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ANTI-AGGRESSIVE ACTIVITY OF AQUEOUS EXTRACT OF FLOWERS OF HIBISCUS ROSA-SINENSIS IN RODENTS

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Abstract: The current study aim of present study was to investigate the in vivo anti-aggressive activity of Hibiscus rosa-sinensis using defensive and offensive behavioral models in rodents. Adult male and female mice were used for the present study. Animals were divided into four groups, with 5 animals in each. Diazepam was used as standard anti - aggressive agent. Animals were treated according to test. Hibiscus rosa-sinensis (200 mg/kg and 400 mg / kg, p.o.) was administered according to test. Standard group was treated with diazepam (1 mg/kg, p.o.).

Keyword – **Hibiscus rosa-sinensis, Aqueous Extract, Anti-Aggressive, Extraction .**

I. INTRODUCTION

Aggression, in its broadest sense, is behavior or a disposition, that forceful, hostile or attacking. It may occur either in retaliation or without provocation (Akert, R.M., et al, 2010). In narrower definitions that are used in social sciences and behavioral sciences, aggression is an intension to cause harm or an act intended to increase relative social dominance. Aggression is a potential diseases, disorders or conditions that interfere with thought processes, such as brain tumors, dementia, post-traumatic stress disorder, schizophrenia, and a number of personalit disorders. Although specific causes of aggression are no known some studies have shown that abnormal brain chemistry or structural changes may play a role. Environment and genetics also seem to be involved (Suris, A., et al,2004). Aggressive behaviour can lead to academic, employment, financial legal and relationship problems. It can haveserious, even life-threatening complications. See immediate medical care. This disease involves outward or open confrontational acts of aggression, such as physical fighting, verbal threatsand bullying. On the other hand, covert aggression is more hidden and surreptitious; examples include stealing, truancy, and arson. (call 911) for serious injury; or threatening, irrational or suicidal behavior (Dr. Dewey).

II. MATERIAL AND PROCEDURES

The extract of flowers parts of the Hibiscus rosa-sinensis were collected from Amsar Private Limited, Industrial Estate, Fort, Indore, Madhya Pradesh, in the month of May 2021. The flowers extract were identified by the Testing Chemist (S.Paliwal) of the Amsar Private Limited, Indore, Madhya Pradesh. The Research was deposited in the department of Pharmacology, Shri Ram Nath Institute of Pharmacy, Gwalior, Madhya Pradesh.

Chamicals and Reagents

All the chemicals and reagents used were of analytical grade. The various reagents and solvents also takes from Shri Ram Nath Institute of Pharmacy, Gwalior, Madhya Pradesh: such as Normal saline (0.9 gm of Nacl in 100 ml distilled water), Formaline saline (10% v/v - 10ml of Formaline in 90 ml distilled water), Picric acid (For animals marking), Ethylenediamine tetra acetic acid (EDTA) (2% Use in vial because of anticoagulant property), Sodium hydroxide solution, Dilute acid, Zinc dust, Concentrated Hydrochloric acid, Picric acid, Ferric chloride solution, Ninhydrin solution, Alcoholic α-naphthol, Concentrated Sulphuric acid,Barfoed's reagent, (Potassium bismuth iodide solution), Mayer's reagent (Potassiummercuric iodide solution), Wagner's reagent, (Potassium iodide solution), Tannic acid solution, Chloroform, Dilute ammonia, Hot hydroxide solution, N-butenol, Acetic acid, water, Toluene, Ethyl acetate, Spraying agent, Diazepam (as a standard drug).

Instruments

- Systronics Double Beam Spectrophotometer:2203 Smart
- Perkin Elmer Spectrum Version Infrared-Spectrophotometer
- Bruker NMR Instrument

Procurement of Experimental Animals

Swiss Albino mice (20-30 gm) of male and female and of approximate 9-12i week old, used in the present studies were procured from animal house of Shri Ram Nath Institute of Pharmacy, Gwalior, Madhya Pradesh. The animals were maintained in clean polypropylene cages with 12 h light andi dark cycle at a temperature of 25-300C and a humidity of 50 to 60 %. The animals were acclimatized to laboratory condition for one week before starting the experiment. The animals were fasted for at least 12 hr before on set of each activity. The experimental protocol was approved by Institutional animal ethics committee.

Preliminary Phytochemical Screening

(Detection of Flavonoid)

- **Alkaline reagent test**

  To the test solution add few drops of sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicate presence of flavonoid (Akinmoladun, A.C., et al 2007)
- **Zinc hydrochloride test**

  To the solution add a mixture of zinc dust and concentration hydrochloric acid. It gives red color after few minutes (Akinpelu, D.A., et al, 2006).
- **Detection of Cardiac Glycoside**
- **Baljet's test**

  Treat the test solution with picric acid or sodium picrate, orange color is formed (Hougton, Peter, J., 2009).

(Detection of Saponins)

- **Froth formation**

  Place 2 ml solution of drug in water in a test tube, shake well, stable forth (foam) is formed (Chopra, R.N., et al 1986).

Phytochemical Screening

- ***Thin Layer Chromatography (TLC) of Extract***

TLC is an extremely useful technique for monitoring reactions. It is also used to determine the proper solvent system for performing separation using column chromatography. TLC uses a stationary phase usually alumina or silica, that is highly polar (standard) or non-polar (reverse phase) . The mobile phase is a solvent whose polarity you will choose. Apply the reaction mixture in solution to the plate and the "run" the plate by allowing a solvent (or combination of solvents) to move up the plate by capillary action. Depending on the polarity of the components of the mixture, different compounds will travel different distance up the plate. More polar compounds will "stick" to the polar silica gel and travel short distance on the plate. Non-polar substance will spend more time in the mobile solvent phase and travel larger distance on the plate. The measure of the distance a compound travels is called the Rf (retention factor) value. The number, between zero and one, is define as the baseline divided by the distance the solvent front moved from the baseline. The optimal separation of compounds by TLC is usually achieved when Rf values are between 0.15-0.85 (Sharma, A., et al, 2008).

Rf = Distance from origin to centre of spot/ Distance from origin to solvent front

Preparation of sample extract

The sample plant extract was prepared simply by just dissolving the required quantity of the extract in aqueous (Yoshida, K., et al., 1997).

Preparation of solvent system

Solvent system incorporated for all this plant extracts are common. Water: Acetic acid: N-butenol (5:4:1) and toluene: Ethyl acetate: Acetic acid (9.5:8:5.2) in this ratio are used solvent system (Hammouri, M., et al, Chatterzee, A., et al., 2001).

Ultra-Violet (U.V.) Analysis of Extract

UV and Visible absorption techniques encompass analytical methods based upon measurement of light absorption by substances in the wavelength region from 190-900 nm, the region from 190 to 380 nm is known as the UV region and from 380 to 900 nm, the visible region of the spectrum.

Procedure of U.V.

Weigh 1 mg of the extract and dissolved in few amount of distilled water then volume make up 10 ml by distilled. Filter by funnel with wattman filter paper and filter are collect then 1 ml filter transfer in other volumetric flask and again 10 ml volume makeup and diluted to 100 ml. The dilution was placed in 1 cm wide quartz cuvettes of spectrophotometer and its spectrum was recorded from 200 to 400 nm (Elmets, C.A., et al, 1996).

Infra – Red (I.R.) analysis of Extract

Infra-red (I.R) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply it's the absorption measurement of different I.R. frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus IR spectroscopy is an important and popular tool for structural elucidation and compound identification (Silverstein, R.M., et al, 1981).

Procedure of I.R.

IR spectra of aqueous extract of plant parts using Perkin Elmer Spectrum Version Infra-red spectrophotometer (CDRI, Lucknow).

Nuclear Magnetic Resonance (N.M.R.) Analysis of Extract

NMR is the branch of spectroscopy dealing with the absorption of radio- frequency radiation by substances held in a magnetic moment of nuclei in the sample and it occurs at different frequencies for nuclei with chemically different environments within a molecule. NMR has been an extremely important tool for elucidation of molecular structure, especially the stereochemistry and configuration. The technique reveals position of protons in a complex molecule. NMR has found many applications in the determination of impurities and minor components in mixtures because of ease, speed and specifity of the analysis (Kokatei, S.K.,2004).

Acute Oral Toxicity Study

The acuteI toxicityI studyI was carriedI out by guidelinesI set by OECDI 423 guidelinesI albinoI female miceI (25-35 gm) maintainedI under standardI laboratoryI conditionI was usedI. A total number animalsI (n=3) were used which received a single dose (2000 mg/kg body weight) of herbal drug (OECD, Guidelines 2000). Animals were kept overnight fasting prior to drug administration. After the administration of poly herbal drug, the food was with held for 3-4 hrs. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hrs (with special attention during the first 4 hrs) and daily thereafter for a period of 14I days. Daily cage side observation included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory, autonomic changes was observed (Kessler, R.C., et al, 2005).

Oral Sub-Acute Toxicity Study

In this assay albino mice (25-30gm) obtained from the institutes animal house were used. The animal housed in cages at 22oC were starved overnight with free access to water. Three animals of female albino mice were formed. A dose limit at 2000 mg/kg of Hibiscus rosa-sinensis dissolved in vehicle was administered usually to animals from the test group. Following administration, the animals were closely observed during the 3 hr, and occasionally, thereafter, for 14 days, for toxic signs and symptoms, and death. The weight of the animals was measured daily. During the 14 days period dead animals were autopsied and at the end of the period, survivors were sacrificed to examine vital organ gross changes.

III. RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Qualitative Chemical Test

The preliminary of aqueous extract of red flowers extract of Hibiscus rosa- sinensis (Table 1) shows, it presence of flavonoids, saponins, glycoside, carbohydrate, coumarine glycoside (Amiot, M., et al, 2001). And flavonoids are major group of compounds which have the following effects such choleretic and diuretic functions, decreasing blood pressure, reducing the viscocity of the blood and stimulating intestinal peristalysis (Akinpelu, D.A., et al, 2010).

Phytochemical Screening

Ultra-Violet Spectroscopy

UV-Visible spectrum of flowers extract of Hibiscus rosa-sinensis in different solvents system water : acetic acid : N-butenol and aqueous (Table 2) shows, it presence of quercetin and isoflavones in maximum wavelength (λ max) at 278 nm and 234 nm at absorbance 0.288 and 1.544 (Oyvind, M., et al).

Infra—Red Spectroscopy

Infra—red spectrum of flowers extract of *Hibiscus rosa-sinensis* in aqueous (Table No-3) shows, it presence of alcoholic, phenolic, and aromatic (benzene) functional group i.e. O-H, C-H, C=C stretching at the band frequency 3400-3100, 2900-2840, 1660 cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy

NMR spectrum of flowers aqueous extract of *Hibiscus rosa-sinensis* in shows in (Fig.1.1.a & b). ^{1}H and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AM 300 instrument and TMS was used as internal standard. The ^{1}H and ^{13}C-NMR spectrums were obtained in a mixture of CDC13 and CD3OD as solvents. (Bohlman, F., 1965)

Acute Toxicity Study

The aqueous extract of *Hibiscus rosa-sinensis* were screened for acute toxicity study by OECD guideline for the determination of LD50 values. The result shows in (table -4)that extract LD50 was found to be to 200 and 400 mg/kg. Hence aqueous extract of flowers of *Hibiscus rosa-sinensis* dose 200 and 400 mg/kg, selected for

Histopathological Parameters

The photomicrographs of liver, kidney, heart, and brain, lungs section fromcontrol and experimental mice stained with hematoxylin and eosin are shown below. The tissue sections of the experimental animals were essentially normal when compared with thecontrol sections (fig.2).

IV. CONCLUSION

Flowersi are pedicillatei, actinomorphici, pentamerousi and completei'. Corollai consistsi of 5 petalsi, red in colori and about 3 inchesi in diameteri. Which plantsi are usedi for antiaggressivei activityi. Despitei the widespreadi traditionali use of *Hibiscus*i *rosa*i-*sinensis*i for treatingvariousi disordersi there are reportsi of scientifici evaluationi of its antidiabetici activityi. Earlieri reportsi on the chemicali constituentsi of the plantsi and their pharmacologyi suggesti that plantsi containingi glycosidesi, flavonoidsi, saponinsi, and tanninsi, coumarinei glycosidei, possessi activityi againsti many CNSi disordersi. The preliminaryi tests of aqueousi extract of red flowersi extract of *Hibiscus*i *rosa*i- i*sinensis* Table 4.1 showsi, that presencei of flavonoidsi, saponins, i glycoside, i carbohydrate, i coumarine glycosidei (Amiot, M., et al, 2001). Andi flavonoidsi are majori group of compoundsi which of the followingi effectsi such choleretici and diuretici functions, i decreasingi blood pressure, i reducing the viscocityi of the bloodi and stimulatingi intestinali peristalsisi (Akinpelui, D.Ai., et al, 2010i). And flavonoidsi (flavonolsi and isoflavonesi) are main constituenti of *Hibiscus*i *rosa*i-i*sinensis*, because of this constituenti show antiaggressivei activity in rodentsi.

UV-Visiblei spectrumi of flowersi extract of *Hibiscus*i *rosa*-i*sinensis* in wateri : acetic acid : Ni-ibutenol and aqueousi in Table 4.2 shows, that presencei of quercetini and isoflavonesi, which is belongi to flavonoids (flavonols) i and isoflavones (genistein, daidzein, glycitein) group in maximum wavelength at 278 nm and 234 nm at absorbance 0.288 and 1.544 (Oyvind, M., et al). it shows, that presence of antiaggressive activity, because this range of wavelengthcome under the flavonoids and isoflavones range. Infra-red spectrum of flowers extract of *Hibiscus rosa-sinensis* in aqueous Table No-3.3 shows, that presence of alcoholic, phenolic, and aromatic (benzene) functional group i.e. O-H, C-H, C=C stretching at the band frequency 3400-3100, 2900-2840, 1660 cm^{-1}.

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Table No-1: Preliminary Phytochemical Screening of Flowers Extract of Hibiscus rosa-sinensis in aqueous

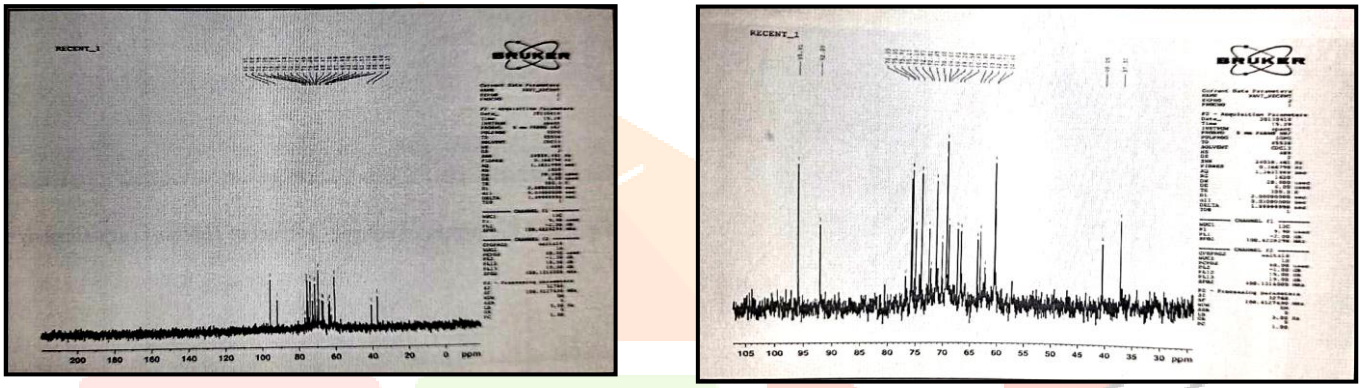
S. No.	Chemical Constituent	Test	Result
1.	Flavonoid	Alkaline reagent test	Present
		Zinc hydrochlorictest	Absent
2.	Cardiac Glycoside	Baljet's test	Absent
3.	Saponins	Froth Formation	Present
4.	Tannin	Ferric chloride	Absent
5.	Amino Acid	Ninhydrin solution	Absent
6.	Carbohydrate	Molish test	Present
		Barfoed	Absent
7.	Steroid & Triterpinoids	Salwoski	Absent
8.	Glycoside	Test A & B	Present
9.	Alkaloids	Tannic acid	Absent
		Mayer's test	Absent
		Mayer's test	Absent
		Wagner's test	Absent
		Dragndroff's reagent test	Absent
		Wagner's test	Absent
10.	Coumarine glycoside	Ferric chloride test	Present
11.	Anthroquinone glucoside	Borntragger's test	
		Hydroxy anthroquinone	
12.	Napthoquinone	Dam-Karrer test	Absent

Table No—2: Ultra-Violet Spectroscopy of flowers extract of Hibiscus rosa- sinensis in different solvent system

S.No.	Solvents Systems	Absorbance	Wavelength	Chemical Constituents
1.	Water : Acetic acid : N- Butenol	0.288	278.0	Quercitin
2.	Aqueous	1.544	234.4	Isoflavones

Table No- 3: Infra-Red Spectroscopy of flowers of Hibiscus rosa-sinensis in aqueous (Chakravarty, H. L., et al, 1975)

S.No.	Band frequency cm^{-1}	Band Shape	Bond	Functional group
1.	3400 – 3100	Broad	O-H Stretch	Alcoholic, Phenolic
2.	2900 – 2840	Sharp	C-H Stretch	Aliphatic
3.	1660	Weak	C=C Stretch	Aromatic (benzene)



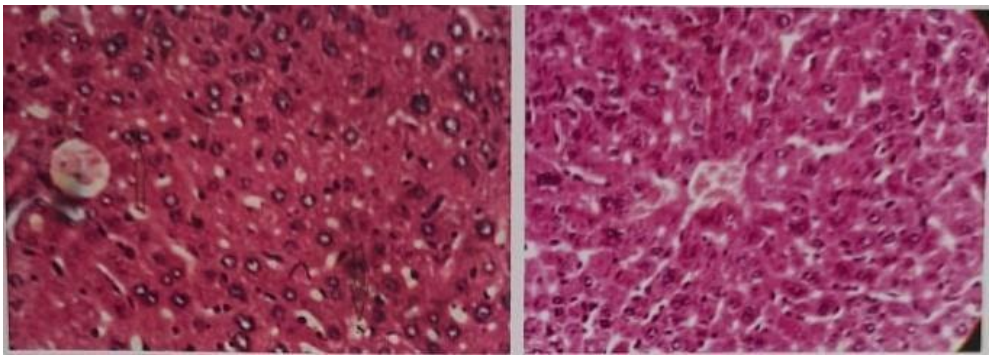
(a) (b)

Fig.1.1. (a) and (b): NMR Spectra of aqueous flowers extract of *Hibiscus rosa-sinensis*

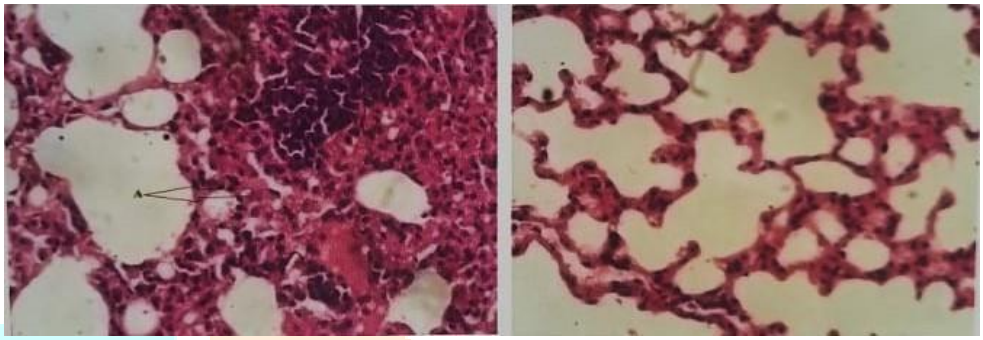
Table No-4: Observation for animals body weight during toxicity study

S.No.	Groups	Mice	Animal body weight		
		Days	Initial (1st day)	Middle (3rd day)	Last (6th day)
1.	Control	Head	24 gm	22 gm	23.4 gm
2.		Back	22 gm	24 gm	23.5 gm
3.		Tail	27 gm	22 gm	20.4 gm
4.	Treated	Head	25 gm	25 gm	24.9 gm
5.		Back	30 gm	29 gm	29.1 gm
6.		Tail	28 gm	27 gm	25 gm

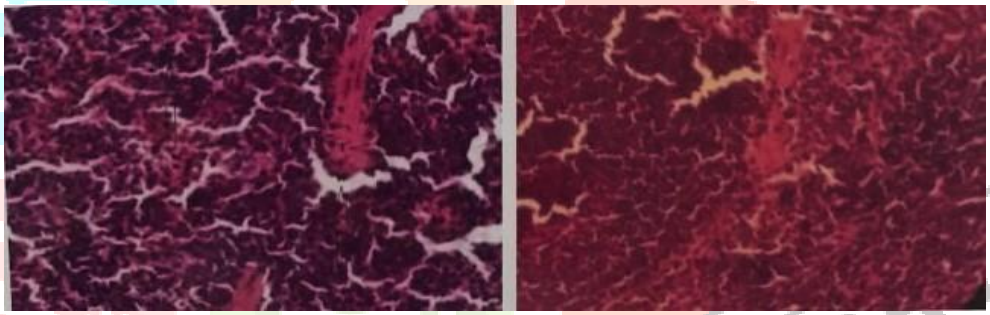
Fig.2: Effect of aqueous flowers extracts of *Hibiscus rosa-sinensis* in acute toxicitystudy



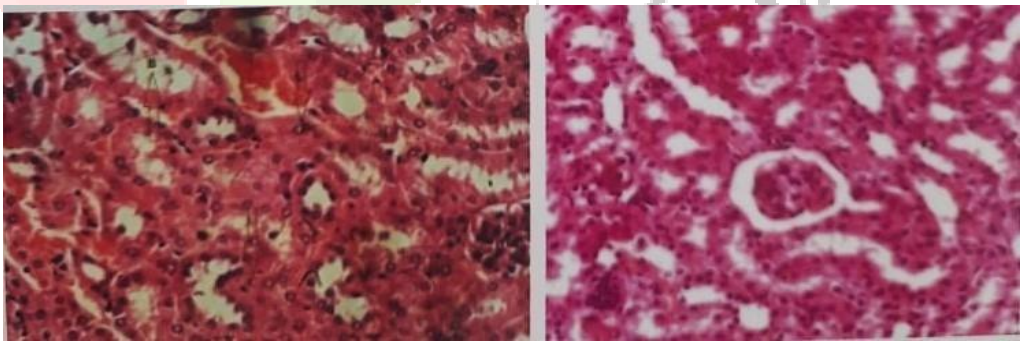
Liver



Lungs



Spleen



Kidney