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ORIGINAL PAPER

Does patchouli oil change blood platelet monoamine oxidase-A activity of adult mammals?

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Abstract Patchouli oil, an essential aroma oil extracted from patchouli leaf during short-term exposure with five and ten drops either inhibited (at 1 or 2 h) or stimulated (at 4 h) the platelet MAO-A activity depending on the dosages of the aroma oil mainly due to inhibition or stimulation of its K_m. The long-term 15 consecutive days exposure (with two or five drops) of patchouli oil, on the other hand, maximally stimulated the platelet MAO-A activity with five drops patchouli oil for 1 h exposure, but further continuation of its exposure with same doses (two or five drops) for 30 consecutive days significantly stimulated (with two drops) and inhibited (with five drops) the platelet MAO-A activity due to stimulation and inhibition respectively of its corresponding both K_m and V_{max}. These results thus suggest that this aroma oil exposure may modulate the blood platelet serotonergic regulation depending on the dose, duration, and conditions of exposure.

Keywords Blood platelet · Monoamine oxidase-A · Patchouli oil

Introduction

The patchouli plant, *Pogostemon cablin* (Blanco) Benth, is a member of the Lamiaceae family. It is a semi-perennial (when cultivated) and well-branched bushy aromatic plant with fragrant leaves having medicinal and aromatic properties [1]. It is native to the subtropical Himalayas, South East Asia, and the Far East and more particularly to the Philippines, and is commercially cultivated in many countries around the globe, namely Brazil, Indonesia (Java, Sumatra), Malaysia, China, Singapore, Hanoi (Vietnam), etc., including India for its internationally important and valuable essential oil called "patchouli oil" [1–3]. The oil as such is the secondary metabolite of the plant [4] and is mainly obtained by hydrodistillation/steam distillation of the shed-dried and well-cured whole plants/leaves [1, 3]. The oil is thick, light yellow to dark brown in color, and well acclaimed for its characteristic musky-sweet, strong spicy, and long-lasting herbaceous–woody–earthy–camphoraceous smell [1].

Patchouli oil has a pronounced mood-uplifting effect on humans, as it reduces tension, insomnia, and anxiety as an anti-depressant [4]. Numerous terpenes and delicate aroma-chemicals of patchouli oil function coherently and thereby exert healing effects in case of 'aromatherapy' [5], where the overall effect of a 'blend' has been reported to be far greater than the cumulative individual effect of each known component of the 'blend' [6]. It has been established that fragrant molecules on inhalation get absorbed via the lipophilic surface of the lungs and transported into the blood stream to exert distinct healing effects and feeling of happiness (i.e., euphoric action) in humans by stimulating the limbic system (the emotional center of the brain) and behave like pharmacologically active substances [7–11] and remain as either unchanged aromatic molecules or their metabolites before being excreted out rapidly from the body via the skin, sweat, urine, feces, or exhalation [12].

Platelet is an important enucleated blood component containing a plasma membrane, platelet contractile protein (surrounded by a glycocalyx outer layer), mitochondria and other organelles having some definite roles in the physiological functions of a system or body [13]. Monoamine oxidases (MAOs) are a family of mitochondrial outer-membrane bound

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enzymes in most cell types of the body including blood platelet mitochondria [14, 15]. These enzymes are classified into MAO-A and MAO-B (the two isoforms) based on their substrate specificities and inhibitor sensitivities [16]. These enzymes (MAO-A and MAO-B) take part in the metabolism (or breakdown) of different monoamines (or neurotransmitters) and thereby regulate the intracellular monoamine stores to exert some effects in the body [17]. Serotonin (5-HT), one of the monoamines and also known as a "happy messenger", is a specific substrate for MAO-A [13, 16, 17]. MAO-A catalyses 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) and thereby plays a role in regulating mood [18]. In different physiological conditions of neurodegenerative diseases like schizophrenia and Alzheimer's, platelet MAO-A activity has been found to be either reduced, e.g., in schizophrenia [19] or increased as in the case of Alzheimer's [20, 21].

A decrease of platelet MAO-A activity is also associated with bipolar depressed patients [22] and with children suffering from attention-deficit hyperactive disorder (ADHD) [23]. On the other hand, a class of drugs called MAO-inhibitors blocks the action of these enzymes to prevent the breakdown of serotonin and norepinephrine etc., causing their concentration to increase in the brain or plasma to exert some healing effects in the system concerned [18]. Moreover, the roles of patchouli oil exposure by inhalation (with varying doses and durations) upon experimental subjects to influence the modulatory action of 5-HT and that of MAO-A have not been well established so far.

Since (a) patchouli oil has anti-depressant and sedative effects [24, 25]; (b) blood platelet count and platelet serotonin are increased in stress-induced physiological changes [24]; (c) serotonin, a well-known happy messenger as well as neuromodulator modulates mood and other associated behaviors as well as sleep [26] and (d) platelet MAO activity has been found to be used as a biomarker for the alcoholic abusing behavior [27], suicidal behavior [28], depression [29], personally traits [30]. etc., it is not unreasonable to assume that the fragrant molecules present in patchouli oil may have an effect on blood platelet MAO-activity, especially MAO-A, in adult rats. The present study deals with the effect of short- and long-term exposure of patchouli oil (containing 32.693% patchouli alcohol) with varying doses and durations on blood platelet mitochondrial MAO-A activity including its kinetic behavior.

Materials and methods

Materials

Pure patchouli oil, double distilled and stored at room temperature (28 ± 0.5 °C); 5-hydroxy tryptamine (5-HT)-HCl and Triton X-100 were purchased from Sigma Chemicals (St. Louis, MO, USA). Ethylene di-amine tetra acetic acid (EDTA), sodium–potassium tartarate, copper sulphate, sodium hydroxide, potassium dihydrogen phosphate, sodium hydrogen phosphate, sodium carbonate, and semicarbazide of analytical grades were purchased from Merck-India (Worli-Mumbai), India.

Animals

Adult male albino healthy rats derived from original Wistar strain in the age group of 4 months (body weight 150–160 g) were used as experimental subjects. All of these rats at the age of 1 week were supplied by registered animals' breeder company (M/S. Chakrabarty Enterprise; Regn. No. 1 443/PO/b/11/CPCSEA). The rats were maintained in a 12-h light–dark cycle room having a constant temperature of 28 ± 0.5 °C and relative humidity of 80 ± 5%. All of the animals were provided with a standard laboratory diet along with water ad libitum. In the course of this study, the guidelines of the ethical committee (Department of Biochemistry, University of Calcutta) were followed with an effort to minimize the number of subjects used including their suffering.

Pure essential oil of patchouli

The planting materials (i.e., the rooted cuttings) of patchouli were collected from M/S. Keva Biotech Private Limited of Hyderabad, India, followed by transplantation in the field and grown (during pre-monsoon to autumn) by using standard agro-technology with the help of trained farmers at Deganga of 24-Parganas (N), West Bengal, India. Aerial portions of the matured plants were carefully harvested after 4½ months after transplantation, then subjected to a wilting and drying process. The dried and tightly packed planting materials (leaves) were then cured under shed for 4 months prior to commercial distillation. The essential oil of patchouli was thereafter recovered from the so-cured leaves by live-steam distillation method under "optimum conditions of distillation" [31] (i.e., packing of raw material in an effectively designed commercial 'distillation plant' with desired bulk density, quality of steam, steam pressure–temperature, flow rate, and effective cooling of mixed vapor followed by effective separation of liquid mixture, leading to a commercially complete distillation of the charged raw materials) with 4.1% oil yield (v/w) containing 32.693% patchouli alcohol (the major constituent responsible for the characteristic 'note' and commercial value of the oil) and 77 other semi-major and minor (both known and unknown) constituents in different percentages [analyzed by GC/MS (gas chromatography/mass spectometry) using an Agilent 7890A GC connected with 5975C MSD (mass selective detector) and FID (flame

ionization detector) connected with a splitter, e.g., column [DB5-MS (60 m × 0.25 mm × 0.25 μm)], oven etc.] as presented in Table 1. The moisture-free clear-pale brown essential oil of patchouli, bearing the characteristic 'note' or smell, was finally preserved under air-tight conditions in an amber-colored glass bottle at 4 °C for subsequent use in the present experiments.

In order to obtain reproducible results following the exposure of the patchouli oil and to ensure the constant composition of this essential oil (in terms of percentages of oil constituents), the same lot of patchouli oil was used in the present study.

Experimental design

For giving exposure to the experimental animals with the oil of patchouli, pure (undiluted) patchouli oil was to place in drops (1 drop = 26.66 μl) in the top-covered specially designed poly propylene transparent exposure box of 37 cm × 25 cm × 17 cm dimensions having a single set of 6.0-mm-diameter holes on each of the four side walls of the rectangular exposure box (Fig. 1). Each set consists of five such holes where two sets of holes were made on the face-to-face walls at the bottom of the exposure box and the other two sets were made just below the top cover of the two opposite walls of the box in the same face-to-face manner (Fig. 1). Depending on the protocol, two or five or ten drops of oils were used for giving exposure to each animal for the specified period of 1 or 2 or 4 h (either single-exposure: for the short-term exposure sets; or, consecutive-exposure at 24-h intervals: for the long-term exposure sets i.e., for 15 or 30 consecutive days). The face-to-face two sets of bottom-level holes of the exposure box were temporarily sealed by blotting paper strips (1.2 × 10 cm) prior to initiating any exposure (using odourless glue/gum) followed by soaking them with two or five or ten drops of aroma oil in 1:1, 2:3, or 5:5 ratio on each strip; whereas the remaining two sets of holes at the top levels kept unsealed for the purpose of ventilation. Each of the above-mentioned 'exposure boxes' was then taken to house the experimental animal for exposure for the specified period of time. Adult male albino rats ($n = 4$–6) of 4-months age (having body weight in the range of 150–160 g) were separately housed in different duly labeled boxes for exposing each of them for the specified period (for both the short-term and long-term exposure). The rats corresponding to the experimental groups were kept in identical conditions in aroma-

Table 1 GC and GC/MS chart of 15 major and semi-major identified components of the patchouli oil used in the present experiment

Serial no.	RT	Composition (% on FID)	Component	CAS number
1	13.413	0.032	Alpha Pinene (*monoterpene*)	000080-56-8
2	15.401	0.087	Beta Pinene (*monoterpene*)	000127-91-3
3	37.419	0.101	Elemene Delta (*sesquiterpene*)	020307-84-0
4	41.124	3.17	Beta Patchoulene and Beta Elemene (*sesquiterpenes*)	000514-51-2, 000515-13-9
5	43.503	3.619	Trans Beta Caryophyllene (*sesquiterpene*)	000087-44-5
6	44.508	12.031	Alpha Guaiene (*sesquiterpene*)	003691-12-1
7	45.921	8.628	Gamma Patchoulene and Alpha Humulene (*sesquiterpenes*)	000505-55-4, 006753-98-6
8	46.620	5.207	Alpha Patchoulene (*sesquiterpene*)	000560-32-7
9	46.759	1.786	Alloaromadendrene (*sesquiterpenoid*)	025246-27-9
10	47.057	1.494	Patchoulene Gamma (*sesquiterpene*)	000508-55-4
11	48.415	2.215	Aciphyllene (*sesquiterpene*)	087745-31-1
12	49.087	15.806	Alpha Bulnesene (*sesquiterpenoid*)	003691-11-0
13	54.008	0.883	Norpatchoulenol (*terpenoid*)	041429-52-1
14	54.333	1.383	Caryophylene oxide (*sesquiterpene*)	001139-30-6
15	60.666	32.693	Patchouli alcohol (*sesquiterpene alcohol*)	005986-55-0

The identified above-mentioned 15 major and semi-major peaks out of 78 peaks of GC of patchouli oil were identified by GC/MS analysis. These identified 15 components, representing 89.135% of the oil and among them the patchouli alcohol, are the primary candidate molecules containing 32.693% of the oil. During the analysis, the following specification were followed: column: DB5-MS (60 m × 0.25 mm × 0.25 μm); oven: initial temperature 70 °C for 0 min and 2 °C/min to 270 °C for 20 min; inlet: injection volume 0.2 μl, temperature 270 °C, split ratio 50:1, constant pressure mode 20 psi, flow 0.89404 ml/min; detector FID: temperature 270 °C; MS acquisition parameters: acquisition mode scan, low mass 50.0, high mass 550.0, MS source 230 °C, MS quad 150 °C. Other details are mentioned in the "Materials and methods" section

RT retention time, *CAS* chemical abstract service

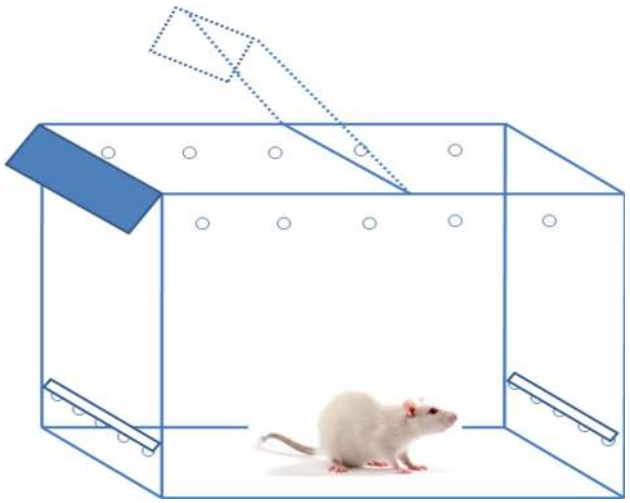


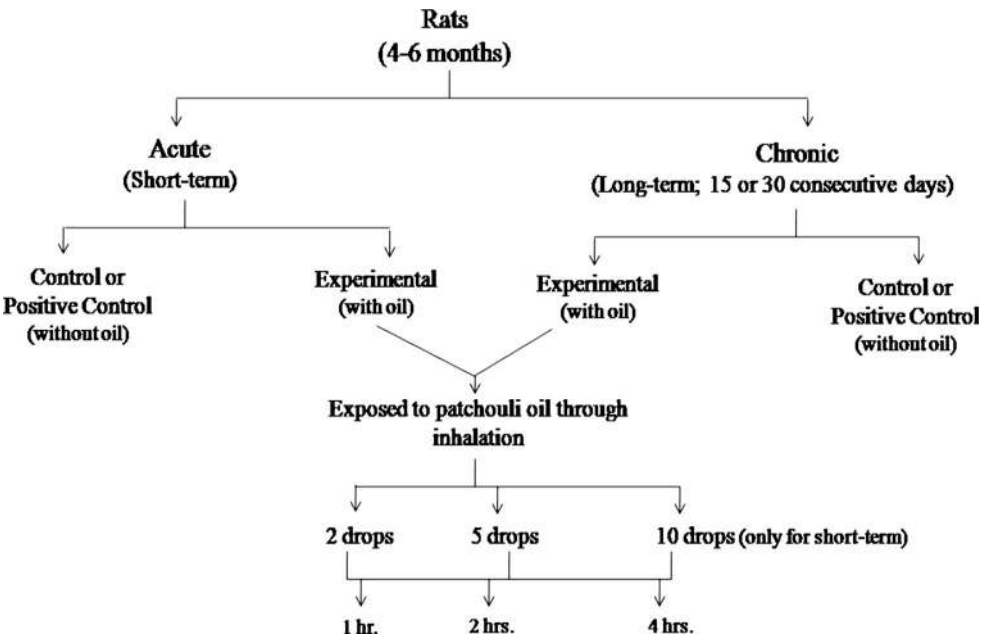
Fig. 1 A schematic diagram of the exposure box (with and without patchouli oil)

free box were considered as control or positive control rats (Fig. 2). All the experimental and control rats were killed between 09:00 and 10:00 h to avoid the circadian effect, if any.

Collection of blood and preparation of platelet-rich plasma (PRP)

Immediately after killing of both control and experimental rats of the specified groups, the blood was collected (with 1% EDTA solution as anticoagulant) under cold conditions (0–4 °C). The PRP was then isolated from the blood following the method of Collins and Sandler [32], duly modified by Banerji et al. [15]. The PRP was used as an enzyme source in the present study.

Fig. 2 A schematic diagram of the experimental design



Assay of monoamine oxidase-A (MAO-A) activity

Monoamine oxidase-A (MAO-A) activity was measured according to the method as described by Dalal and Poddar [33] using 0.24 mM 5-HT (serotonin) as the substrate with 100 µg enzyme. The kinetic study of MAO-A was performed with varying concentrations of serotonin (0.05–0.4 mM per 100 µg enzyme).

Protein estimation

The protein content was estimated spectrophotometrically against bovine serum albumin (BSA) as standard by following the method of Lowry et al. [34].

Statistical analysis

Significance of the statistical analysis of the data were assessed by analysis of variance (ANOVA) with a post hoc Tukey's test. $p < 0.05$ was considered as significant.

Results

The changes of rat blood platelet mitochondrial MAO-A activity and its kinetic parameters (K_m and V_{max}) with short-term (single) exposure of patchouli oil

Figure 3 depicts that single exposure of patchouli oil significantly inhibited the blood platelet MAO-A activity with (i) 2-drops at 1 h (48.01%, $p < 0.001$) and 4 h (31.68%, $p < 0.001$); (ii) five drops at 1 h (81.49%, $p < 0.001$)

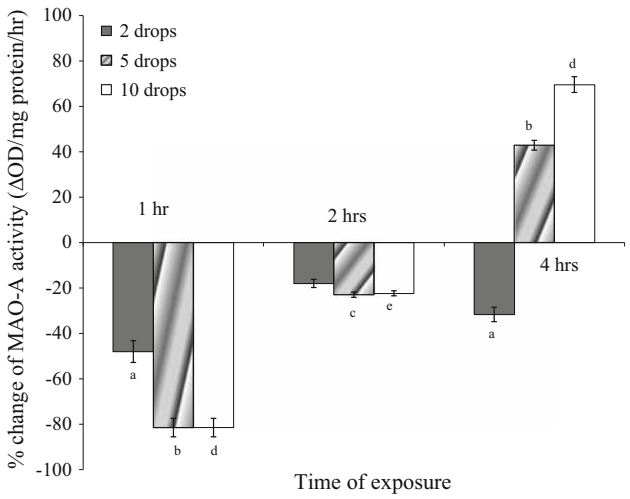


Fig. 3 Blood platelet MAO-A activity (percentage change) in short-term patchouli oil exposure (1, 2, and 4 h) with different dosages (drops) in adult male albino rats Results (percent change) are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. MAO-A activity was measured using serotonin as a substrate. Patchouli oil exposure was given at different dosages (2, 5, and ten drops). The control value of young rat blood platelet MAO-A activity (ΔOD/mg protein/h) is 1.61 ± 0.033. Other details are described in the "Materials and methods" section. Significant difference was from control young rats with (i) two drops $^{a}p < 0.001$, (ii) five drops $^{b}p < 0.001$, $^{c}p < 0.01$ and (iii) ten drops $^{d}p < 0.001$, $^{e}p < 0.01$

maximally, 2 h (22.98%, $p < 0.01$) and (iii) ten drops at 1 h (81.49%, $p < 0.001$) and 2 h (22.36%, $p < 0.01$); but stimulated the same with (i) five drops at 4 h (42.86%, $p < 0.001$) and (ii) ten drops at 4 h (69.56%, $p < 0.001$) with respect to their corresponding control rats.

Figure 4 and Table 2 show that during single exposure of patchouli oil with five drops for 1 h inhibited the blood platelet MAO-A activity with a significant increase in its only K_m (238.10%, $p < 0.001$) and an apparent decrease in its V_{max} (11.48%, $p > 0.05$) with respect to the kinetic parameters (K_m and V_{max}) observed in the corresponding control rats. The kinetic study of patchouli oil (for 4-h exposure with ten drops) induced increase of blood platelet MAO-A activity revealed that there was a significant decrease in its K_m (42.86%, $p < 0.001$) with an apparent increase in V_{max} (3.35%, $p > 0.05$) in comparison to the kinetic parameters (K_m and V_{max}) of the corresponding control rats.

The effect of long-term exposure (15 and 30 consecutive days) of patchouli oil on rat blood platelet mitochondrial MAO-A activity and its kinetic parameters (K_m and V_{max})

It is evident from Fig. 5a that the blood platelet MAO-A activity was increased in long term (15-consecutive days) exposure with (i) two drops of patchouli oil after 1 h (92.64%, $p < 0.001$) and 2 h (84.47%, $p < 0.001$), but apparently decreased after 4 h (9.94%, $p > 0.05$) and (ii) five drops of patchouli oil after 1 h (197.80%, $p < 0.001$), 2 h (164.60%, $p < 0.001$), and 4 h (36.34%, $p < 0.001$) in comparison to their corresponding control rats. Figure 5b shows that 30 consecutive days of long-term exposure of patchouli oil with (i) two drops significantly stimulated the blood platelet MAO-A activity after 1 h (164.91%, $p < 0.001$), 2 h (148.32%, $p < 0.001$) and 4 h (107.32%, $p < 0.001$) with respect to their corresponding control rats; whereas (ii) five drops of patchouli oil exposure for 30 consecutive days significantly inhibited the blood platelet MAO-A activity at 1 h (72.73%, $p < 0.001$), 2 h (45.34%, $p < 0.001$) and 4 h (20.59%, $p < 0.01$) in comparison to their corresponding control rats.

Figure 6A and Table 3 show that the patchouli oil exposure with 5 drops for 1 h for a period of 15 consecutive days increased the blood platelet MAO-A activity due to their significant reduction in K_m (52.38%, $p < 0.001$) and significant stimulation in V_{max} (92.82%, $p < 0.001$) in comparison to the kinetic parameters (K_m and V_{max}) observed in the corresponding control rats. Figure 6B and Table 3 revealed that the blood platelet MAO-A activity was increased during the exposure of patchouli oil for 30 consecutive days with 2 drops for 1 h with a significant decrease in K_m (52.38%, $p < 0.001$) and significant increase in V_{max} (52.63%, $p < 0.001$) with respect to the kinetic parameters (K_m & V_{max}) as were observed in the corresponding control rats.

Discussion

The present study reveals the changes in scenario of blood platelet MAO-A activity due to inhalation of patchouli oil at different doses and durations under short- and long-term conditions in male adult rats. The inhalation of essential oils is an alternative promising, simple, non-invasive administration method without any adverse side effect of administration [35]. The inhalation of fragrant molecules act as either a sedative or stimulatory agent through both (i) the central nervous system (CNS) with the involvement of olfactory bulb of the brain through the olfactory sensory neurons in the olfactory epithelium and (ii) the respiratory system by the absorption of the molecules to the blood or circulation [36]. The mechanism by which the patchouli oil vapors get absorbed into the body is still under investigation [37, 38]. Based on our present study, it is clear that either the patchouli oil vapor as a blend or its most abundant volatile component, patchoulol, exerts an effect on blood platelet MAO-A activity (Tables 2, 3; Figs. 3, 4, 5, 6). The possibility of absorption of the patchouli oil vapor

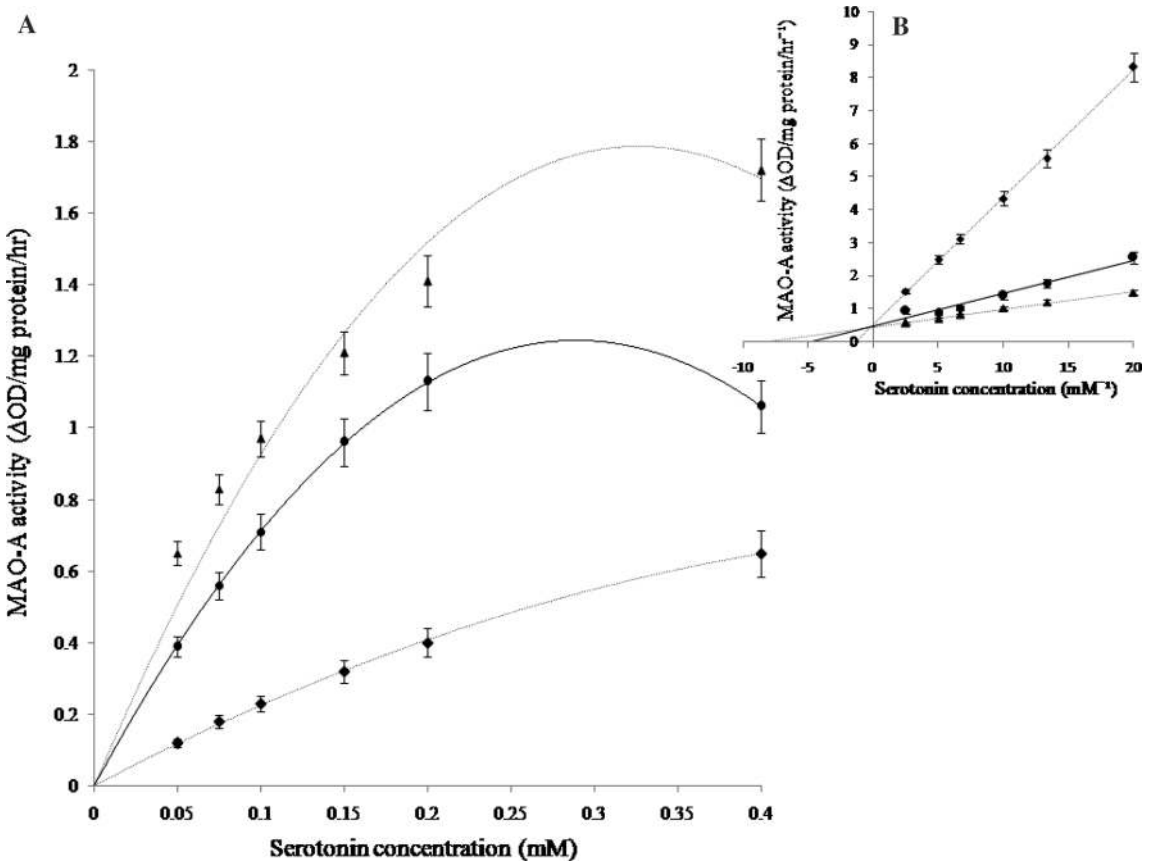


Fig. 4 **A** Effect of various concentrations of serotonin (0.05–0.4 mM) on blood platelet MAO-A activity in control and short-term single patchouli oil-exposed rats. Results are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. The *filled circle* represents the control, the *filled diamond* represents five drops patchouli oil of 1-h single exposure, and the *filled triangle* represents ten drops patchouli oil of 4-h single exposure. Other details are the same as were described in the legend of Fig. 3. **B** Lineweaver–Burk plot of blood platelet MAO-A activity in control and short-term patchouli oil-exposed rats. Lineweaver–Burk plot was drawn from the progress curve with varying substrate concentration as presented in **A**

Table 2 Effect of short-term patchouli oil exposure on blood platelet MAO-A kinetic parameters in young male rats

Kinetic parameters (%) of MAO-A activity	Conditions of patchouli oil exposure		
	Control (without exposure)	Short-term	
		Five drops for 1 h	Ten drops for 4 h
K_m (mM)	100.00 ± 4.29	338.10 ± 11.91[a]	57.14 ± 5.16[a]
V_{max} (ΔOD/mg protein/h)	100.00 ± 4.31	88.52 ± 4.78	103.35 ± 5.26

Results (%) are expressed as mean ± SEM ($n = 4$–6). The percent changes were calculated with respect to the control. The control value of K_m (mM) is 0.21 ± 0.009 and V_{max} (ΔOD/mg protein/h) is 2.09 ± 0.09. V_{max} and K_m values were calculated from Lineweaver–Burk plot (Fig. 3). Kinetic studies were carried out using varying concentrations (0.05–0.4 mM) of serotonin. Other details are the same as described in the legend of Figs. 3 and 4

Significantly different from corresponding control rats [a] $p < 0.001$

through the lungs may not be unlikely as it is also an essential oil [7–11, 24] and due to inhalation a major portion of inhaled molecules goes to lungs, though further studies are needed. In addition, the possibility of absorption of the patchouli (aroma) oil vapor through olfactory epithelium cannot be ignored, as the sensation of smelling occurs through this site [39]. The absorbed patchouli oil (blend or only patchoulol), transported through the blood capillaries of the olfactory epithelium (epithelium is blood capillary enriched tissue) into the circulation,

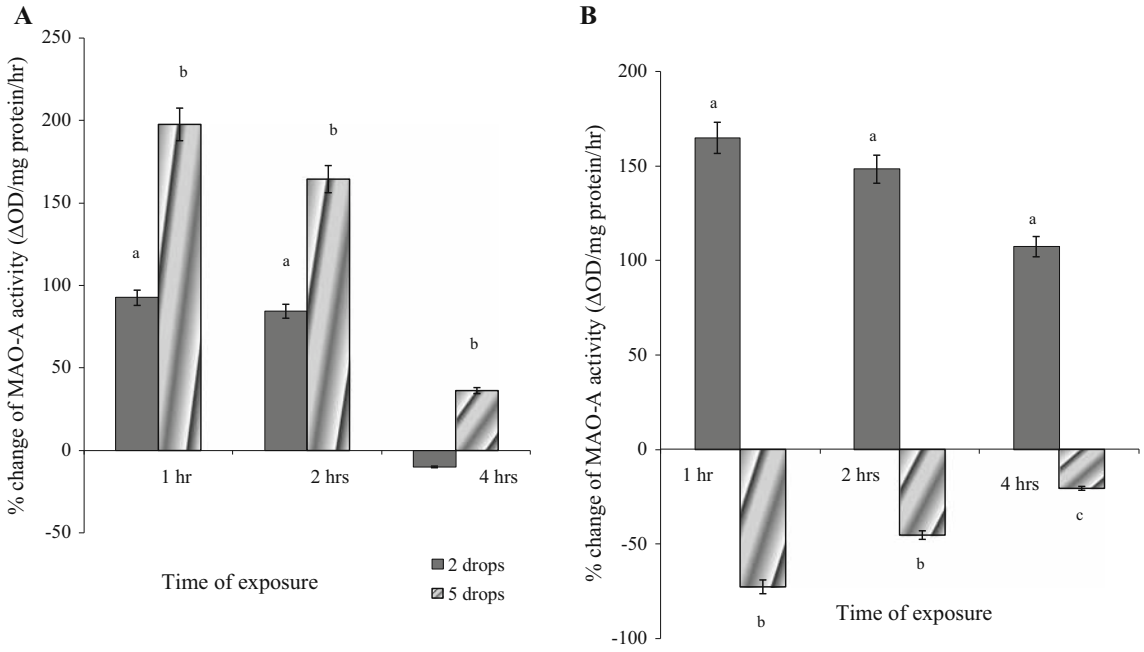


Fig. 5 Blood platelet MAO-A activity (percentage change) in long-term [15 A and 30 B consecutive days] patchouli oil exposure with different dosages (drops) in adult male albino rats. Results (percent change) are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. MAO-A activity was measured using serotonin as a substrate. Patchouli oil exposure was given at different dosages (two and five drops). The control value of young rat blood platelet MAO-A activity (ΔOD/mg protein/h) is 1.61 ± 0.033. Other details are described in the "Materials and methods" section. Significantly different from control young rats of long-term **A** 15 consecutive days with (i) two drops $^{a}p < 0.001$, (ii) 5 drops $^{b}p < 0.001$ and **B** 30 consecutive days with (i) two drops $^{a}p < 0.001$, (ii) five drops $^{b}p < 0.001$, $^{c}p < 0.05$

independently of olfaction and metabolize in vivo to norpatchoulene [12] and excreted out through urine, though it requires further to confirm. It is well known that patchouli oil may show a stimulatory or sedative activity depending on the dose(s) and duration(s) of patchouli oil inhalation [1, 37]. The inhalation of essential oils (patchouli oil, pepper oil, rose oil, estragon oil, fennel oil, grape fruit oil, etc.) individually or as a "blend" has been found to be associated with the inhalation of their fragrant molecules as such [7–11, 24]. It has also been reported that in aromatherapy, the oil of patchouli is used to calm nerves, control appetite, relieve depression and stress, and elevate the mood [1], and these kinds of effects are regulated by the steady-state level of 5-HT [18, 27–30]. However, nothing is known about these sorts of actions of patchouli oil in CNS, the role of 5-HT degrading enzyme, i.e., MAO-A activity in blood platelet has not yet been studied.

The short-term (single) exposure of patchouli oil for 1 h irrespective of the doses (two, five, and ten drops) has inhibited the blood platelet mitochondrial MAO-A activity, maximally with the five and ten drops with the inhibition of its (MAO-A) binding affinity ($1/K_m$) (Figs. 3, 5; Table 2). This inhibition of blood platelet MAO-A activity may elevate the blood platelet serotonin concentration [14, 40, 41] as much as similar or little greater (unpublished) than the amount of serotonin after i.v. injection [42] that may exert a positive effect in the membrane permeability of blood–brain barrier to transport the 5-HT from blood to brain [42–45] and hence may increase the brain serotonin concentration to influence the serotonin-mediated anti-depressant-like behavior of patchouli oil [46], which is under investigation for further clarification. In this context, it may be mentioned that Meszaros et al. [40] though have studied blood platelet MAO-B activity and they have also suggested the possibility of an increase in blood serotonin concentration due to a decrease of blood MAO-A activity [47]. This inhibition of blood platelet MAO-A activity has been found to be minimized with the increase of exposure time (from 1 to 2 h) probably by minimizing these scenario discussed during the 1-h patchouli oil exposure (Fig. 3). The extension of exposure time up to 4 h has shown that the higher doses (five and ten drops) stimulated the blood platelet MAO-A activity, which has been found to be maximum with ten drops (Figs. 3, 5; Table 2) with the activation of its (MAO-A) only binding affinity ($1/K_m$) towards substrate (Fig. 5; Table 2). The blood platelet MAO-A activity has been found to remain inhibited even up to the extension for 4-h single exposure with the same drop (two drops) of patchouli oil under similar conditions (Fig. 3), suggesting

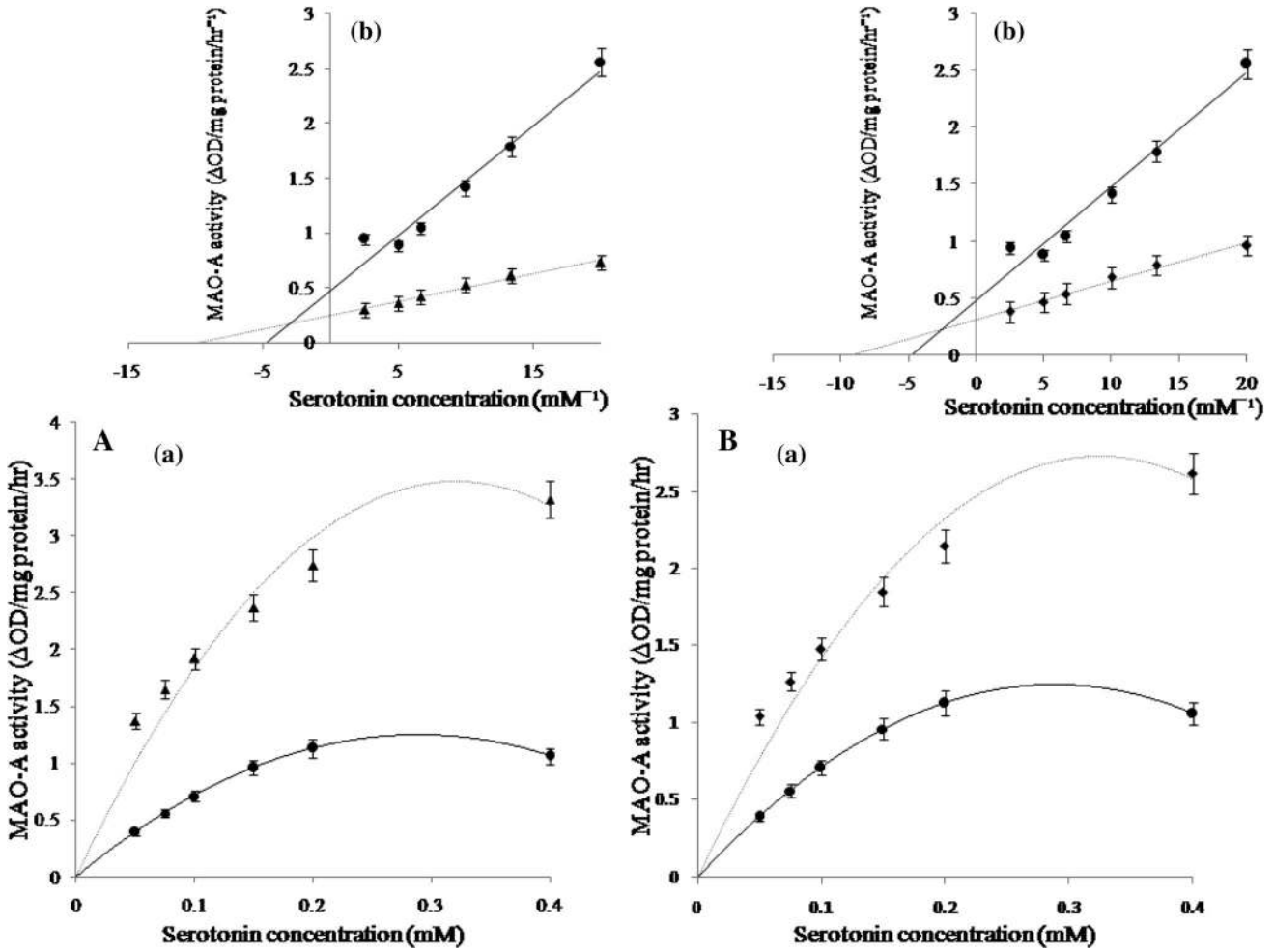


Fig. 6 (a) Effect of various concentrations of serotonin (0.05–0.4 mM) on blood platelet MAO-A activity in control and long-term [A 15 and B 30 consecutive days] patchouli oil-exposed rats. Results are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. The *filled circle* represents control, the *filled triangle* represents five drops of patchouli oil of 1-h exposure for 15 consecutive days, the *filled diamond* represents two drops of patchouli oil of 1-h exposure for 30 consecutive days. Other details are the same as described in the legend of Fig. 5. **(b)** Lineweaver–Burk plots of blood platelet MAO-A activity of control and long-term patchouli oil-exposed rats. Lineweaver–Burk plots were drawn from the progress curves with varying substrate concentration as presented in *a* of **(A)** and **(B)** respectively

that the extension of exposure time from 1 to 4 h with two drops of patchouli oil may have a similar effect on the blood platelet mitochondrial MAO-A activity and may be due to the excretion of the parent component of patchouli oil or its metabolite [12] with the extension of exposure time from 2 to 4 h. The activation of blood platelet MAO-A activity during 4-h short-term (single) exposure with five and ten drops of patchouli oil may decrease the blood serotonin concentration, which in turn may inhibit the 5-HT transportation to the brain through blood–brain barrier (BBB) [40–44], as discussed earlier, though further study is needed. The kinetic studies of blood platelet MAO-A activity during the short-term (single) exposure of patchouli oil (either 1 or 4 h with five or ten drops) have shown that the enzyme activity decreases or increases mainly due to the inhibition or stimulation of only its (MAO-A) substrate binding affinity (Table 2) with apparent change in its (MAO-A) catalytic activity (V_{max}) (Fig. 5; Table 2). This observation indicates that during the short-term (single) exposure of patchouli oil, the blood platelet mitochondrial MAO-A activity changes (inhibition or stimulation) qualitatively (Fig. 5; Table 2) [48, 49] and may be due to the masking or unmasking of the substrate binding site, respectively [15].

Activation of blood platelet mitochondrial MAO-A activity with the short-term exposure (4 h) of patchouli oil (five drops) has been found to be potentiated during the long-term (15 consecutive days) exposure (1 or 2 h) of patchouli oil; but during the long-term (15 consecutive days) exposure for 4 h the said potentiation has turned back

Table 3 Effect of long-term (15 and 30 consecutive days) patchouli oil exposure on blood platelet MAO-A kinetic parameters in young male rats

Kinetic parameters (percent) of MAO-A activity	Conditions of patchouli oil exposure	
	Long-term	
	Five drops for 1 h per day for 15 consecutive days	Two drops for 1 h per day for 30 consecutive days
K_m (mM)	47.62 ± 1.91[a]	47.62 ± 0.95[a]
V_{max} (ΔOD/mg protein/h)	192.82 ± 3.83[a]	152.63 ± 3.35[a]

Results (%) are expressed as mean ± SEM ($n = 4$–6). The percent changes were calculated with respect to the control. The control value of K_m (mM) is 0.21 ± 0.009 and V_{max} (ΔOD/mg protein/h) is 2.09 ± 0.09 of 15 consecutive days and for 30 consecutive days. The control value of K_m (mM) is 0.22 ± 0.01 and V_{max} (ΔOD/mg protein/h) is 2.11 ± 0.02. V_{max} and K_m values were calculated from Lineweaver–Burk plot (Fig. 4). Kinetic studies were carried out using varying concentrations (0.05–0.4 mM) of serotonin. Other details are the same as described in the legend of Figs. 5 and 6

Significantly different from corresponding control rats [a] $p < 0.001$

to almost the similar activation level as has been found in case of short-term (single exposure for 4 h) exposure with five drops. This observation with the long-term exposure, unlike the short-term exposure of patchouli oil, may be either due to the different components of patchouli oil or may be due to the most abundant component and/or its metabolites. Patchoulol or patchouli alcohol is the most abundant component (32.693%) of patchouli oil as per the present GC and GC/MS analysis (Table 1) and it is metabolized in vivo and excreted through urine [12]. During the long-term exposure, the patchoulol with other components may have a chance to metabolize with time and the formed metabolites may change or potentiate the blood platelet MAO-A activity (Fig. 4) by the activation of both catalytic activity (V_{max}) and binding affinity ($1/K_m$) towards substrate (Fig. 6; Table 3). Further, the short-term (single exposure for 4 h) patchouli oil (two drops)-induced inhibition of blood platelet MAO-A activity has been found to be stimulated during the long-term (15 consecutive days) exposure (1 or 2 h) of patchouli oil and inhibited with the 4-h exposure; but this potentiation does not exceed the potentiated level as has been found with five drops of patchouli oil under similar conditions.

This activation may be due to the time-dependent exposure of patchouli oil and the metabolism of its major component molecule (Table 1), patchoulol, within the body and further minimization may be due to the over-production of metabolites or over-accumulation of individual components of patchouli oil within the circulation during 4 h for 15 consecutive days of long-term exposure, though further study is needed. This activation of blood platelet MAO-A activity, irrespective of the dosages, may decrease the blood platelet serotonin concentration and may impair the transportation of serotonin to the brain, as has been discussed previously during short-term exposure. This patchouli oil (two drops)-induced potentiation of blood platelet MAO-A activity has been found to be further potentiated with the increase of duration of exposure from 15 to 30 consecutive days, which has again turned back to almost the potentiated level, as has been found in the 15 consecutive days of exposure. This potentiation of MAO-A activity may be due to the activation of both catalytic activity (V_{max}) and substrate binding affinity ($1/K_m$) of the enzyme (Fig. 6; Table 3). This particular dose (two drops) for 30 consecutive days for 1 h shows a stimulatory effect than the stimulatory effect observed for 15 consecutive days with similar dose (two drops) and time (1 h) of exposure. From this observation, it may be stated that the patchoulol concentration or its metabolite may have a stimulatory effect on blood platelet MAO-A activity with the short-term (1 h) exposure for either 15 or 30 consecutive days of exposure (Fig. 5). Interestingly, the extension of exposure time from 1 to 4 h has been found to minimize the blood platelet MAO-A activity, maybe due to the longer exposure time (2 or 4 h) to catabolize the patchoulol or clear the metabolites from the circulation [12, 50] though it needs to be confirmed. The increase of dose from two to five drops for 30 consecutive days and after 1 h of last inhalation may attain the threshold level of patchoulol or its metabolites to decrease the blood platelet MAO-A activity and attain the level of inhibition as has been observed during short-term (single) exposure from 1 to 4 h (Figs. 3, 4). So, from the present study, it has been found that the patchouli oil exposure plays a phasic role on the blood platelet mitochondrial MAO-A activity depending on the doses (two and five drops), time (1, 2, and 4 h) and duration [under short-term (single) exposure and long-term (15 and 30 consecutive days)] of exposures. Unlike the short-term exposure, the blood platelet MAO-A activity during the long-term exposure has been found to be increased with the increase of both V_{max} and K_m (Figs. 3, 4, 5, 6; Tables 2, 3), suggesting the blood platelet

mitochondrial MAO-A enzyme changes both quantitatively and in a qualitative manner, though further studies at the level of pharmacokinetic and pharmacodynamic effects of patchouli oil and/or its major component, patchoulol, are needed to clarify this.

Finally, it may be concluded that patchouli oil exposure under (a) short-term showed biphasic response depending on the dose and duration of exposure, (b) long-term also showed dual action depending on the dose and period of treatment. The short-term patchouli oil exposure with lower dose showed physiological beneficial effect by activation of blood platelet serotonergic activity, whereas the exposure for short-term with higher dose and longer period with lower as well as higher dose, patchouli oil produced toxic effects by reducing blood platelet serotonergic activity in the physiological response. So, this aroma oil (patchouli oil), extracted from *Pogostemon cablin,* inhalation for short-term as well as long-term (30 consecutive days) with lower dose (two or five drops/day) may be used to uplift the serotonergic system in young adults.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Rumya HG, Palanimuthu V, Rachna S (2013) An introduction to patchouli (*Pogostemon cablin* Benth.)—a medicinal and aromatic plant: it's importance to mankind. Agric Eng Int CIGR J 15:243–250
2. Lawrence BM (1981) Patchouli oil: progress in essential oils. Perfum Flavorist 6:73–76
3. Bunrathep S, Lockwood GB, Songsak T, Ruangrungsi N (2006) Chemical constituents from leaves and cell cultures of Pogostemon cablin and use of precursor feeding to improve patchouli alcohol level. J Sci Asia 32:293–296
4. Yahya A, Yunus RM (2013) Influence of sample preparation and extraction time on chemical composition of steam distillation derived patchouli oil. Sci Verse Sci Direct Proc Eng 53:1–6
5. Healy MA, Aslam M (2002) Homoeopathic medicine and aromatherapy. In: Evans WC (ed) Pharmacognosy, 15th edn, pp 463–466
6. Bruna FMTA, Camila PB, Klinsmann C dos S, Lidiane NB, Vera LMR, José MS, Ana AHF, Ary FJ (2014) Effect of inhaling *Cymbopogon martinii* essential oil and geraniol on serum biochemistry parameters and oxidative stress in rats. Biochem Res Int **(article ID 493183, 7 pages)**
7. Tetsuya M, Takuya Y, Youhei Z, Kousei Y, Mitsuyuki I (2014) Reduction of the chronic stress response by inhalation of hiba (*Thujopsis dolabrata*) essential oil in rats. Biosci Biotechnol Biochem 78:1135–1139. doi:10.1080/09168451.2014.918492
8. Almeida RN, Motta SC, de Brito Faturi C, Catallani B, Leite JR (2004) Anxiolytic-like effects of rose oil inhalation on the elevated plusmaze test in rats. Pharmacol Biochem Behav 77:361–364
9. Fukada M, Kano E, Miyoshi M, Komaki R, Watanabe T (2012) Effect of "rose essential oil" inhalation on stress-induced skin-barrier disruption in rats and humans. Chem Senses 37:347–356
10. Shen J, Niijima A, Tanida M, Horii Y, Maeda K, Nagai K (2005) Olfactory stimulation with scent of lavender oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 383:188–193
11. Shen J, Niijima A, Tanida M, Horii Y, Maeda K, Nagai K (2005) Olfactory stimulation with scent of grapefruit oil affects autonomic nerves, lipolysis and appetite in rats. Neurosci Lett 380:289–294
12. Bang L, Ourisson G, Teisseire P (1975) Hydroxylation of patchoulol by rabbits, semisynthesis of nor-patchoulenol, the odour carrier of patchouli oil. Tetrahedron Lett 26:2211–2214
13. Tipton KF, Boyce S, O'Sullivan J, Davey GP, Healy J (2004) Monoamine oxidases: certainties and uncertainties. Curr Med Chem 11:1965–1982
14. Banerjee S, Poddar MK (2013) Platelet monoamine oxidase-A activity and aging: effect of carnosine. J Physiol Sci 63:279–285
15. Banerji A, Poddar MK, Ghosh JJ (1977) Action of Δ^9-tetrahydrocannabinol on membrane-bound monoamine oxidase activity. Taxicol Appl Pharmacol 40:347–354
16. Johnston JP (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem Pharmacol 17:1285–1297
17. Benedetti MS, Keane PE (1980) Differential changes in monoamine oxidase A and B activity in the aging rat brain. J Neurochem 35:1026–1032
18. Young SN, Leyton M (2002) The role of serotonin in human mood and social interaction: insight from altered tryptophan levels. Pharmacol Biochem Behav 71:857–865
19. Baron M, Levitt M, Perlman R (1980) Low platelet monoamine oxidase activity: a possible biochemical correlate of borderline schizophrenia. Psychiatry Res 3:329–335
20. Adolfsson R, Gottfries CG, Oreland L, Wiberg A, Winblad B (1980) Increased activity of brain and platelet monoamine oxidase in dementia of Alzheimer type. Life Sci 27:1029–1034
21. Alexopoulos GS, Liberman KW, Yang RC (1984) Platelet MAO activity in primary degenerative dementia. Am J Psychiarty 141:97–99
22. Bongioanni P, Mondino C, Boccardi B, Borgna M, Castagna M (1996) Monoamine oxidase molecular activity in platelets of Parkinsonian and demented patients. Neurodegeneration 5:351–357
23. Parnetti L, Reboldi GP, Santucci C, Santucci A, Gaiti A, Brunetti M, Cecchetti R, Senin U (1994) Platelet MAO-B activity as a marker of behavioural characteristics in dementia disorders. Aging Milano 6:201–207
24. Haze S, Sakai K, Gozu Y (2002) Effects of fragrance inhalation on sympathetic activity in normal adults. Jpn J Pharmacol 90:247–253
25. Perry N, Perry E (2006) Aromatherapy in the management of psychiatric disorders—clinical and neuropharmacological perspectives. CNS Drugs 20:257–280
26. Huwig-Poppe Ch, Voderholzer U, Backhaus J, Riemann D, Konig A, Hohagen F (2012) The tryptophan depletion test: impact on sleep in healthy subjects and patients with obsessive–compulsive disorder. In: Huether G, Kochen W, Simat TJ, Steinhart H (eds) Tryptophan, serotonin and melatonin: basic aspects and application. Springer, New York

27. Wargelius H-L, Fahlke C, Suomi SJ, Oreland L, Higley JD (2010) Platelet monoamine oxidase activity predicts alcohol sensitivity and voluntary alcohol-intake in rhesus monkeys. Upsala J Med Sci 115:49–55
28. Verkes RJ, Kerkhof GA, Beld E, Hengeveld MW, van Kernpen GMJ (1996) Suicidality, circadian activity rhythms and platelet serotonergic measures in patients with recurrent suicidal behavior. Acta Psychiatr Scand 93:27–34
29. Asor E, Ben-Shachar D (2012) Platelets: a possible glance into brain biological processes in schizophrenia. World J Psychiatry 2:124–133
30. Oreland L, Hallman J (2009) Monoamine oxidase activity in relation to psychiatric disorders: the state of the art. Nordic J Psychiatry 42:95–105
31. Karim MF (2011) Optimum-range of distillation period (ORDP) per batch of different aromatic raw-materials are generally different, minimum and crop-specific as well: an outstanding fact for industrial application (abstr). In: Book of abstracts, third international congress on aromatic and medicinal plants (CIPAM-2011), Cagliari, p 74
32. Collins GGS, Sandler M (1971) Human blood platelet MAO. Biochem Pharmacol 20:289–297
33. Dalal A, Poddar MK (2010) Involvement of high plasma corticosterone status and activation of brain regional serotonin metabolism in long term erythrosine induced rearing motor hyperactivity in young adult male rats. Toxicol Mech Methods 20:287–297
34. Lowry OH, Rosenbrough NJ, Farr AL, Randale RJ (1951) Protein measurement with folin-phenol reagent. J Biol Chem 193:265–275
35. Fujiwara Y, Ito M (2015) Synergistic effect of fragrant herbs in Japanese scent sachets. Planta Med 81:193–199
36. Muchtaridi M, Tjiraresmi A, Febriyanti R (2015) Analysis of active compounds in blood plasma of mice after inhalation of cajuput essential oil (*Melaleuca leucadendron* L.) Indones J Pharm 26:219–227
37. Ito K, Ito M (2011) Sedative effects of vapor inhalation of the essential oil of *Microtoena Patchoulii* and its related compound. J Nat Med 65:336–343
38. Ito K, Akahoshi Y, Ito M, Kaneko S (2016) Sedative effects of inhaled essential oil components of traditional fragrance *Pogostemon cablin* leaves and their structure-activity relationships. J Tradit Complement Med 6:140–145
39. Guyton AC, Hall JE (2011) Textbook of medical physiology, 12th edn. Saunders, Elsevier Press, New York
40. Meszaros Z, Borcsiozky D, Mate M, Tarcali J, Szombathy T, Mekes K, Magyar K (1998) Platelet MAO-B activity and serotonin content in patients with dementia: effect of age, medication and disease. Neurochem Res 23:863–868
41. Banerjee S, Poddar MK (2016) Can carnosine prevent the aging-induced changes of blood platelet and brain regional monoamine oxidase-A mRNA in relation to its activity? Int J Pept Res Ther 22:471–480
42. Winkler T, Sharma HS, Stålberg E, Olsson Y, Dey PK (1995) Impairment of blood–brain barrier function by serotonin induces desynchronization of spontaneous cerebral cortical activity: experimental observations in the anaesthetized rat. Neuroscience 68:1097–1104
43. Meltzer CC, Pollock BG, Kupfer DJ (1998) Serotonin in aging, late-life depression and Alzheimer's disease: the emerging role of functional imaging. Neuropsychopharmacology 18:407–430
44. Sharma HS, Dey PK (1986) Influence of long-term immobilization stress on regional blood–brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. J Neurol Sci 72:61–76
45. Sharma HS, Westman J, Cervos Navarro J, Dey PK, Nyberg F (1996) Probable involvement of serotonin in the increased permeability of the blood–brain barrier by forced swimming. An experimental study using Evans blue and ^{131}I-sodium tracers in the rat. Behav Brain Res 72:189–196
46. Abbott NJ (2000) Inflammatory mediators and modulation of blood–brain barrier permeability. Cell Mol Neurobiol 20:131–147
47. Celada P, Sarrias MJ, Artigas F (1990) Serotonin and 5-hydroxyindoleacetic acid in plasma. Potential use as peripheral measures of MAO-A activity. J Neural Transm (Suppl) 32:149–154
48. Gerhard M (1974) Determination of Michaelis constants and inhibitor constants. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol 1, 2nd English edn. Academic Press, New York, pp 146–151
49. Masciandaro GBC, Rouchi V (2000) Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilizers. Biol Fert Soils 32:479–483
50. Baser KHC, Buchbauer G (2015) Handbook of essential oils: science, technology, and applications, 2nd edn. CRC Press, Boca Raton. ISBN: 781466590465.