that the antidepressant-like effects of the ALK was not affected by prazosin (an α_1-receptor antagonist). However, atomoxetine (selective noradrenaline re-uptake inhibitor) was inhibited by prazosin pretreatment. Stone et al. [32], reported that activation of brain α_1-adrenoceptors is instrumental in the excitation of mood

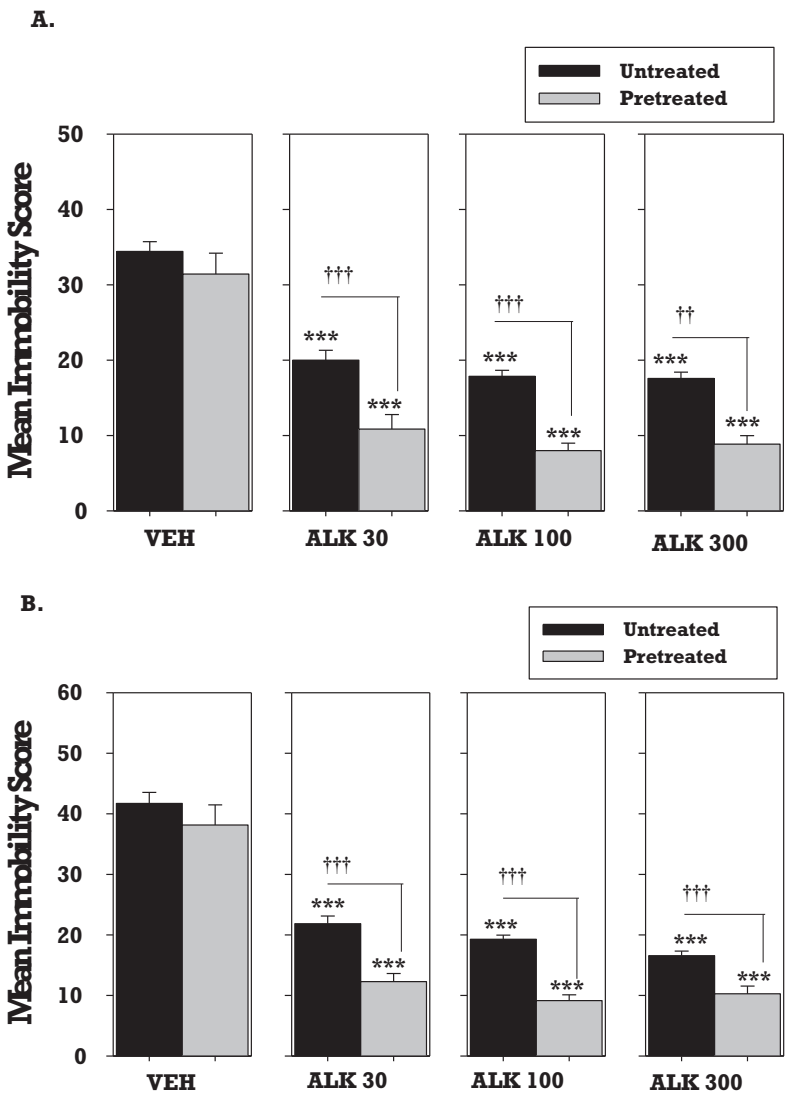

Fig. 9. Effects of pre-treatment of mice with L-NAME on ALK-induced (30, 100 and 300 mg/kg) reduction in immobility score in the (A) TST and (B) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle is indicated as: $^{***}P < 0.0001$; (One-way ANOVA followed by Newman-Keul's test). ††† $P < 0.001$; †† $P < 0.01$; † $P < 0.05$; significant difference between drug pretreated and untreated group (Two-Way ANOVA with Bonferroni *post hoc* test).

associated behaviours and its desensitization or blockade has been implicated in depressive illness. The inability of prazosin to affect the antidepressant-like effect of ALK suggests that activity of ALK is not dependent on activation α_1-adrenoceptors. This finding may be revolutionary since most antidepressant drugs that antagonize α_1-adrenoceptors as part of their mechanism(s) of action are known to cause priapism (a medical emergency) as well as orthostatic hypotension [35]. Though too early, this finding may provide an opportunity to confirm whether ALK does not produce any of these side effects that militate against antidepressant drug compliance and treatment outcomes. The study also revealed that yohimbine (α_2-receptor antagonist) potentiated the antidepressant-like effect of ALK. Presynaptic α_2-adrenergic receptors regulate NA release (autoreceptors) and therefore when antagonized leads to an increase in noradrenaline release. Similarly, if presynaptic α_2-adrenergic receptors are essential for the antidepressant-like effect of a drug, blockade of the receptor would be expected to potentiate rather than reduce its effects on improving mood disorders, however, the opposite is observed if postsynaptic α_2-adrenergic receptors are involved [38]. The present results suggest that blockade of pre-synaptic α2-adrenergic receptors may play a major role in the observed antidepressant-like effect of ALK.

The present experiments also examined the role of serotonin in the acute behavioural effects of ALK in mice models of depression by using a drug that interferes with the neurotransmitter synthesis. Depletion of serotonin by pre-treating mice for 3 days with a tryptophan hydroxylase inhibitor, *para*-chlorophenylalanine (*p*CPA), decreased the antidepressant-like effect of ALK, FLX and IMI, indicating that the observed antidepressant activity is dependent on the enhancement of serotoninergic

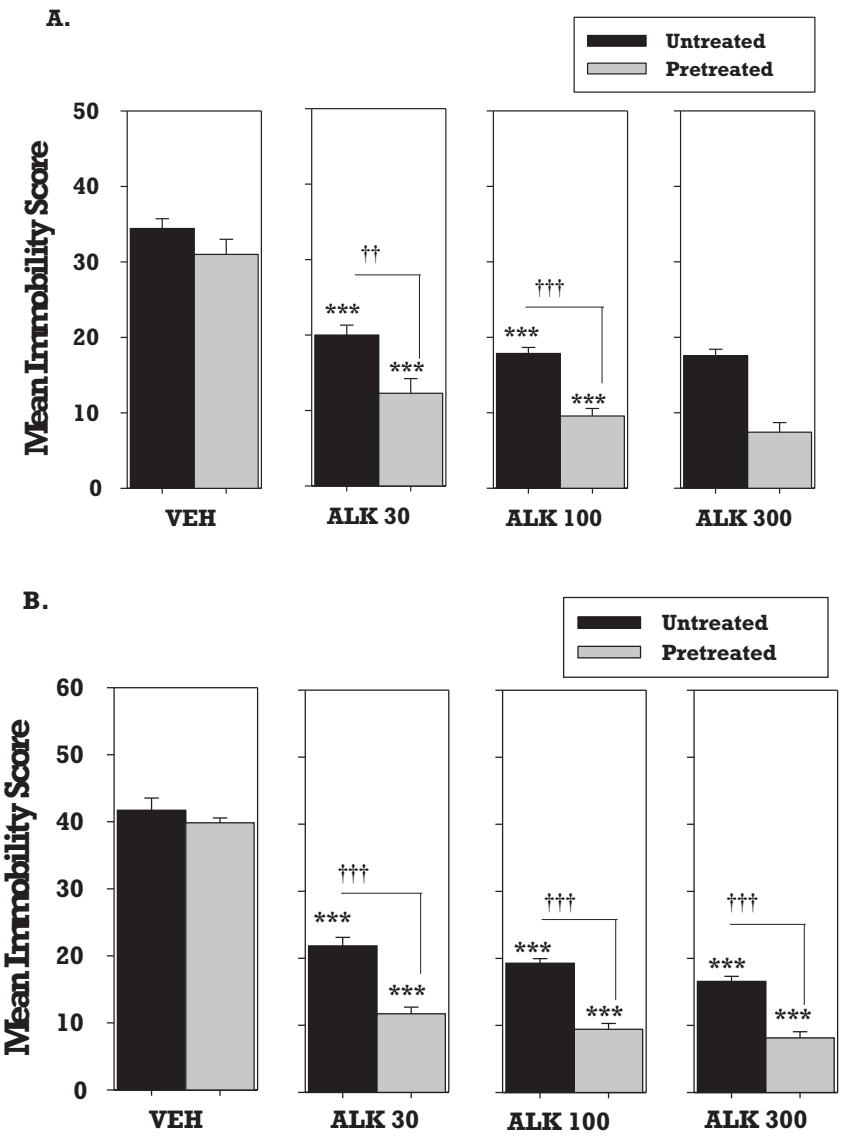

Fig. 10. Effects of pre-treatment of mice with methylene blue on ALK-induced (30, 100 and 300 mg/kg) reduction in immobility score in the (A) TST and B) FST. Each column represents the mean ± SEM (n=7). Significantly different from vehicle is indicated as: $^{***}P < 0.0001$; (One-way ANOVA followed by Newman-Keul's test). $\dagger\dagger\dagger P < 0.001$; $\dagger\dagger P < 0.01$; significant difference between drug pretreated and untreated group (Two-Way ANOVA with Bonferroni *post hoc* test).

neurotransmission. The inhibition of the antidepressant effect of fluoxetine in *p*CPA-treated mice agrees with reports that fluoxetine demonstrate its acute effects via the selective blockade of the serotonin transporter, thus, increasing extracellular serotonin in the synaptic cleft [8]. The study thus indicates that aside catecholamines, the presence of serotonin is also essential in the antidepressant-like effect of ALK.

Both clinical and preclinical studies support the role of 5-HT_2 receptors in depression neurobiology and by extension antidepressant effects of drugs (Opal et al. [3]). In the present study, the reduction in immobility score elicited by ALK in TST and FST was blocked by the pre-treatment of mice with cyproheptadine (a 5-HT_2 receptor antagonist). Several clinical studies have shown that antidepressants with affinity for 5-HT_{2A} receptors augment the clinical response to selective serotonin reuptake inhibitors (SSRIs) in treatment-resistant depressive patients [29]. Desensitization of 5-HT_{2C} receptors is also reported following chronic SSRI treatment. This result further confirms the role of the 5-HT system in the mode of action of ALK.

A plethora of studies have demonstrated that nitric oxide (NO) plays an essential role in the modulating neurotransmission of the nervous system. This has led to pharmacological exploitation of the nitrergic system and contributed to novel therapeutic agents in the treatment of depression [9]. The study demonstrated that pretreatment of mice with L-arginine, a

nitric oxide synthase (NOS) substrate, significantly reduced the antidepressant-like effect of ALK. This suggests that increased levels of nitric oxide in the brain negatively affects the antidepressant effect of ALK. It is plausible the mechanism of antidepressant action of ALK is dependent on competitive inhibition of nitric oxide synthesis, and/or action. Previous studies have demonstrated that the antidepressant-like effect of some classical antidepressants such as imipramine and paroxetine are blocked by pre-treatment with L-arginine [11].

Furthermore, the antidepressant-like effect of ALK was potentiated by pretreating with nitro-L-arginine-methyl ester (L-NAME), a nonselective nitric oxide synthase inhibitor, or methylene blue, an inhibitor of both NOS and sGC. Several studies have shown that NOS inhibitors exert antidepressant-like activity in animal models of depression (Mutlu et al. 2009). In addition NOS inhibitors are known to augment the behavioural effect of tricyclic antidepressants (TCAs) such as imipramine and selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, but not noradrenaline reuptake inhibitors (NRIs) in FSTs [34]. Based on this observation, it has been suggested that serotoninergic mechanisms contribute to antidepressant-like effects of the NOS inhibitors. It has been reported that NO can inactivate tryptophan hydroxylase, the rate limiting enzyme in the synthesis of serotonin thus leading to depletion of serotonin levels and subsequently impairment of serotonin neurotransmission. In addition, ineffective doses of NOS inhibitors have been shown to potentiate the antidepressant effect of TCAs, SSRIs but not SNRIs in the FST [37]. From the current study, reduction of the immobility time elicited by ALK may be due to inhibition of NO synthesis as well as inhibition of the action of soluble guanylate cyclase, the nitric oxide receptor responsible for the conversion of GTP to cGMP. Inhibition of sGC, therefore, reduces synthesis of cGMP, which is also implicated in depression.

Heiberg et al. [21] demonstrated that excessive cGMP levels may generate some depressive symptoms and decreasing cGMP levels may show antidepressant-like actions. Phosphodiesterase enzyme is responsible for the degradation of cGMP. Therefore inhibiting the enzymatic activity of phosphodiesterase may cause an upsurge in the levels of cGMP and thus produce depressive-like conditions [4]. The reversal of the anti-immobility effect of ALK by the PDE_5 inhibitor sildenafil demonstrates that ALK exerts its antidepressant-like effect via the reduction in cGMP levels, either through enhancing its breakdown or inhibiting its synthesis. This finding corroborates reports where sildenafil attenuated the antidepressant-like effects of antidepressant agents [23]. Therefore, the results support the assertion that the inhibition of cGMP synthesis may be a significant drug target for antidepressant agents.

The findings of this research, to the best of our knowledge, is novel since it is the first time 5HT, catecholamines and nitric oxide pathways are being attributed to the antidepressant-like effect of the hydroethanolic extract of the stem bark of *T. monadelpha*.

Conclusion

The present study demonstrated that total alkaloids from *Trichilia monadelpha* possess antidepressant-like effect in mice model, and the anti-depressant effect is mediated *via* enhancement of serotoninergic, noradrenergic and L-arginine-NO-cGMP pathway.

Ethics approval

Ethical approval for this research was obtained from the National Institutional Animal Care and Use Committee, Noguchi Memorial Institute for Medical Research, University of Ghana, with protocol number 2014-02-4N.

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Declaration of Competing Interest

The authors declare they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2020.e00422.

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