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Potentiation of the Response of $GABA_A$ Receptors by Bangladeshi Medicinal Plants

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As part of the search for new sources of mental health supplements, alcohol extracts of 11 Bangladeshi medicinal fruits and plants were investigated for neuropharmacological effects in mice and on ionotropic γ-aminobutyric acid receptors ($GABA_A$ receptors). The extracts of *S. caseolaris*, *T. bellirica*, *S. cumini*, and *T. arjuna* significantly potentiated the GABA-induced response of $GABA_A$ receptors expressed in *Xenopus* oocytes, though the extracts alone induced no response. In mice, administration of these extracts prolonged pentobarbital-induced sleeping time. Potentiation of $GABA_A$ receptor response reportedly generates anxiolytic, sedative, sleep-inducing and anesthetic activities in the human brain. Thus, these extracts may have potential regarding the development of a supplement with tranquilizing and sleep-inducing effects that is beneficial for mental health.

Keywords: Bangladeshi fruit, $GABA_A$ receptor, mangrove tree, pentobarbital-induced sleeping time, tranquilizer

Introduction

As society ages and the amount of lifestyle-related stress increases, there is increasing demand for the development of supplements which improve physical and mental health. Therefore, it is important to find new botanical resources for use as active ingredients for supplements or drugs.

Over 1000 of the estimated 5000 species of phanerogams found in Bangladesh, Southeast Asia are regarded as having useful chemical constituents (Goni, 2003). A variety of these plants have been used traditionally as astringents, antiseptics, tonics, febrifuges and fish poison as they possess active compounds such as alkaloids, polyphenols, saponins, tanic acids, resins, waxes and fragrant compounds (Goni, 2003). However, despite the consumption and use of these herbs and fruits, little scientific data clarifying their physiological activities are available. There is some information available relating to their chemical constituents (Goni, 2003; Sadhu, *et al.*, 2006) and biological properties (Scartezzini *et al.*, 2005; Abdille *et al.*, 2004; Lee *et al.*, 2005). Our laboratory has recently reported the antioxidative, antiamylase, antiglucosidase and antihistamine release activities of some Bangladeshi fruits (Hossain *et al.*, 2008).

Various neurotransmitter receptors are involved in defining mental state, particularly the ionotropic γ-aminobutyric acid receptors ($GABA_A$ receptors), which are the main inhibitory neurotransmitter receptors in the human brain (Hossain *et al.*, 2007). These heteropentamers composed of various α, β, γ, δ and ε subunits were found to be expressed in *Xenopus laevis* oocytes (Trauner *et al.*, 2008). The potentiation of the response of these receptors by drugs such as benzodiazepine, pentobarbital and anesthetics induces tranquilizing, sleep-inducing or anesthetic responses in humans (Nicholls, 1994; Chebib & Johnston, 2000; Harrison *et al.*, 2000). It was found that fragrant compounds such as terpinen-4-ol, 1-octen-3-ol, and linalool potentiated the response of $GABA_A$ receptors expressed in *Xenopus* oocytes after injection of the receptor poly(A)$^+$RNA or cRNA (Aoshima & Hamamoto, 1999; Aoshima *et al.*, 2001; Aoshima *et al.*, 2006; Hossain *et al.*, 2002a, 2003, 2004, 2007; Hossain *et al.*, 2002b). Initially, $GABA_A$ receptors expressed in *Xenopus* oocytes injected with rat whole brain mRNA were used for measurements, but fragrances had similar effects on receptor response to that obtained on injecting cRNA of the α_1 and β_1 subunits

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of bovine $GABA_A$ receptors (Aoshima *et al.*, 2001). The potentiation site for alcohol, anesthetics and pentobarbital is present in $GABA_A$ receptors composed of only α and β subunits (Mihic *et al.*, 1997; Whiting *et al.*, 2000), though the γ subunit is necessary for potentiation of the $GABA_A$ receptor-mediated response by benzodiazepine (Gunther *et al.*, 1995). $GABA_A$ receptors composed of α_1 and β_1 subunits were used in the present study.

Fragrant compounds may modulate mood through potentiation of the $GABA_A$ receptor response after being absorbed into the brain because hydrophobic compounds are easily absorbed through the blood-brain barrier in the same way as tranquilizers, sleeping drugs, and anesthetics. $GABA_A$ receptor channels are modulated not only by clinically important drugs such as benzodiazepines, barbiturates and various general anesthetics, but also by several compounds of plant origin including flavonoids, such as methyl-apigenin (Sheghart, 1995) or eogonin (Hui *et al.*, 2002), polyacetylenes, (Baur *et al.*, 2005), monoterpenes, such as borneol (Granger *et al.*, 2005), and thymol (Garcia *et al.*, 2006). Perez *et al.* (1998) found neuropharmacological properties in the fruit of *Solanum nigrum* which possesses potential CNS-depressant action.

In this study, 11 Bangladeshi fruits and trees traditionally used as medicines were screened for potentiating effects on the response of the $GABA_A$ receptor together with prolonging effects on pentobarbital-induced sleeping time. The aim of this study was to find medicinal plants which can be used in the food and pharmaceutical industries for preparation of functional foods, drinks, supplements and drugs with tranquilizing and sleep-inducing activities.

Materials and Methods

Plant materials The 11 plants investigated in the present study and their traditional uses are presented in Table 1. Five of the samples were collected from the world's largest mangrove forest, in Bangladesh. The other six were collected from a local market in Khulna city, Bangladesh. They were all taxonomically identified by experts at the Bangladesh National Herbarium or authenticated at Forest and Wood Technology Discipline, Khulna University, Bangladesh. The plant materials were cut into small pieces and dried in the sun. The dried materials were ground into a powder with a grinder and stored separately in an air tight container in a cool, dark and dry place.

Preparation of extracts of the plants About 400 g of powdered material was placed in a clean, flat bottomed glass container (4 L) and soaked in 1.3 L of 80% ethanol for *Derris uliginosa*, *Sonneratia caseolaris*, *Embelica officinalis*, *Terminalia bellirica*, *Terminalia chebula*, *Terminalia arjuna*, *Syzigium cumini* and *Dillenia indica*. For *Avicennia officinalis*, *Hibiscus tiliaceous* and *Manilkara zapota*, 80% methanol was used instead of 80% ethanol. The container was sealed for a period of 7 days with occasional shaking and stirring. The whole mixture then underwent coarse filtration through a piece of clean, white cotton followed by filtration through Whatmann filter paper. The filtrate was concentrated using a rotary evaporator (Bibby RE200, Sterlin Ltd., Caerphilly, UK) to obtain the crude extract. The sample yields were 12 to 15% (w/w). These crude extracts

Table 1. Plant samples and their uses[a].

Local name	Name of the plant	Family name	Part used	Medicinal use
White mangrove (Kala Baen)	*Avicenia officinalis* Linn	Avicenniaceae	Leaves	Anti-allergy and diet.
Derris (Pan lota)	*Derris uliginosa*	Leguminosae	Leaves	Anti-allergy and fish poison
Beach hibiscus	*Hibiscus tiliacious* Linn	Malvaeceae	Leaves and Stems	Scorpion-sting and snake-bite.
Sapodilla (Sofeda)	*Manilkara zapota* (L.) Royen	Sapotaceae	Bark	Tonic and febrifuge
Mangrove apple (Orali)	*Sonneratia caseolaris* Linn	Sonneratiaceae	Leaves	Astringent and antiseptic
Amla (Amloki)	*Embelica officinalis* (Gaertn.)	Euphorbiaceae	Fruits	Astringent, diuretic and laxative.
Black Myrobalan (Horitoki)	*Terminalia chebula* (Gaertn.) Retz.	Combretaceae	Fruits	General tonic, astringent and purgative.
Arjuna Myrobalan (Arjun)	*Terminalia arjuna* Roxb	Combretaceae	Bark	Cardiac tonic, astringent and febrifuge
Beleric Myrobalan (Bohera)	*Terminalia bellirica* Roxb	Combretaceae	Fruits	Laxative, astringent and tonic.
Black berry (Jam)	*Syzygium cumini* Linn	Myrtaceae	Fruits	Diet, diarrhea and ringwarm.
Elephant apple (Chalta)	*Dillenia indica* Linn	Dilleniaceae	Fruits	Astringent and pain killer

[a] The Botanical sources and uses were collected from Goni (2003) and Balasooriya *et al.*, (1982).

(20 mg) were dissolved in ethanol (1 mL) for experiments.

To investigate the effect of hydrophobic components in *S. caseolaris* on $GABA_A$ receptor response, a pentane extract of *S. caseolaris* was prepared by adding 1 g of ethanolic *S. caseolaris* extract to 40 mL pentane followed by 24 h vigorous shaking. The pentane phase was obtained after filtration through filter paper. The pentane was then evaporated using a suction evaporator, and the solid taken as a pentane fraction. This solid was dissolved in ethanol (20 mg/mL) and stored at 4°C in a refrigerator and tested in the same way as the other extracts.

Preparation of cRNA and Xenopus oocytes The cRNA of the α_1 and β_1 subunits of bovine $GABA_A$ receptors were synthesized from cloned bovine $GABA_A$ receptor cDNA with RNA polymerase (Promega, Madison, WI) according to the manufacturer's instructions. The cloned cDNA was provided by Prof. Eric A. Barnard at the Medical Research Council Center, London, UK.

Adult female frogs (*Xenopus laevis*) were purchased from Hamamatsu Seibutsu Kyozai Co., (Hamamatsu, Japan). The oocytes were dissected from adult frog ovaries that had been kept in ice for 1 h. They were manually detached from the inner ovarian epithelium and follicular envelope after incubation in a collagenase (type I, 1 mg/mL; Sigma, Tokyo, Japan) solution for 1 h according to the procedure of Kusano *et al.* (1982). The oocytes were microinjected with cRNA in sterilized water and then incubated in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$ and 0.41 mM $CaCl_2$ in 5 mM Tris at pH 7.6) containing 25 mg/L penicillin and 50 mg/L streptomycin at 15 to 18°C for 2 to 7 days before electrophysiological measurements (Aoshima *et al.*, 2001).

Electrophysiological measurements of the response The membrane current of the receptors evoked by GABA was measured by the voltage clamping method with a voltage clamp amplifier (TEV-200A, Dagan Co., Minneapolis, MN) according to the procedure described in a previous paper (Mitou *et al.*, 2008). To examine the agonistic activities of the extracts alone, 40 μg/mL of each extract was dissolved in normal frog Ringer's solution (115 mM NaCl, 1 mM KCl and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) were applied to the oocytes expressing $GABA_A$ receptors. Responses induced by 20 μM GABA were taken as a positive control. To examine the effect of the extracts on the GABA-elicited responses of the $GABA_A$ receptors, GABA dissolved in normal frog Ringer's solution with or without the extract being tested was applied to oocytes expressing the $GABA_A$ receptors. The respective solution was selected by switching a valve in the flow system and the electrical responses induced by a mixture of 10 μM GABA and 40 μg/mL of extract were measured. The control response was obtained by perfusing a 10 μM GABA solution without extract and was taken as 100%. Ethanol at high concentrations potentiates the response of $GABA_A$ receptors but the effect of ethanol present in the extracts is negligible (Aoshima *et al.*, 2001). The measurement was repeated several times in the same oocyte and control values were recorded after every two or three measurements. Values were expressed as the mean of four experiments. Student's *t*-test was used to evaluate the significance of differences between the mean values of the sample and those of the control.

The response (%) was analyzed with the assumption of a simple equilibrium between the active compound and the receptor:

$$\text{Response} - 100 = (V_m - 100)[\text{compound}]/(K_p + [\text{compound}])$$

where [compound], K_p and V_m are the concentration of the compound, the dissociation constant and the maximum potentiation of the receptors when all the receptor potentiation sites are occupied by the compound (Aoshima *et al.*, 2001).

Measurement of pentobarbital-induced sleep in mice Male ddY mice aged 4 weeks and weighing 15 to 30 g were purchased from Kyudo Co., Ltd. (Tosu, Japan). They were housed in Plexiglas cages (10 mice/cage) with a stainless-steel mesh top and excelsior bedding (Clea Japan, Tokyo, Japan). Commercial solid (Clea Japan) and tap water were available ad libitum. The cages were placed in a room artificially illuminated by fluorescence lamps on a 12L:12D schedule (light period: 07:00-19:00), at a temperature of 25 ± 1°C (Umezu, 1999). All experiments proceeded in accordance with the guidelines of the Ethics Committee for Experimental Animals of the Yamaguchi University, Japan, which essentially follows the National Institute of Health Guide for Care and Use of Laboratory Animals.

Pentobarbital-induced sleep was measured as reported by Koda *et al.* (2003). In the present study, two types of extract administration were performed: intraperitoneal injection and oral administration. In the case of intraperitoneal injection, pentobarbital was dissolved in a physiological solution of sodium chloride. Body-weight was measured with a weighing scale. The extracts (10 – 100 mg/kg) were dissolved in olive oil and administered to mice intraperitoneally 30 min before intraperitoneal injection of pentobarbital (50 mg/kg). Olive oil without the extract was administered intraperitoneally as a control. The volume of sample injected was 1 mL/100 g (or 0.2 mL/20g mouse). Oral administration of the extracts was performed by suspending water supply overnight followed by provision of ad libitum access to water containing the extract (2 mg/mL) 5 hours prior to the administration of pen-

tobarbital. Average administration dose of the extracts was estimated from the volume of water with extract consumed. Sleeping time was measured as the time between disappearance and recovery of the righting reflex. To examine the effect of the extracts alone on the behavior of mice, the extracts (100 mg/kg) dissolved in olive oil were administered to mice intraperitoneally and the behavior was observed for 2 h.

Five or six measurements were made for each sample. Student's *t*-test was used to evaluate the significance of differences between the mean values of the sample and those of the control.

Results

Potentiation of the response of $GABA_A$ receptors $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNA of the α_1 and β_1 subunits of the bovine $GABA_A$ receptors as shown in Fig. 1a and Fig. 2a. Plant extracts (40 μg/mL) dissolved in frog Ringer solution induced no response

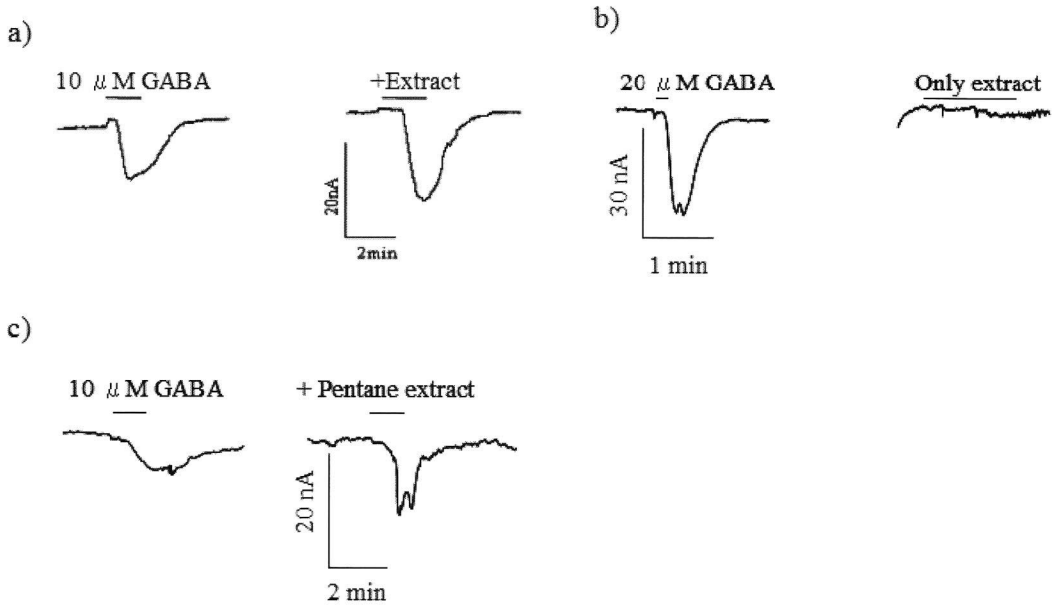

Fig. 1. Effect of the extract of *S. caseolaris* on the response of *Xenopus* oocyte $GABA_A$ receptors, expressed by injection of receptor cRNA. Currents were measured with a voltage clamp at –40 mV. An inward current is shown as a downward curve. Two responses in a given panel were obtained from the same injected oocyte, but responses in panels a, b and c represent different oocytes.

a) Potentiation of $GABA_A$ receptor response by 40 μg/mL extract with 10 μM GABA. The upper bars indicate the timing of application of 10 μM GABA or the mixture of GABA and extract (40 μg/mL).

b) Receptor response induced by the ethanol extract of *S. caseolaris* only. *S. caseolaris* (40 μg/mL) extract was applied to the oocyte expressing $GABA_A$ receptors. The response induced by 20 μM GABA was shown as a positive control.

c) Potentiation of $GABA_A$ receptor response by *S. caseolaris* in the pentane phase (40 μg/mL) in the presence of 10 μM GABA.

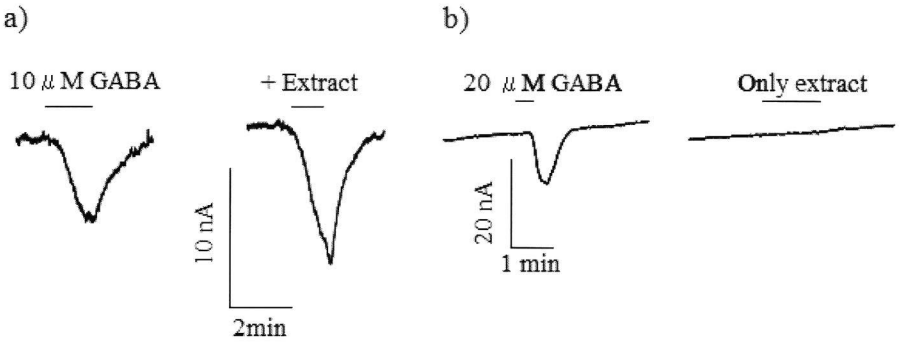

Fig. 2. Effect of the extract of *T. bellirica* on the response of *Xenopus* oocytes $GABA_A$ receptors expressed by injection of receptor cRNA.

a) Potentiation of $GABA_A$ receptor response by ethanol extract of *T. bellirica* (40 μg/mL) in the presence of 10 μM GABA.

b) Receptor response induced by the ethanol extract of *T. bellirica* (40 μg/mL) only.

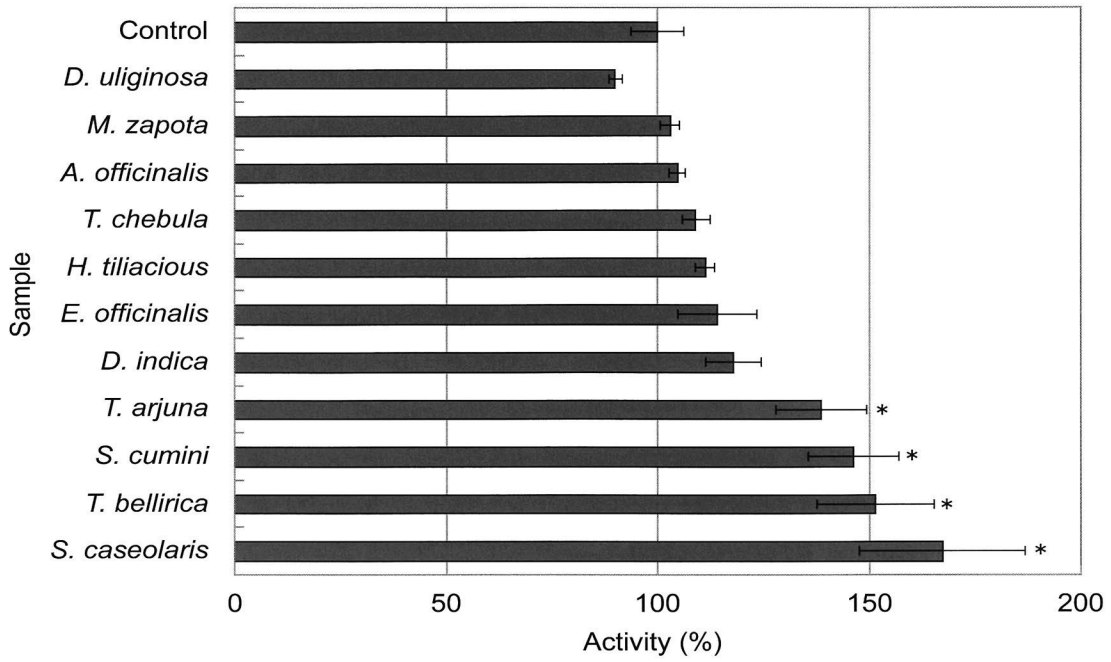

Fig. 3. Effect of various plant extracts (40 μg/mL) on the 10 μM GABA-induced potentiation of $GABA_A$ receptor response. $GABA_A$ receptors were expressed in *Xenopus* oocytes by injecting cRNA prepared from cDNA for the α_1 and β_1 subunits of bovine $GABA_A$ receptors. The control response was obtained by perfusing 10 μM GABA solution without extract and was taken as 100%. Data are the mean ± SD (bars) of four experiments. $^*P < 0.05$, Student's *t*-test of the mean values of the sample and those of the control.

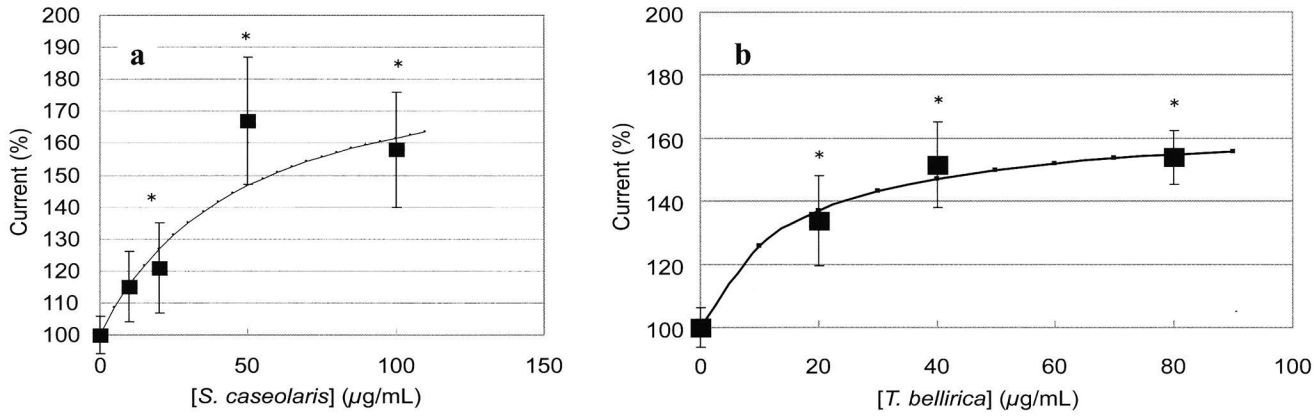

Fig. 4. Dose-potentiation of the extracts of *S. caseolaris* (**a**) and *T. bellirica* (**b**). The extracts at various concentrations were applied simultaneously with 10 μM GABA. The control response was obtained by perfusing the GABA solution without extract and was taken as 100%. The theoretical curve was drawn using the values, $K_p = 48$ μg/mL and $V_m = 191\%$ (*S. caseolaris*) and $K_p = 15.5$ μg/mL and $V_m = 165.36\%$ (*T. bellirica*) on the basis of a simple model (Aoshima *et al.*, 2001). The data are shown as mean ± SD (bars) of four experiments. $^*P < 0.05$, Student's *t*-test for comparison between the mean values of the sample and those of the control.

when they were applied to the injected oocytes (Fig. 1 b and Fig. 2 b), indicating the absence of GABA in the extracts. However, addition of 40 μg/mL of the extract of *S. caseolaris*, *T. arjuna*, *T. bellirica*, or *S. cumini* to the 10 μM GABA solution significantly potentiated the response of the $GABA_A$ receptors as shown in Fig. 3. The extracts of *D. indica*, *E. officinalis*, *H. tiliacious* and *T. chebula* tended to potentiate the response, while that of *D. uliginosa* showed slight inhibitory activity.

The dose-potentiation curves of the extracts of *S. caseolaris* and *T. bellirica* are shown in Fig. 4a and b. The dissociation constant (K_p) and maximum potentiation of the receptors (V_m) when all potentiation sites of the receptors were occupied by the compound were estimated to be 48 μg/mL and 191% and 15.5 μg/mL and 165.36% respectively, with the assumption of a simple equilibrium between the compound and the receptor (Aoshima *et al.*, 2001). When the pentane extract of *S. caseolaris* was applied, potentiation

of receptor response was observed in the pentane phase (Fig. 1c), indicating that active components are lipophilic.

Pentobarbital-induced sleeping time in mice Pentobarbital induces sleep by potentiating the response of $GABA_A$ receptors (Nicholls, 1994). As compounds which potentiate the response have been shown to prolong sleeping time in mice given pentobarbital (Koda *et al.*, 2003; Hossain *et al.*, 2007), we examined the effects of intraperitoneal administration of several extracts (100 mg/kg) on pentobarbital-induced sleep (50 mg/kg) (Fig. 5a). Fig. 5b illustrates the close relationship (R-squared value: 0.922) observed between the extract-associated potentiation of $GABA_A$ receptor response (Fig. 3) and the extension of pentobarbital-induced sleeping time in mice (Fig. 5a). The co-administration of the extracts of *S. caseolaris*, *T. bellirica*, *S. cumini* or *T. arjuna* with pentobarbital prolonged the sleeping time significantly. Oral administration of these extracts had similar effects on sleeping time, as shown in Fig. 5c. The dose-dependence of the

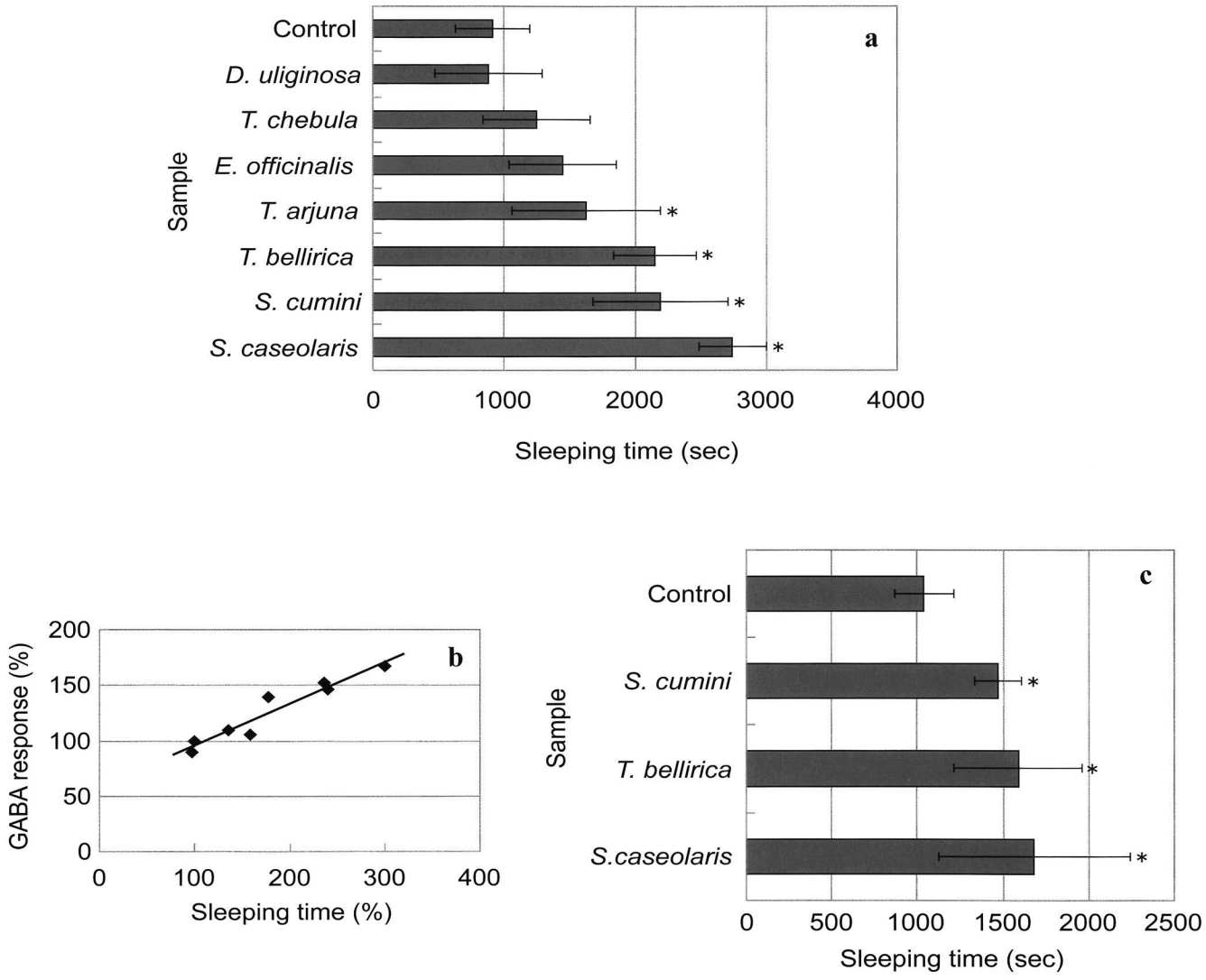

Fig. 5. **a**) Effect of extracts on pentobarbital-induced sleeping time in mice. Pentobarbital (50 mg/kg) was injected into mice intraperitoneally 30 min after intraperitoneal injection of the extracts (100 mg/kg). Sleeping time was calculated as the time between disappearance and recovery of the righting reflex. The data are shown as mean ± SD (bars) for five experiments. The extract of *D. uliginosa* was used as a negative control. $^*P < 0.05$, Student's *t*-test for comparison between the mean values of the sample and those of the control.
b) Relationship between the potentiation of the response of $GABA_A$ receptors (Fig. 3) and the extract-associated extension of pentobarbital-induced sleeping time in mice (Fig. 5a). R-squared value of this relationship was calculated to be 0.922.
c) Effect of the oral administration of extracts on pentobarbital-induced sleeping time in mice. The mean oral administration doses of *S. caseolaris*, *S. cumini* and *T. bellirica* extract were 5.8 mg, 5.4 mg and 6.8 mg per mouse, respectively. As the average mouse weight was approximately 20 g, this represents administration of approximately 300 mg/kg of extract over 5 h.

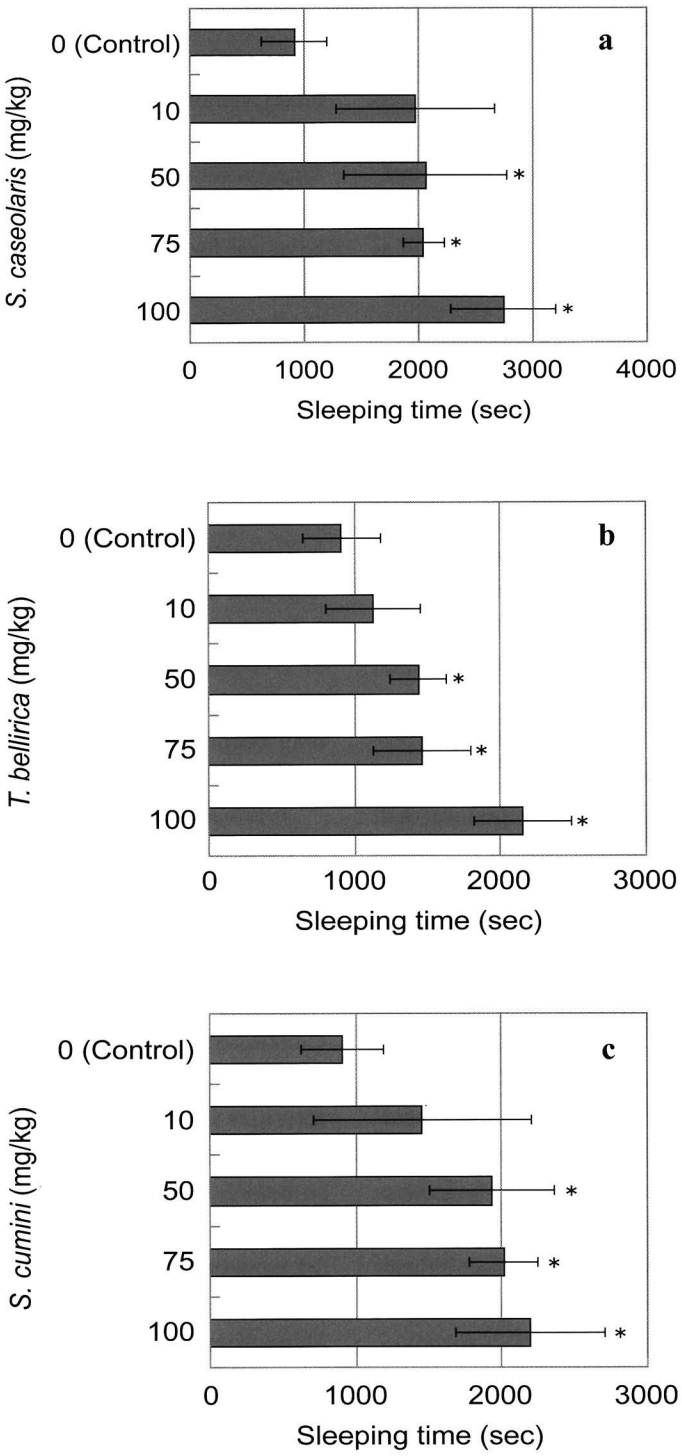

Fig. 6. Dose-dependent effects of *S. caseolaris* (**a**), *T. bellirica* (**b**) and *S. cumini* (**c**) extracts on pentobarbital-induced sleeping time in mice. Pentobarbital (50 mg/kg) was injected into mice intraperitoneally 30 min after intraperitoneal injection of the extracts (10 - 100 mg/kg). $^{*}P < 0.05$, Student's *t*-test for comparison between the mean values of the sample and those of the control.

effects on sleeping time of three extracts was measured as shown in Fig. 6a, b and c. These findings suggest that these extracts act on $GABA_A$ receptors and potentiate their response *in vivo* as well. The co-administration of the extract of *E. officinalis* or *T. chebula* with pentobarbital prolonged sleeping time slightly, while that of *D. uliginosa* did not prolong it at all (negative control) as shown in Fig. 5a.

Discussion

$GABA_A$ receptors are major inhibitory neurotransmitter receptors in the brain responsible for various neurological states such as anxiety, wakefulness and seizures. Potentia-

tion of the responses of $GABA_A$ receptors causes tranquilizing and sleep-inducing effects on the brain, like that of benzodiazepines or pentobarbitals. Therefore, natural products which potentiate the response of $GABA_A$ receptors could be used to reduce anxiety and mental disorders (Hossain *et al.*, 2007).

Recently in Japan, GABA and glycine have been used as food additives with claims that they will induce mental relaxation as they are agonists of major inhibitory neurotransmitter receptors in the brain. However, it is unlikely that GABA and glycine added to foods act on $GABA_A$ and glycine receptors in the central nervous system. This is because neurotransmitters, including GABA and glycine, are usually incorporated selectively into the brain by special transporters and do not pass through the blood-brain barrier freely because of their hydrophilicity. There is a possibility that GABA in the blood acts on metabotropic (G protein-coupled) GABA receptors ($GABA_B$ receptors) in the peripheral nervous system, inhibiting the release of noradrenaline from sympathetic nerves and decreasing blood pressure in hypertensive rats or humans (Hayakawa *et al.*, 2002). Conversely, hydrophobic compounds, such as fragrant compounds, will be incorporated into the brain and act on $GABA_A$ receptors in the central nervous system, as they pass through the blood brain barrier easily.

The addition of alcoholic extracts of *S. caseolaris*, *T. bellirica*, *S. cumini* and *T. arjuna*, which are medicinal fruits and trees used in Bangladesh, to a GABA solution potentiated the response of $GABA_A$ receptors expressed in *Xenopus* oocytes, though the extracts themselves induced no response. The methanol extracts of *A. officinalis*, *H. tiliaceous*, and *M. zapota* did not induce significant responses, possibly because these plants include few effective components. The pentane extract of *S. caseolaris* also potentiated the response, indicating that the active component(s) in the extract are hydrophobic. Moreover, the extracts of *S. caseolaris*, *T. bellirica*, *S. cumini* and *T. arjuna* prolonged pentobarbital-induced sleeping time additively, when administered to mice both intraperitoneally 30 min before the administration of pentobarbital and orally 5 h before the administration of pentobarbital. A close relationship (R-squared value: 0.922) was observed between the potentiation of the response of $GABA_A$ receptors (Fig. 5b) and the extension of pentobarbital-induced sleeping time in mice given by the extracts (Fig. 5a), suggesting that active components are incorporated into the brain and act on the $GABA_A$ receptors. The possibility cannot be excluded that the extracts inhibit the decomposition of pentobarbital in the liver and thus increase sleeping time in mice, but it is unlikely as these plants have been used as traditional medicines for a long time and no toxicity has been reported. These extracts have considerably less effect on $GABA_A$ receptors than synthetic drugs such as benzodiazepine and pentobarbital (Nicholls, 1994; Chebib & Johnston, 2000; Harrison *et al.*, 2000), but their side effects may also be much weaker. Thus, when consumed they will induce only slight mental relaxation and pose little risk. Therefore, these extracts may be of use for the development of supplements which improve mental health as tranquilizers by potentiating the response of $GABA_A$ receptors.

S. caseolaris is a small tree found in tidal creeks and mangrove swamps in Bangladesh. The fruit is used as a poultice on sprains and swellings. The fermented juice of the fruit is useful in arresting hemorrhage and stop-bleeding treatment of piles (Kirtikar & Basu, 1987). The fruits of *T. bellirica* and *S. cumini* are used as medicinal treatments for hepatitis, coughing and hoarseness, and for anti-dysentery, inflammation, and diabetes mellitus, respectively. The bark of *T. arjuna* is used to treat hypotension, and as a cardiac tonic and febrifuge (Goni, 2003). Since these plants have long been used as medicinal products, they should be safe when used as supplements.

It remains necessary to identify the effective components of these active extracts. As $GABA_A$ receptors composed of α_1 and β_1 subunits were used for this study, a benzodiazepine-like compound is unlikely to be such a component (Gunther *et al.*, 1995).

Conclusion

The extracts of 11 Bangladeshi plants were screened for activities beneficial to mental health. Pentobarbital injection after both intraperitoneal and oral administration of *S. caseolaris*, *T. bellierica*, *S. cumini* and *T. arjuna* potentiated the response of $GABA_A$ receptors and significantly extended the pentobarbital-induced sleeping time in mice. These findings suggest that these extracts have tranquilizing activities. These extracts may thus serve as sources of new supplements for the improvement of mental condition. It is necessary to clarify the extract components responsible for these activities, and further examination of these medicinal plants for other beneficial effects is considered worthwhile.

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