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Study of Sedative, Anxiolytic, CNS – Depressant and Skeletal Muscle Relaxant Effects of Methanolic Extract of *Hibiscus Rosa-Sinensis* on Laboratory Animals

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Abstract:
Aim: Methanolic extract of *Hibiscus rosasinensis* (MEHR) shows sedative, anxiolytic, skeletal muscle relaxant and CNS – depressant effect.
Methods: *Hibiscus rosasinensis*, found in south Asia, was extracted by methanol and the practical yield of extract was found to be 7.2%w/w.
Models:
1. Anxiolytic action was studied by the open field behavior model in which it significantly increase in rearing and crossing, elevated plus-maze model in which MEHR significantly increase the entry in both arm, dark & light field model in which MEHR significantly increase the time spent in dark field, compared with control-CMC solution [using diazepam (25 mg/kg, i.p.)].
2. Sedative effect was seen by Phenobarbital induce sleep model.
3. Skeletal muscle relaxant effect was studied using rota rod model, in which MEHR significantly increase the time of fall, compared with control-CMC solution [using diazepam (25 mg/kg, i.p.)].
4. CNS - depressant effect was studied using Actophotometer model in which MEHR significantly decrease the number of cut-off, compared with control-CMC solution [using diazepam (25 mg/kg, i.p.)].

Conclusion: The MEHR shows seadative, anxiolytic, skeletal muscle relaxant and CNS – depressant effects.
Keywords: Hibiscus, sedative, anxiolytic, skeletal muscle relaxant, rota-rod and CNS – depressant effects, actophotometer, etc.

INTRODUCTION: [1,2,5]

Biological Name: The dried ripe flowers, leaves of *Hibiscus rosa-sinenis* belongs to family **Malvaceae.**[1]

Source: Widely spread all over the world, majorly in tropical and subtropical areas.[2]

Description: The leaves are alternate, simple and ovate to lanceolate, often with a toothed or lobed margin. The flowers are large, conspicuous, trumpet-shaped, with five or more petals, ranging from white to pink, red, orange, purple or yellow, and from 4–18 cm broad. [2, 6]

Synonyms: Aloala [Hawai]; red hibiscus; China rose [English]; da hong hua (big red flower) [China]; Jasud [Gujarati], japapushpam [India]; shoeflower [Jamaica]. [5]

Nomenclature: Hibiscus is taken from the Greek "hibiscos," a name for mallow. [6]

Pharmacological Actions: Abortifacient[5]; analgesic[5]; antidiarrhoic[5]; antiestrogenic[1]; antifungal[5]; anti-infectious; anti-inflammatory; antipyretic; astringent[5]; CNS depressant[1]; constipating[1]; contraceptive; demulcent[5]; dentifrice[6]; diuretic[6]; expectorant[5]; hemostat; hypoglycemic[5]; hypotensive; hypothermic; insect attractant[1]; promotes hair growth and color[1,2,,5,6]; purgative[1]; refrigerant[5]; relaxes spasm[1]; soothes irritated tissue.[5]

Collection of materials and Method for extraction:

The herb of Hibiscus was collected from local region in Rajkot district of Gujarat & morphological & microscopy of pant was authentified by pharmarcognosy department of R.K. College of pharmacy. The leaves were separated and dried between 55 ° to 60° C and then pulvirized to very fine powder. The powder was extracted using Soxhlet apparatus

using methanol as a solvent. The % yield was found to be 7.2%W/W.

Animals:

Male Swiss albino rat of weighing 220-280 g were used for the study.

The animals were procured from Animal House , Department of Pharmacology, R.K.College of Pharmacy, Rajkot, India. The animals were place at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water ad libitum.

Experiments were carried out between 09:00 an d 14:00 h.

All the experimental procedures and protocols used in the study were reviewed and approved by the Institutional AnimalEthical Committee(IAEC) and care of laboratory animals was taken as per the guidelines of Committee for the purpose of control and supervision of experiments on animals(CPCSEA), Govt. of India (Registration No.1131/ac/07/CPCSEA.)

EXPERIMENTAL MODELS AND STUDIES (METHODS):

The study includes the sedative, anxiolytic, skeletal muscle relaxant effect and CNS-depressant effect of Methanolic extract of *Hibiscus rosa-sinensis* [MEHR].

A] For Sedative effect:

1. Phenobarbital induced sleep model:

The 18 animals (mice) divided in to 3 groups containing 6 animals each.

[Normal: 0.5ml 1% CMC solution, i.p.; standard: Phenobarbital: 5 mg/kg, i.p.; test: MEHR: 200 mg/kg, p.o.]. Observe the onset of time and duration of action. [3,4,9,11]. From the given data in table: 1 we can say that drug possesses sedative effect.[Chart 1]

B] For Anxiolytic effect:

1. Open-field behavior model:

Instrument: The apparatus consisted of a wooden box (60 X 60 X 60 cm). The arena of the open field was divided into 16 squares (15 X 15 cm): the four inner squares in the center and 12 squares in the periphery along the walls. The experimental room was a sound attenuated, dark room.

Method: The 18 animals (mice) divided in to 3 groups containing 6 animals each. [Normal: 0.5ml 1% CMC solution, i.p.; standard: diazepam: 25 mg/kg, i.p.; test: MEHR: 200 mg/kg, p.o.]. Allow the animal to freely move in the model and note the number of crossing and number of rearing. From the given data table 2 the number of crossing increases in test compare to normal. [3,4] [Chart 2]

2. Elevated Plus model:

Instrument: The EPMT apparatus consisted of four arms elevated 30 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. Two of the arms were enclosed with high walls (30 X 7 X 20 cm), and the other arms were connected via a central area (7 X 7 cm) to form a plus sign.

Method: The 18 animals (mice) divided in to 3 groups containing 6 animals each. [Normal: 0.5ml 1% CMC solution, i.p.; standard: diazepam: 25 mg/kg, i.p.; test: MEHR: 200 mg/kg, p.o.]. Allow the animal to freely move in the model and note the number of entry in open arm and close arm. From the given data table 3 the number entry in open arm increases in test compare to normal. [3,4] [Chart 3]

3. Dark and light field model:

Instrument: It consists of open top wooden box. Two distinct chambers, a black chamber (25 cm long X 35 cm wide X 35 cm deep), painted black and made dark by covering its top with black plywood, and a bright chamber (25 cm long X 35 cm wide X 35 cm deep), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. The two chambers were connected through a small open doorway, (7.5 cm long X 5 cm wide) situated on the floor level at the center of the partition.

Method: The 18 animals (mice) divided in to 3 groups containing 6 animals each. [Normal: 0.5ml 1% CMC solution, i.p.; standard: diazepam: 25 mg/kg, i.p.; test: MEHR: 200 mg/kg, p.o.]. Allow the animal to freely move

in the model and note the number of entry in light field as well as count the time spent in light field. From the given data table 4a & 4b the number entry as well as time spent in light field increases in test compare to normal. [7] [Chart 4 a & b]

C] For CNS-depressant effect:

1. Actophotometer:

The 18 animals (mice) divided in to 3 groups containing 6 animals each. [Normal: 0.5ml 1% CMC solution, i.p.; standard: diazepam: 25 mg/kg, i.p.; test: MEHR: 250 mg/kg, p.o.]. Allow the animal to freely move in the model and note the number of cut off (crossing) of lesser for 2 minutes. From the given data in table 5 the number of cut off decreases in test compare to normal. [3,4,10] [Chart 5]

D] For Skeletal muscle relaxant effect:

1. Rota rod model:

Instrument: Rota rod apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non-slippery surface. This rod was divided into two equal sections by two disks, thus enabling two mice to walk on the rod at the same time at the speed of 25 rpm.

Method: The 18 animals (mice) divided in to 3 groups containing 6 animals each. [Normal: 0.5ml 1% CMC solution, i.p.; standard: diazepam: 25 mg/kg, i.p.; test: MEHR: 200 mg/kg, p.o.].

The animal is put on the rotating rod and the time required to fall down from the rod is measured. From the given data, in table 6 the time required to fall down is lesser in test compared to normal. [3,4,11] [Chart 6]

Statistical Analysis: Results were expressed as mean ± SEM. Difference in means were compared using one way analysis of variance (ANOVA) followed by Tukey's test. $P<0.001$ were considered statistically significant.

Result:

Activity and Model	Parameters	Control		Standard		Test	
		Before Reading ±SEM	***After Reading ±SEM***	***Before Reading ±SEM***	***After Reading ±SEM***	***Before Reading ±SEM***	***After Reading ±SEM***
Sedative (Phenobarbital induce sleep)	Onset of action (Minutes)	---	---	---	26.5±0.84	---	33.16±1.25
	Duration of action (Minutes)	---	---	---	187.66±4.75	---	159±3.41
Anxiolytic (open field model)	Number of crossing	18.75±1.1	21.5±1.5	19±1.04	48.5±1.73	16±1.42	35.83±0.97
Anxiolytic (Elevated plus model)	Entries in open arm	8.16±0.49	8.5±0.27	7.5±0.52	18.33±2.12	8±0.44	14±0.89
Anxiolytic (Light/Dark model)	Number of Entry in light area	10.5±0.64	11±0.70	9±0.40	20.5±0.6	10.66±0.4	18±0.40
	Time spent in light area (seconds)	25±0.70	24±0.70	24±0.70	112±0.9	22±0.70	72.5±0.93
CNS-depressant (Actophotometer)	Number of cut-off	52.5±0.76	53±0.63	51.5±0.8	9.5±0.62	51±0.96	20±0.36
SKM-relaxant (Rota-rod model)	Time of fall (Seconds)	---	246.6±3.3	---	5.83±0.60	---	17.5±0.80

Images of Models used in work:

OPEN FIELD MODEL

ELEVATED PLUS MODEL

LIGHT DARK MODEL

ACTOPHOTOMETER

ROTAROD

Table 1: Sedative effect of MEHR on rat using Phenobarbital induced sleep model

For Sedative: phenobarbiatal induced sleep model

Sr. No.	Group	Control (Seconds)		Standard (Seconds)		Test (Seconds)	
		Onset of time	Duration of action	Onset of time	Duration of action	Onset of time	Duration of action
1	Head	---	---	26	190	30	155
2	Tail	---	---	27	180	34	154
3	Back	---	---	24	189	29	148
4	Head back	---	---	30	200	35	167
5	Back tail	---	---	27	169	34	160
6	No mark	---	---	25	198	37	170
Average		---	---	26.5	187.66	33.16	159
SD		---	---	2.073	11.60	3.06	8.34
SEM		---	---	0.84	4.75	1.25	3.41
variance		---	---	6.25	66.91	8.66	63.33

Table 2: Anxiolytic effect of MEHR on rat using Open field model

For Anxiolytic action: Open field model (number of crossing in 5 minutes)

Sr. No.	Group	Control		Standard		Test	
		Before	After	Before	After	Before	After
1	Head	15	17	22	51	20	39
2	Tail	18	21	20	54	18	36
3	Back	22	26	16	46	12	33
4	Head back	18	20	18	45	16	36
5	Back tail	19	23	20	49	15	36
6	No mark	20	22	18	46	14	35
Average		18.667	21.5	19	48.5	15.833	35.833
SD		2.3381	3.0166	2.0976	3.5071	2.8577	1.9408
SEM		1.169	1.5083	1.0488	1.7536	1.4289	0.9704
variance		5.46	9.1	4.4	12.3	8.16	3.76

Table 3: Anxiolytic effect of MEHR on rat using Elevated plus model

For Anxiolytic action: Elevated Plus method (number of entry in open arm 5 minutes)

Sr. No.	Group	Control		Standard		Test	
		Before	After	Before	After	Before	After
1	Head	9	8	7	15	7	14
2	Tail	8	9	6	14	8	13
3	Back	9	9	9	19	9	15
4	head back	7	8	8	19	9	15
5	back tail	9	9	7	17	8	14
6	No mark	7	8	8	26	7	13
Average		8.16	8.5	7.5	18.33	8	14
SD		0.98	0.54	1.04	4.274	0.89	0.89
SEM		0.49	0.27	0.52	2.137	0.44	0.44
variance		0.96	0.3	1.1	18.26	0.8	0.8

Table 4 a: Anxiolytic effect of MEHR on rat using Light Dark model

For Anxiolytic action: Light Dark model (number of entry in light field 5 minutes)

Sr. No.	Group	Control		Standard		Test	
		Before	After	Before	After	Before	After
1	Head	11	10	10	22	10	18
2	Tail	12	13	9	20	12	18
3	Back	10	11	9	21	11	19
4	No mark	9	10	8	19	10	17
5	back tail	11	12	8	21	10	16
6	No mark	10	10	10	20	11	20
Average		10.5	11	9	20.5	10.66	18
SD		1.048	1.26	0.89	1.04	0.81	1.41
SEM		0.52	0.63	0.44	0.52	0.40	0.70
variance		1.66	2	0.66	1.66	0.91	0.66

Table 4 b: Anxiolytic effect of MEHR on rat using Light Dark model

For Anxiolytic action: Light Dark model (Time spent in light field 5 minutes)

Sr. No.	Group	Control		Standard		Test	
		Before	After	Before	After	Before	After
1	Head	25	23	24	115	21	73
2	Tail	26	22	23	113	22	75
3	Back	24	25	22	112	23	74
4	No mark	23	24	25	110	22	71
5	back tail	27	26	26	110	20	72
6	No mark	25	24	24	112	24	70
Average		25	24	24	112	22	72.5
SD		1.41	1.41	1.41	1.89	1.41	1.87
SEM		0.70	0.70	0.70	0.94	0.70	0.93
variance		1.66	1.66	1.66	4.33	0.66	2.91

Table 5: CNS -depressant effect of MEHR on rat using Actophotometer model

For CNS-Depressant: Actophometer model (number of cut-off in 2 minutes)

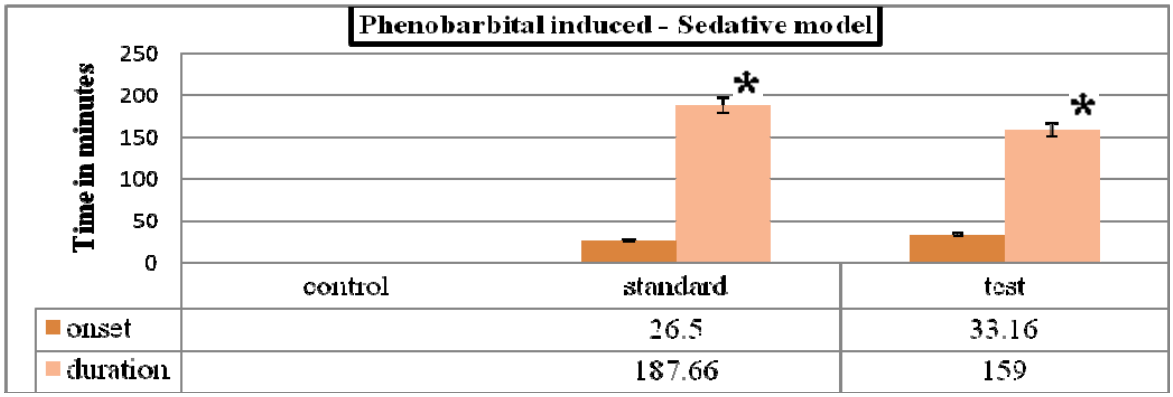
Sr. No.	Group	Control		Standard		Test	
		Before	After	Before	After	Before	After
1	Head	50	51	49	9	51	19
2	Tail	52	52	52	10	55	20
3	Back	55	54	51	8	50	21
4	head back	51	52	50	8	50	19
5	back tail	53	54	52	10	52	21
6	No mark	54	55	55	12	48	20
Average		52.5	53	51.5	9.5	51	20
SD		1.87	1.54	2.07	1.51	2.36	0.89
SEM		0.76	0.63	0.84	0.62	0.96	0.36
variance		3.5	2.4	4.3	2.3	5.6	0.8

Table 6: Skeletal Muscle Relaxant effect of MEHR on rat using Rota-rod model

For Skelatal muscle relaxant: Rota rod model (time required to fall down)

Sr. No.	Group	Control (seconds)	Standard (seconds)	Test (seconds)
1	Head	240	5	17
2	Tail	250	6	15
3	Back	260	8	21
4	head tail	250	5	17
5	back tail	240	7	18
6	No mark	240	4	17
Average		246.66	5.83	17.5
SD		8.16	1.47	1.97
SEM		3.34	0.60	0.80
variance		66.66	2.16	3.9

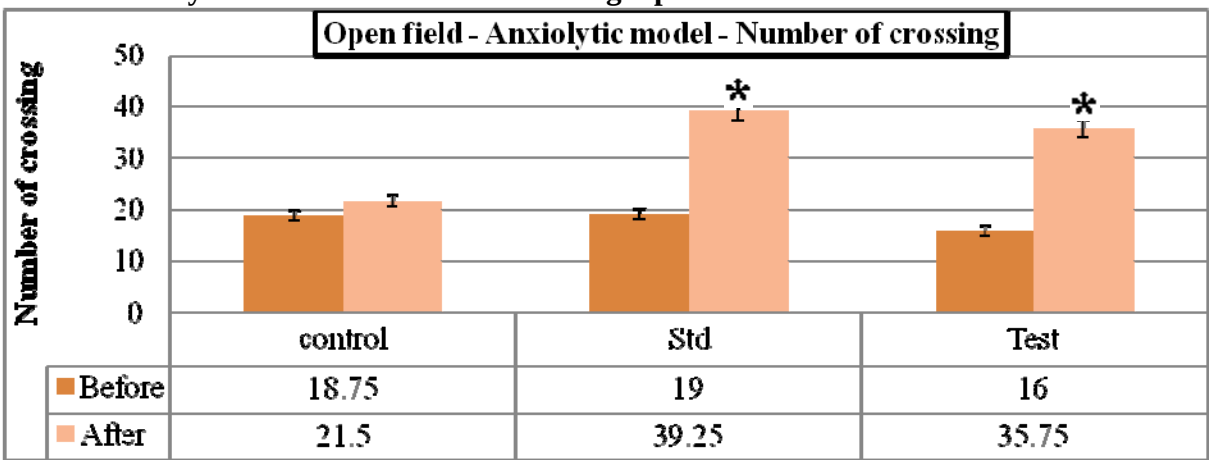
Chart 1: Sedative effect of MEHR on rat using Phenobarbital induced sleep model



	control	standard	test
onset		26.5	33.16
duration		187.66	159

* indicate significant difference from control (p<0.001)

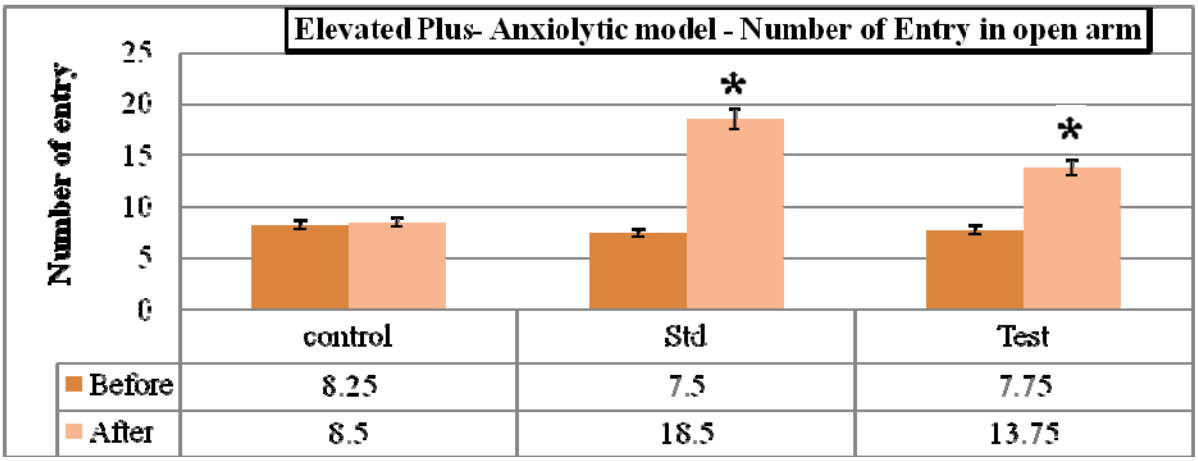
Chart 2: Anxiolytic effect of MEHR on rat using Open field model



	control	Std	Test
Before	18.75	19	16
After	21.5	39.25	35.75

* indicate significant difference from control (p<0.001)

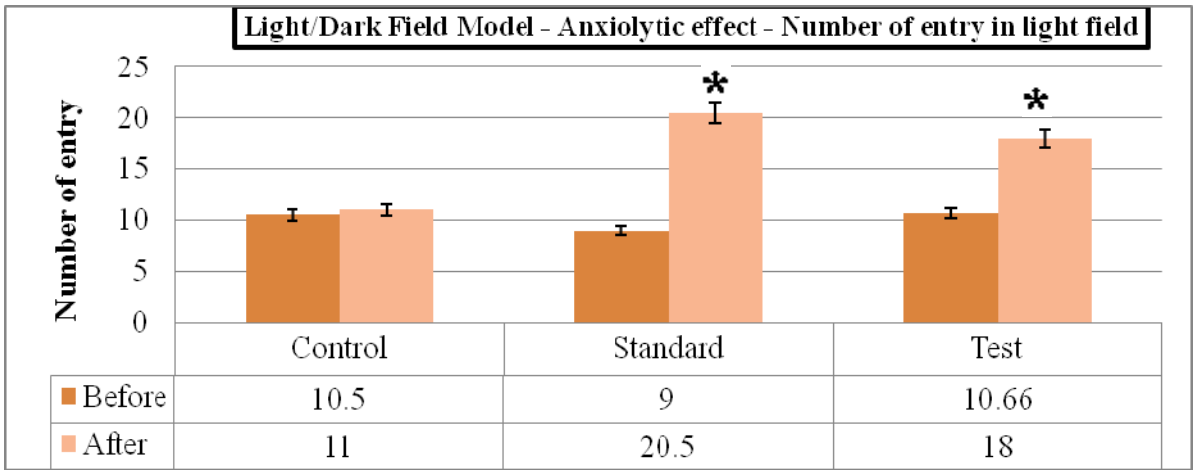
Chart 3: Anxiolytic effect of MEHR on rat using Elevated plus model



	control	Std	Test
Before	8.25	7.5	7.75
After	8.5	18.5	13.75

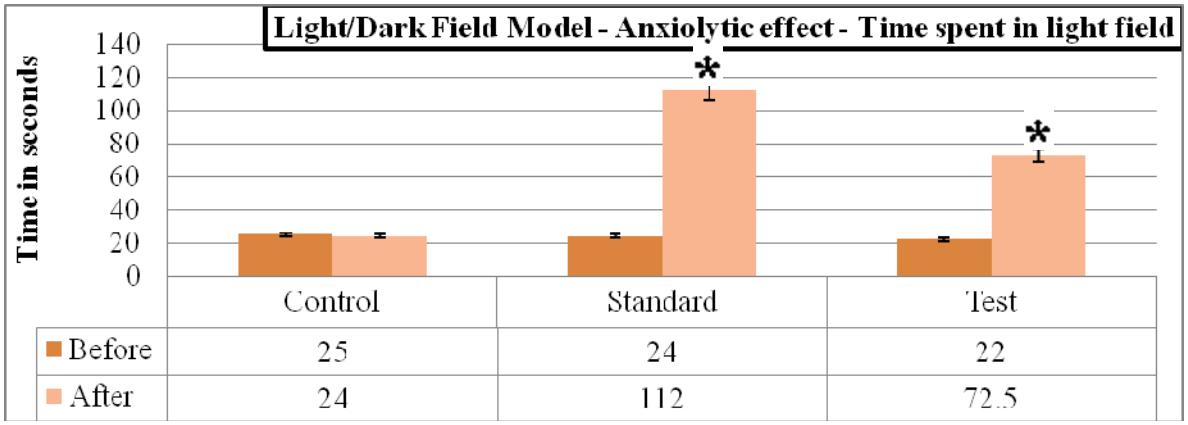
* indicate significant difference from control (p<0.001)

Chart 4 a: Anxiolytic effect of MEHR on rat using Light Dark Field model



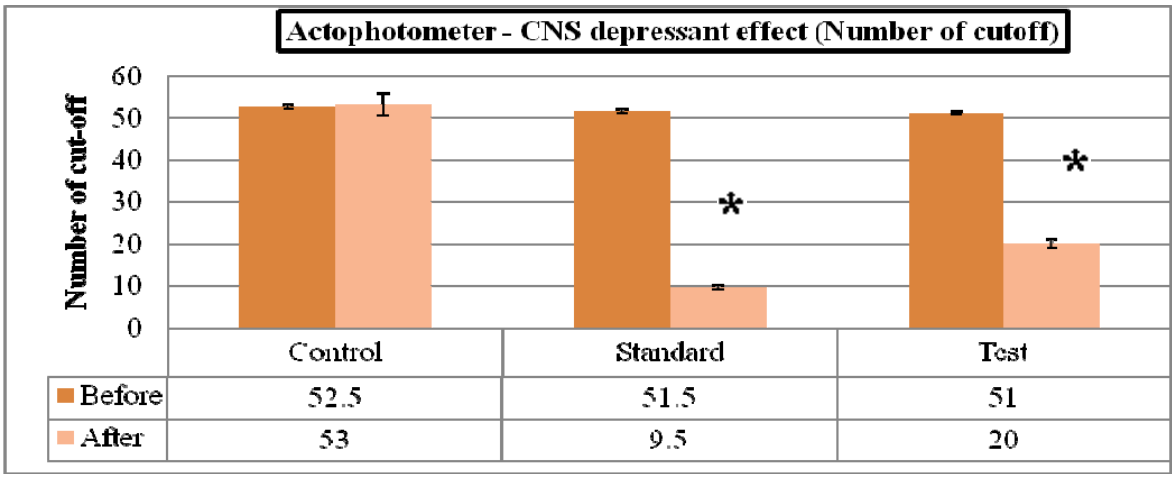
* indicate significant difference from control ($p<0.001$)

Chart 4 b: Anxiolytic effect of MEHR on rat using Light Dark Field model



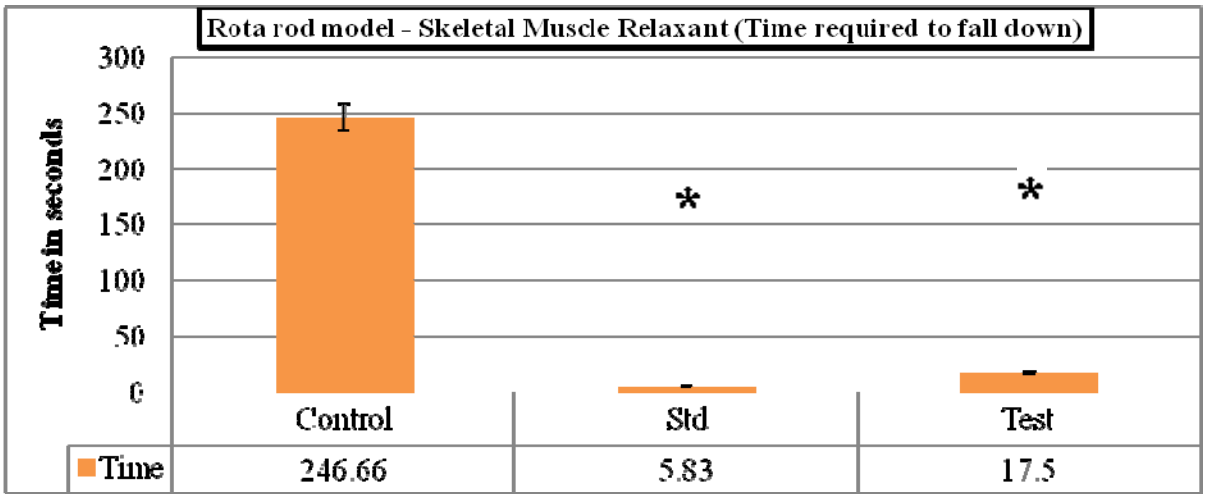
***** indicate significant difference from control ($p<0.001$)

Chart 5: CNS -depressant effect of MEHR on rat using Actophotometer model



* indicate significant difference from control ($p<0.001$)

Chart 6: Skeletal Muscle Relaxant effect of MEHR on rat using Rota-rod model



* indicate significant difference from control ($p<0.001$)

DISCUSSION:

The result of our study shows methanolic extract of flower of *Hibiscus rosa-sinensis* can markedly reduce fall of time (in Rota-rod model), decrease in onset of time as well as increase in duration of action (in phenobarbital induced sleep model), decrease number of cut-off (in Actophotometer) and increase in number of crossing (in open-field behavior model), increase time spent in light field as well as increase number of entry in close and open arm (in elevated plus model).

In conclusion, our data indicates that MEHR can possess Sedative, anxiolytic, CNS - depressant and skeletal muscle relaxant activities.

The MEHR contains flavanoids (hibiscitin), phenolic content as well as terpenoid compounds like β – sitosterol, caemphesterol, etc, which are probably responsible for the actions.[5]

CONCLUSION:

The methanolic extract of *Hibiscus rosa-sinensis* can increase the duration of action so it possesses sedative effect.It can also increase the number of crossing in open field model, increase the time spent in open arm, entry in open arm (in elevated plus model) as well as increase the time spent in light field (in Light Dark field) thus we can conclude that it can also possesses anxiolytic action.

The decrease in number of cut-off in actophotometer model indicates that it can possess CNS-depressant.It can also decrease the time of fall from the rotating rod in rota-rod model, thus it can possesses skeletal muscle relaxant action.

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