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Anxiolytic and sedative effects of extracts of *Hibiscus sabdariffa* Linn (family Malvaceae)

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Summary

Aqueous (A), hydroalcoholic (AE) and ethanolic (E) extracts and fractions of dried calyxes of *Hibiscus sabdariffa* were evaluated for anxiolytic property using elevated-plus maze (EPM), and sedative properties using ketamine-induced sleep in animal models. The extracts exhibited a dose-dependent increase in the time spent in the open arm with ethanol extracts having the best anxiolytic activity. The extracts at A5mg/kg, AE5mg/kg and A50mg/kg did not cause an increase in time spent in the open arms ($p<0.05$) but other doses significantly did when compared with the vehicle control. The fractions of the hydroalcoholic extracts showed no significant anxiolytic activity. Neither the extracts nor the fractions significantly reduced or increased latency to sleep after a single dose except AE300 ($p<0.01$). There was significant reduction in onset of sleep, and increase in sleeping time with A and AE extracts with multiple doses at A300, AE50 and AE300mg/kg dose groups ($p<0.001$, $p<0.01$ and $p<0.05$ respectively). A reduction in sleeping time after several daily doses of ethanol extracts doses was observed. A single dose of one of the fractions (EAC at 50mg/kg) caused a significant reduction ($p<0.05$) in sleep duration. The study showed that extracts of *Hibiscus sabdariffa* possess anxiolytic and sedative effects which become more pronounced with administration of repeated doses of the extracts.

Keywords: *Anxiolytic, sedative, Hibiscus sabdariffa extracts*

Résumé

Les extraits aqueuse (A), hydroalcoliques (AE) et éthanoïque (E) et les fractions des calyxes sèches d'*Hibiscus sabdariffa* étaient évalués pour leur propriétés anxiolytiques utilisant le maiis plus élevé (EPM), et les propriétés sédatives utilisant la ketamine-induisant le sommeil aux animaux. Les extraits démontraient une dose dépendent croissante de temps mis dans les groupe ouvert avec les extraits méthanoïques ayant la meilleure activité anxiolytique. Les extraits a la dose de A5mg/kg, AE5mg/kg et A50mg/kg ne causaient pas d'augmentation da temps mis dans ce groupe ($p<0.05$) mais d'autres doses augmentaient significativement lorsque comparé le groupe de contrôle. Les fractions des extraits hydroalcoliques ne démontraient d'activité anxiolytique significative. Ni les extraits, ni les fractions ne réduisaient ou n'augmentaient significativement le temps du sommeil après un dose unique à l'exception du AE300 ($p<0.01$). Il y avait une réduction significative du début du sommeil et augmentait le temps du sommeil avec les extraits A et AE à des doses multiples de A300, AE50 et AE300mg/kg dosage par groupe ($p<0.001$, $p<0.01$ et $p<0.05$ respectivement). La réduction de temps de sommeil après plusieurs doses journalières d'extraits du méthanol était observée. Une dose unique de la fraction (EAC de 50mg/kg) causait une réduction significative de la durée du sommeil ($p<0.05$). Cette étude démontrait que les extraits d'*Hibiscus sabdariffa* possèdent des effets anxiolytiques et sédatifs qui deviennent plus intense avec l'administration des doses répétées des extraits.

Introduction

Beverages made from the dried calyx of *Hibiscus sabdariffa* is taken in many parts of the world (Nigeria – Zobo; Sudan/Arabic Countries – Karkade and Mexico – rosselle juice). Several studies conducted have shown that these extracts possess antihypertensive properties in man and rats [1-4], *in vivo* anti inflammatory and *in-vitro* antimicrobial [5], antioxidant activity and inhibition of tumor-promoting activity [6,7] in animal models.

Studies in human shows that the beverage prepared from the dried calyx as a soft drink [8] increases the clearance of paracetamol by 11% [9],

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reduces chloroquine bioavailability [10] while it causes significant reduction in the blood pressure of hypertensive patients [4] and reduces the excretion of diclofenac, a nonsteroidal anti-inflammatory drug in healthy volunteers [11]. There is no report on interaction of the beverage or extract from this plant with hypnotics/sedatives.

There is no literature on the activity of the extracts of the plant parts on the central nervous system. Though none of above uses are particularly indicative of sedative or anxiolytic activity, sedatives and anxiolytic agents are adjuvant therapy in management of hypertension. This study was therefore designed to investigate if extracts of the dried calyx of *Hibiscus sabdariffa* possess anxiolytic or sedative properties which might contribute to its management in hypertension.

Materials and methods

Plant material

The calyx of *Hibiscus sabdariffa* L. (family Malvaceae) was authenticated at the Forestry Research Institute of Nigeria, Ibadan. The calyx were further dried and pulverised. A 100g each of the powdered plant material was extracted twice with one litre of water, ethanol: water (1:1) or absolute ethanol over an 8-hour period. The extract obtained was filtered and evaporated *in vacuo* and weighed. The residue was reconstituted in water to make suitable concentrations.

The hydroalcoholic extract was partitioned successively into dichloromethane, ethylacetate and butanol (Thomas Baker Chemicals PVT Ltd, Mumbai, India).

The anthocyanin content of the dried calyx was determined indirectly through estimation of cyanindin-3-glucoside using a colorimetric method based on the ability of anthocyanins to produce a colour at pH 1.0 that disappears at pH 4.5 [12].

Animals

Female albino mice weighing 18-24g were housed under standard environmental conditions with access to water and commercial mice pellets *ad libitum*. The mice were divided into groups of five animals per group. Each group was administered with 5, 50 or 300mg/kg body weight of aqueous, 50%ethanol or 100% ethanol extracts as prepared above. An insoluble fraction, EAC, and the residual water-soluble fraction, RWSF, were also administered.

Elevated plus maze

The elevated plus maze (EPM) consisted of two open arms (35cm X 5cm) and two closed arms (30cm X 5cm X 15cm) extending from a common central platform (5cm X 5cm) elevated 60cm above floor level. The floors and walls of the closed arms were black, while the open arms were white and at right angles to each other. Each mouse was administered the appropriate dose of the extract or fraction orally 1 hour before being placed on the EPM. Briefly, each mouse was placed on the centre platform facing the closed arms. The time spent in each arm and the number of entries into either arm was noted. The mouse was taken as entering an arm if all the paws are inside the border line separating the arm and the central platform. The ambulatory activity of each mouse was measured for exactly 5 minutes using a stopwatch. The apparatus was wiped clean with a cloth to remove any odour before the next mouse was placed in the EPM.

Fourteen groups of animals were used for the extracts/fractions at oral doses of 5, 50 or 300mg/kg of the aqueous, 50% ethanol or 100% ethanol extracts, with intraperitoneal (ip) diazepam (0.5mg/kg) and water as controls. The extracts and water were administered orally one hour and diazepam 30 minutes before placement in the central platform. The insoluble fraction (EAC) was administered at 50 and 100mg/kg while the residual water-soluble fraction (RWSF) was administered at 100mg/kg dose.

Ketamine-induced sleeping time

Doses of extracts/fractions showing good anxiolytic activity – 50 or 300 mg/kg body weight of the aqueous, 50% ethanol and ethanol extracts, EAC (50 and 100mg/kg) and RWSF at 100mg/kg - were evaluated with the modification of a method by Rabbani *et. al.*[13]. Eleven groups of five swiss albino mice per group were administered with 100mg/kg ketamine hydrochloride (Themis Medicare Ltd, Mumbai, India) intraperitoneally after one hour administration of oral extracts (aqueous, 50% ethanol or ethanol extracts) at 50 or 300mg/kg body, and 50, 100mg/kg of EAC and 100mg /kg RWSF, water and intraperitoneal diazepam (1mg/kg, positive control). The time between administration of ketamine and the loss of righting reflex in the mouse was taken as the onset of sleep, while the time interval between the loss of righting reflex and regaining of the reflex was considered as duration of sleep. The experiment was repeated seven days after daily administration of doses of the extracts.

Statistical analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) with Dunnet post hoc test. Level of significance was set at $p<0.05$. All data were expressed as mean± SEM.

Results

Ethanol was observed to extract the least amount of anthocyanin (as cyanidine-3-glucoside) of 1.23mg/g of plant material, while 50% ethanol and water extracted higher anthocyanins - 3.83mg/g and 3.22mg/g respectively.

Table 1: Anxiolytic effects of extracts and fractions of *Hibiscus sabdariffa* using elevated plus maze (n=5)

Extract / Fraction / Control	Open Arm		Closed Arm	
	Number of entries	Time spent (minutes)	Number of entries	Time spent (minutes)
A 5mg/kg	4.5 ± 2.52	71.25 ± 57.21	4.75 ± 2.63	228.75 ± 57.21
A 50mg/kg	4.4 ± 2.30	104.4 ± 79.54	4.8 ± 2.39	193.6 ± 82.67
A 300mg/kg	8.2 ± 2.39*	132.2 ± 27.48	7.4 ± 2.51	167.6 ± 27.25
AE 5mg/kg	3.2 ± 2.59	107.4 ± 116.26	2.8 ± 2.59	194.6 ± 118.31
AE 50mg/kg	8.6 ± 2.41*	131.8 ± 28.63	8.8 ± 2.49	168.2 ± 28.63
AE 300mg/kg	10.2 ± 3.19*	141.2 ± 26.55	9.8 ± 3.03*	158.8 ± 26.55
E 5mg/kg	7.2 ± 1.92	88.6 ± 24.84	7.8 ± 2.05	209.4 ± 24.16
E 50mg/kg	7.6 ± 3.21	110.6 ± 49.70	7.2 ± 2.68	189.4 ± 49.70
E 300mg/kg	8.2 ± 1.79*	151.8 ± 14.77*	7.8 ± 1.64	148.2 ± 14.77
EAC 50mg/kg	6.8 ± 2.5	256.2 ± 67.87**	6.2 ± 2.3	44.6 ± 3.7
EAC 100mg/kg	7.6 ± 2.2	214.4 ± 22.89**	7.8 *± 2.10	86.4 ± 15.7
RWSF 100mg/kg	6.8 ± 1.8	207.7 ± 112.7**	4.7 ± 1.2	93.3 ± 20.0
DZP	6.0 ± 2.35	201.4 ± 97.54**	4.6 ± 4.28	98.6 ± 97.5
Water	3.8 ± 2.39	43.6 ± 12.6	3.4 ± 3.30	256.4 ± 12.6

**p<0.05 compared with vehicle*
A – Aqueous extract
E – Ethanol Extract
RWSF – Residual water-soluble fraction
***p<0.001 compared with vehicle*
AE – 50% Ethanol extract
EAC – Insoluble fraction of the 50% ethanol extract
DZP – Diazepam

Elevated plus maze

Mice treated with ip diazepam (DZP) spent significantly more time in the open arm (201± 9.88 seconds) compared with the control (43.6 ± 3.55 seconds). There was a dose-dependent increase in the time spent in the open arm with the extracts. The best activity was obtained with E300mg/kg, EAC and RWSF. The fractions, EAC and RWSF significantly increased ($p<0.01$) the time spent in the open arm compared with the vehicle control, water. The time spent in the open arms by the animals administered the fractions was comparable to what was obtained by animals administered diazepam. DZP however, did not show any statistically significant difference in the number of times of entry into the open arms when compared with the vehicle treated group. The extracts and the fractions tended to increase the number of entries into the open arms showing increase in mobility when compared with the vehicle control. There was no significant difference with the time spent in the closed arm comparing the vehicle-treated group with the extract/fraction groups except for AE300mg/kg. Summary of the results is shown in table 1.

Ketamine-induced sleep

The extracts did not show dose-dependence activity in either their ability to induce sleep or the duration of sleep (Table 2). Diazepam, the positive control, caused a near four-fold increase in the sleeping time compared with the vehicle treated groups ($p<0.001$). AE50mg/kg and EAC50mg/kg increased sleeping time with single dose administration. There was also a significant increase in the sleeping time with the A50, A300, AE50 and AE300mg/kg dose groups after seven daily doses ($p<0.001$, $p<0.001$, $p<0.01$ and $p<0.05$ respectively). The ethanol extracts caused a reduction in sleeping time after repeated doses of the extract when compared with single dose. The

fractions also did not exhibit any significant difference in the onset of sleep when compared with the vehicle group. EAC at 50mg/kg showed a significant increase in sleeping time ($p<0.05$), when compared with the control.

produced by diazepam, thus showing a better profile as an anxiolytic agent without unwanted sedative activities with single doses. Though, this study might have been limited by the non-blinding nature of

Table 2: Effect of the extracts of *Hibiscus sabdariffa* on ketamine-induced sleeping time (n=5)

Treatment	Onset of sleep (seconds) after n doses (MEAN ±SEM)		Duration of sleep (seconds) after n doses (MEAN ±SEM)	
	Dose n = 1	Dose n = 7	Dose n = 1	Dose n = 7
A50	76.6 ± 7.34	70.5 ± 6.7	873.2 ± 77.38	1924.5 ± 56.2**,^{c}
A300	86.0 ± 9.62	60.8 ± 5.94	597.5 ± 91.3^c	1710.4±173.49**,^{c}
AE50	78.2 ± 11.48	55.0 ± 2.57	1250.2 ± 290.65*^{b}	2011.2 ± 253.1**,^{b}
AE300	114.2 ± 13.53*, ^{a}	71.4 ± 4.04^a	950.8 ± 67.43	1185.2 ± 68.34
E50	80.75 ± 10.09	72.2 ± 5.60	1065.5 ± 65.12^b	551.00 ± 34.84^b
E300	81.8 ± 12.47	70.8 ± 4.35	1070.8 ± 144.03^a	745.4 ± 26.16^a
EAC 50	68.75 ± 3.09	ND	1270.3 ± 206.9*	ND
EAC 100	85.67 ± 7.29	ND	1084.7 ± 96.3	ND
RWSF 100	108.67 ± 13.54	ND	657.5 ± 113.39	ND
Diazepam	60.2 ± 14.09	ND	2949.8 ± 239	ND
Vehicle	68.2 ± 15.66	ND	773.4 ± 130.14	ND

Key:

**p<0.05 compared with vehicle*
***p<0.01 compared with vehicle*
****p< 0.001 compared with vehicle*
a - p<0.05 comparing single and multiple doses
b – p< 0.01 comparing single and multiple doses
c – p< 0.001 comparing single and multiple doses
A – Aqueous extract
AE – 50% Ethanol extract
E – Ethanol Extract
EAC –Insoluble fraction of the 50% ethanol extract
RWSF – Residual water-soluble fraction
ND – Not determined

Discussion

The use of herbs as beverages for relaxation is an old practice. The aims of this study were to evaluate some of the properties that may be present that make the water extract of *Hibiscus sabdariffa* acceptable as a beverage, and makes it useful in management of hypertension. As far as we know, this is the first published report on the anxiolytic and sedative effects of extracts of the plant. Various types of herbal medicines have been used as anxiolytic drugs in different parts of the world, but it is interesting to note that *Hibiscus sabdariffa* had no folkloric use as a sedative.

As expected, diazepam produced significant increases in open arm time and in number of entries into the open arms. These data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce robust anxiolytic effects in a variety of anxiolytic screening procedures including EPM procedures [13,14,15]. In this study, the sedative effects of the plant extracts was lower than those evaluation of the number of entries into and out of the elevated plus maze, efforts were made to ensure objectivity in the assessment of the time spent in the arms of the EPM with the use of a stopwatch.

The ability of some of the extracts to increase ketamine-induced sleeping time shows that they may possess some hypnotic/sedative effects which become more pronounced with administration of several doses of the extracts.

The results showed that all the extracts, to varying degrees, have the ability to reduce aversion to the open arms using EPM when compared to control animals, an activity which was also displayed by the fractions. All, except the lower doses for the aqueous and 50%ethanol extracts increased the number of times of entry into the open arms. In addition to mild anxiolytic effects, some of the extracts also have ability to increase the sleeping time induced with ketamine, but with less sedative effects when compared with diazepam. This effect was increased with daily doses in some of the extracts. There was however no increase in latency to sleep in both the

extracts and the fractions after single and several daily doses. The effects on the sleeping time have been shown to be dependent on the solvent for extraction, and the number of doses given. It was observed that for the aqueous and hydroalcoholic extracts, there was increase in sleeping time with increased number of doses, while with ethanol extracts, there was a reduction in the sleeping time with increased number of doses. The anxiolytic and sedative effects might contribute to the total antihypertensive effects observed in hypertensive patients treated with the beverages prepared from the dried calyx of the plant [4].

Conclusion

This study showed that at high doses, the ethanolic extracts and some fractions of the hydroalcoholic extracts of *Hibiscus sabdariffa* possess anxiolytic and sedative effects in animal models. Administration of several daily doses caused a significant increase in sedative effects with aqueous and hydroalcoholic extracts and a significant reduction with ethanolic extract. As desirable as these effects may be, there is a need for caution especially for patients who may be coadministering this beverage with hypnotic/ sedative agents.

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