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Evaluation of Antiulcer Activity of Ethanolic Extract of *Madhuca longifolia flowers* in Experimental Rats

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Abstract- Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, the present study was carried out to investigate antiulcer activity of ethanolic extract of *Madhuca longifolia* flowers in pylorus ligated ulceration in the albino rats .The ethanolic extract of ***Madhuca longifolia* flowers** at doses of 100,200,300 mg/kg b.w produced significant ($p<0.01$) inhibition of the gastric fluid volume, free acidity, total acidity. In conclusion the antiulcer properties of the extract may be attributed to the presence of phytochemicals like flavonoids (quercetin), alkaloids and tannins present in the plant extract with various biological activities.

Index Terms- ***Madhuca longifolia***, Ethanol, Free acidity, Total acidity.

I. INTRODUCTION

Peptic ulcers are a deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa. For decades it was believed that the excessive secretion of gastric acid caused gastrointestinal ulcerations, but many patients presenting such ulcerations had normal acid secretion rates. Then, researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanisms. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth.

Peptic ulcer therapy has undergone many strides over the past few years and a number of drugs are available for treatment. These drugs are broadly classified into two groups, those that decrease or counter acid pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defence factors.These drugs act by different mechanisms, most of the commonly used drugs are H_2 blockers (ranitidine, famotidine etc.,), M_1 blockers (pirenzepine, telenzepine etc.,).proton pump inhibitors (omeprazole, lansaprazole etc.,) decrease secretion of acid while drugs like sucralfate and carbanoxolone promote mucosal defence. Recently the role of these drugs on the defensive factors gaining importance.

It is now assumed that these drugs ultimately balance the aggressive factors (acid, pepsin, *H.Pylori*, bile salt) and defensive factors (mucin secretion, cellular mucus, bicarbonate secretion). Although these drugs have brought about remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects (arrythmiasis, impotence, gynaecomastia) and danger of drug interactions during ulcer therapy .hence search for an ideal anti ulcer drug continuous and has also been extended to herbal drug in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Madhuca longifolia (Koen.) Macbr. (Syn. *Bassia longifolia* J. Koenig ex. L. *M. longifolia* (Koen.) Macbr. var. *longifolia*) , is a large, shady, deciduous tree, both wild and cultivated, dotting much of the Central Indian landscape. The tree is valued for its flowers, fruits, seeds and timber. The expectorant flowers are used to treat chest problems such as bronchitis. They are also taken to increase the production of breast milk. The distilled juice of the flowers is considered a tonic, both nutritional and cooling. The tree wins in fame due to the liquor distilled from the flowers, which is used to make vinegar. The leaves are applied as a poultice to relieve eczema. In Indian folk medicine, the leaf ash is mixed with ghee (clarified butter) to make a dressing for wounds and burns. Mahua preparations are used for removing intestinal worms, in respiratory infections, and in cases of debility and emaciation. The astringent bark extract is used for dental-related problems, rheumatism and diabetes.

The butter cup fruit-seeds, generally ellipsoidally shaped, measures from 1.5 to 2.0 cm and 1.3 to 1.6 cm across the length and breadth, respectively. *Madhuca longifolia* fruit is valued for its seed which yield high quantity of fat (Ca.50%), commercially known as Mahua butter or mowrah butter, and has many edible and medicinal applications.The semi-solid mahua fat is used in cooking, adulderation of ghee, and manufacturing chocolates. The seed fat has emuluscent property; it is used for skin diseases, rheumatism, headache, laxative, piles and sometimes used as galactogugue. Besides its edible and medicinal uses, *Madhuca longifolia* fats can also be utilized in the manufacture of laundary soaps and lubricants.Moreover, the seed cake is reported to have insecticidal and pesticidal property and also used for fishing. The timber is useful in various ways, but neglected. The medicinal properties attributed to this plant are stimulant, demulant, emollient, heating and astringent.

II. MATERIALS AND METHODS

Plant material

Plant parts of *Madhuca longifolia* were collected from 25-30 year old trees, from a temple owned grove, in a Village, Rajendrum Arcot of Thanjavur district, Tamilnadu.The identity of the plant specimens was confirmed by the use of local Floras and standard references.The botanical identity was also authenticated by Dr.M.Jegadeesan, Professor and Head, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur. Herbarium specimen of *Madhuca longifolia* are deposited at Tamil University Herbarium (TUH288).

Preparation of extracts

Fresh flowers of *Madhuca longifolia* were collected and macerated with 50% ethanol for 7 days with occasional shaking to get alcoholic extracts. The alcoholic extracts were concentrated in a rotary flash evaporator and dried in desicator.

III. TOXICOLOGICAL STUDIES

Gross behavioural and acute oral toxicity studies

Gross behavioural and acute oral toxicity studies (LD_{50}) of the extracts were determined as suggested by Turner.The mice weighing between 30-40 g were selected. The mice were grouped into 6 mice per group, each extract at different dose levels (100, 200, 300, 400, 500 mg/kg b.w.). Dissolved in distilled water were administered once orally per dose level to the overnight fasted animals. The group receiving water (1 ml/kg) was kept as control. The animals were subjected to primary screening studies at ½, 1, 2 and 4 hours respectively and finally overnight mortality was recorded. Behaviour of the animals and any other toxic symptoms were also observed for 24, 48 and 72 hours and the animals were kept under observation upto 14 days. After administration to find out delayed mortality if any.

Swiss adult albino male rats (b.w. 180-220 g) were employed for pharmacological evaluations of anti-inflammatory, antiulcer and analgesic activities; while swiss adult albino male rats were purchased from Sri Venkateswara Enterprises, Bangalore and maintained under standard experimental conditions (Temperature 27 ± 2°C, relative humility 60±5% and 12 hours light/dark cycle) and the animals were maintained as per the rules in the guidelines of CPCSEA. The animals were housed in standard microlon boxes and were given standard laboratory diet (Amrut Laboratory Animal Feed, Sangli-416436) and water ad libitum. All pharmacological experiments involving animals described in the present work were carried out and get approved by Local Animal Ethical Committee of Department of Pharmacology, Periyar College of Pharmacy for Women, Trichy.

ANTI-ULCER ACTIVITY (Modified pyloric ligated (Shay) rat model)

The experimental procedures of Shay *et al.* (1945), modified by Okabe *et al.* (1982) were used.

Rats weighing 180-220 g were divided into groups of six animals each and were placed in cages with grating floor to avoid coprophagy and fasted for 48 hours allowing the access to water. One group received water (1 ml/kg) and was served as control (Ranitidine (30 mg/kg) were selected as standard drugs and as given to a groups, for comparison. For the test group, each extract the animals were grouped into three, receiving the drug at a dose level of 100, 200 and 300 mg/kg body weight.

Under light ether anesthesia, the abdomen was opened by a small mid-incision now the xiphoid process, pyloric portion of stomach was slightly lifted out and legated avoiding traction to the pylorus or damage to its blood supply. The stomach was placed carefully and the abdomen wall closed by interrupted sutures. The test drugs were administered twice daily, orally, for two days and other drugs were administered once daily orally for two days prior to and one hour before to pyloric ligation. The animals were deprived of both food and water during the post-operative period. Four hours after the ligation, animals were sacrificed.

The stomach was excised carefully keeping the oesophagus closed, opened along the greater curvature and the luminal contents were removed as described.The gastric contents were collected in a beaker and centrifuged at 1000 rpm for 10 minutes as recommended.The samples were analysed for gastric volume, pH, free and total acidity, sodium and potassium output as recommended.Bio-medical estimations, like total proteins, total hexoses, hexosamine, fucose, sialic acid and pepsin were also done. The mucosa was flushed with saline and stomach pinned on a frog board and scored. The scoring is done as described by Laurence and Bacharach (1964).

Ulcer Score

Ulcer Score	Descriptive Observation
0	Normal rugal pattern
1	Alteration in normal rugal pattern
2	Scattered haemorrhage lesions
3	Haemorrhage lesions and ulcers
4	Penetrating and perforating ulcers

After completed the experimental regimen, the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuge for 5 min at 2000 × g and the supernatant was separated. The volume, pH, and total acidity, free acidity, sodium and potassium of gastric fluid were determined in flame photometer.

Dissolved mucosubstances were estimated in 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was dissolved in 1 ml of 0.1 *N* NaOH or 1 ml of 0.01N H_2SO_4.The former was used for the estimation of protein, total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid. The ratio of total carbohydrate (TC) (Sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity.

IV. PREPARATION OF HOMOGENATE

The stomach was then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 3000 × g for 20 min using centrifuge. The clear supernatant was used for the assays of lipid peroxidation (MDA content), endogenous antioxidant enzymes (Cu/Zn) Superoxide dismutase (SOD) and catalase (CAT)), and reduced glutathione (GSH). The sediment was resuspended in

ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes (Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase) and proteins.

ESTIMATION OF STOMACH CONTENTS

DETERMINATION OF GASTRIC JUICE VOLUME AND pH

The volume and pH of centrifuged gastric juice were measured by pipette and digital pH meter. The volume was expressed as ml

DETERMINATION OF TOTAL AND FREE ACIDITY

The total and free acidity were determined by titrating with 0.01N NaOH using phenolphthalein and Topfer's reagent or methyl orange.

Reagents

1. 0.01N NaOH
2. Phenolphthalein
3. Topfer's reagent or methyl orange

Procedure

Pipette 1ml of filtered gastric contents into a small beaker, add 2 to 3 drops of Topfer's reagent or methyl orange and titrate with 0.01 N NaOH until all trace of the red colour disappears and the colour is yellowish orange. Note the volume of alkali added that indicate free acidity. Then add 2 or 3 drops of phenolphthalein and continue titrating until a definite red tinge reappears. Note the total volume of alkali added that indicate total acidity.

The results expressed as Meq/l

GASTRIC MUCOSAL DEFENSIVE STUDY

DETERMINATION OF PEPSIN ACTIVITY

The pepsin activity was determined by using BSA as a substrate.

Reagents

1. 10% TCA
2. 2.5 N NaOH
3. Folins phenol reagent (1:2)
4. BSA standard (0.5% BSA in 0.01N HCl)

Procedure

A mixture of gastric juice (0.1 ml) and 0.5% BSA in 0.01 N HCl (1 ml) was incubated at 37°C for 20 min and the reaction was stopped by adding 10% TCA (2 ml). After denaturation of protein by heating in boiling water bath for 5 min the precipitate was removed by centrifugation (3000 rpm/10 min). A total of 1 ml of the supernatant was mixed with 0.4 ml of 2.5N NaOH and 0.1 ml of Folin's reagent and total volume was adjusted to 10 ml with distilled water. Read 640 nm after 20 min.

The results were expressed in terms of μmole of L-tyrosine liberated.

ESTIMATION OF SODIUM

Sodium was estimated by colorimetric method

Reagents

1. Precipitating reagent: Magnesium Acetate 533 mMol/1
2. Coloring reagent: Potassium Ferrocynide 15 mMol/1 and Uranyl acetate 22 mMol/1.
3. Standard reagent: 150 mMol/1

Procedure

To the standard tube, about 1ml of precipitating reagent, 0.01 ml of standard reagent were taken. 1ml of precipitating reagent and 0.01 ml of sample was taken for the test mixed well and allowed to stand at room temperature for 5 minutes then centrifuged at 2000-3000 rpm for 2 minutes to obtain clear supernatant.

About 0.02 ml of supernatant was taken from each tube. To this 1 ml of colouring agent was added. Blank consists of 0.02 ml of precipitating reagent and 1 ml of colouring reagent mixed well and allowed to stand at room temperature for 5 minutes which was read at 530 nm. Standard also conducted similar way.

The values were expressed as Meq / L.

ESTIMATION OF POTASSIUM

Potassium was estimated by the method of Maruna & Trinders

Reagents

1. Boron reagent: Sodium Tetraphenyl Boron 30 mMol/1
2. Sodium hydroxide: 50 mMole/1
3. Standard reagent: 5 mMol/1

Procedure

To 0.05 of sample and 1ml of boron reagent were added. 0.1 ml of standard reagents and 1ml of boron reagent was added for standard. All the tubes were mixed well, incubated at room temperature for five minutes. The color developed was read at 620 nm colorimetrically within 10 minutes.

The values were expressed as Meq / L

ESTIMATION OF HEXOSE

Hexose level was estimated by the method of Niebes

Reagent

1. Orcino1- Sulphuric acid reagent
2. Solution A: 60 ml of concentrated sulphuric acid was mixed with 40 ml of distilled water.

Solution B: 1.6 g of orcinol was dissolved in 100 ml of distilled water.

Prepare reagent: 7.4 ml of solution A was mixed with 1 ml of solution B before use.

3. Standard: 5 mg of each of galactose and mannose were dissolved in 100 ml of distilled water (100μg/ml).

Procedure

0.5 ml of the neutralized solution was made upto 1 ml with distilled water and added 8.5 ml ice-cold orcinol reagent. The mixture was heated at 80°C for 15 minutes, cooled and left in the dark for 25 minutes for colour development. Then absorbance was read at 540 nm in a colorimeter. Standard solutions containing 25-100 μg of hexose were treated in a similar manner.

The hexose content was expressed as μg/ml

ESTIMATION OF HEXOSAMINE

Hexosamine content was estimated by the method of Wagner .

Reagent

1. Acetyl acetone reagent

Solution A: Trisodium phosphate 0.1M
Solution B: Potassium tetraborate 0.5N
3.5 ml of acetyl acetone was added to mixture of solution A and solution B in the ration of 98:2(v/v)

2. Ehrlich's reagent

320 mg of p-dimethy; aminobenzaldehyde was dissolved in 21 ml of isopropanol and 3 ml of concentrated HCl.

3. Standard: Galactosamine was prepared in the concentration range of 100 μg/ml in water.

Procedure

0.5 ml of the neutralized sample was made upto 1 ml with distilled water. Standard galactosamine (in the range of 10-4- μg) was also made upto 1 ml with distilled water. 0.6 ml of acetyl acetone reagent was added to all the tubes and heated in a boiling water bath for 30 minutes. After cooling, 2 ml of Ehrlich's reagent was added and the contents were shaken well. The pink colour developed was measured at 540 nm against the reagent blank.

Hexosamine content was expressed as μg/ml.

ESTIMATION OF FUCOSE

Fucose level was estimated by the method of Dische and Shettles

Reagent

1. Sulpuric acid: water mixture (6:1)
2. Cysteine reagent
3. Standard: 20 mg of methyl pentose was dissolved in 100 ml of distilled water.

Procedure

0.05 ml of the neutralized sample and 5 ml of sulphuric acid: water mixture was added and heated in a boiling water bath for 10 minutes. After cooling the tubes, 0.1 ml of cysteine reagent was added. The colour developed after 150 minutes was read at 420 nm. The standard was also treated in a similar manner.

The fucose level was expressed as μg/ml.

ESTIMATION OF SIALIC ACID

Sialic acid level was determined by the method of Warren

Reagents

1. Periodic acid 0.25M
2. 4% Sodium meta arsenite
3. Thiobarbituric acid
4. Acidified butanol
5. Standard sialic acid: 10 mg of N-acetyl neuraminic acid was dissolved in 100 ml of distilled water.

Procedure

0.5 ml of the neutralized sample was taken along with the standards (in the range of 10-40 μg). Blank contained 0.5 ml of 0.1 N sulphuric acids. 0.25 ml of periodate was added to all tubes at 37°C. After 30 minutes, 0.25 ml of arsenite solution was added to inhibit the reaction. Contents were mixed well and 2 ml of thiobarbituric acid was added and the tubes were heated in a boiling water bath for 6 minutes. After cooling, the pink colour developed was extracted into 5 ml of acidified butanol phase and was measured at 540 nm against a reagent blank.

The sialic acid content was expressed as μg/ml

V. STATISTICAL ANALYSIS

The raw data of the present study were subjected to simple statistical analysis to draw meaningful interpretation and conclusion.

1. The standardization values of study drugs were expressed in percentage (w.w.).
2. For Pharmacological Studies

The Mean ± SEM and student's 't' test are computed for all the biochemical estimations, to find out statistical significance at 1% and 5% probability levels.

The data were computed and analysed using Statistical Package for Social Sciences (SPSS) software version 11.5.

VI. RESULT AND DISCUSSION

The alcoholic of flowers of *Madhuca longifolia* were tested for their acute toxicity in Swiss albino mice. The acute oral toxicity was carried out as per OECD, revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (OECD, 2001). To determine acute toxicity of single oral administration of the extract, in a dose of 2000 mg/kg body weight were given. The extracts were prepared as a suspension by titrating with water and 1% gum acacia.

The test substances were administered as a single dose by intra gastric tube. Prior to dosing, animals were kept for 12 h of fasting. The animals were weighed and test substances were administered.

After administration of the extract, the animals were observed individually for 4 h and thereafter 14 days for any mortality and their behavioural pattern. The parameters noted were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion.

In the acute toxicity study, the alcoholic extracts of *Madhuca longifolia* did not show significant toxic effects when observed for the parameters during the first four hours and followed by daily observations for 14 days and no mortality was also observed. The extracts were found to be safe at the tested dose level of 2000 mg/kg body weight indicating the high margin of safety of these extracts.

Alcoholic extract of *Madhuca longifolia* flowers was administered orally to rats in different dosages (100, 200 and 300 mg/kg b.w.) for their antiulcer activity in pyloric ligated (shay) model (Table1). Aggressive factors like free acidity, total acidity, protein and pepsin, ulcer score was found to be significantly decreased in a dose dependent manner. The protective factors like hexoses, hexosamine, fucose and sialic acid were found to be significantly increased in a dose dependent manner. The isolated

rat stomach treated with 300 mg/kg b.w. of alcoholic extracts of *Madhuca longifolia* flowers showed maximum antiulcer activity.

In the present investigation, the alcoholic extract of flowers of *Madhuca longifolia* exhibited a significant gastro protective effect. It has been postulated that histamine might be involved in the formation of pylorus-ligated ulcers and plays a mediating role in the gastric secretion stimulated by gastrin, vagal excitation and cholinergic agents.Thus the effect of the alcoholic extracts of flowers of *Madhuca longifolia* on gastric lesions induced by pylorus ligation could be due to the histamine inhibition and or scavenging the free radical. This gastro-protective effect of the extract can be attributed to the various bioactive principle detected in the ethanolic extract, pylorus ligation-induced ulcers are due to autodigestion of the gastric musoca and break down of the gastric mucosal barrier. In our present study alcoholic extract of *Madhuca longifolia* flowers showed maximum antiulcer activity at a dose of 300 mg/kg b.w. Aggressive factors like free acidity, total acidity, protein, pepsin and ulcer score were found to be significantly decreased in a dose dependent manner. The protective factors like hexoses, hexosamine, fucose and sialic acid were found to be significantly increased in a dose dependent manner. Thus the active principle in the extract might be enhancing the mucosal defensive factors (Hexose, Hexosamine, Fucose, Sialic acid) leading to increased mucus production protecting the surface epithelial cells.

Survey conducted in Australia and US indicated that respectively 48.5 and 34% of the respondents used at least one form of unconventional therapy, including herbal medicine.The most medicinal plants are enriched with bioflavonoids, which have antioxidant activity. Flavonoids as antioxidants exhibited several biological effects such as antiulcer, anti-hepatotoxic, anti-inflammatory, antiallergic, antidiabetic and antipyretic actions.Peptic ulcer is the most common gastro intestinal disorder in clinical practice. Considering the several side effects (arrythmias, impotence, gynaecomastia and haematopoeitic changes) of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer Currently, proton pump inhibitors such as omeprazole are extensively used to control increased acid secretion and acid related disorders including gastroesophageal reflux disease, Zollinger-Ellison syndrome and gastro duodenal ulcers caused by stress (stress related erosive syndrome), non-steroidal anti-inflammatory drugs and by *H. pylori*.Although, histamine-H_2 receptors blockers (ranitidine, famatidine etc.) the proton pump inhibitors (omeprazole, lansoprazole etc.) have been used for the efficient management of gastric hypersecretion and gastroduodenal ulcers, several adverse effects of these drugs have also been reported.

Madhuca longifolia flower extracts exhibited antiulcer activity by increasing hexosamine and carbohydrate protein ratio and decreasing pepsin content. This result in the increase in mucous secretion. The importance of mucous secretion as a response to gastric mucosal trauma has long been recognised.Apart from antiulcer effect the *Madhuca longifolia* flower, they possess significant antipyretic, anti-inflammatory, analgesic, antidiabetic and wound healing activity.Drugs with multiple mechanism of protective action, including antioxidant properties may be one way forward in minimizing tissue injury in human diseases.Although in most of the cases the aetiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism.Studies have shown that alteration in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus-ligation induced ulceration in rats.

The preliminary phytochemical analysis of *Madhuca longifolia* extract showed the presence of alkaloids, flavonoids, triterpenoids,carbohydrates and glycosides. The significant increase in the antiulcer activity of *Madhuca longifolia* could be attributed to the presence of flavonoids (quercetin), alkaloids, tannins,saponin glycosides and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus,bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.So the antiulcer activity of *Madhuca longifolia* may be attributed to its flavonoids content. The results of the present study suggest that the ethanol extract of *Madhuca longifolia* may be beneficial in the treatment of gastric lesions. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

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Table 1
Effect of Ethanalic extract of *Madhuca longifolia* flowers in modified pyloric ligated (Shay) rat model

S. No.	Groups	Drug Dose mg/kg	Ulcer score	pH	Gastric volume (ml)	Protein (µg/ml)	Pepsin (µg/ml)	Free acidity (meq/l)	Total acidity (meq/l)	Na^+ (mg/l)	K^+ (mg/l)	Hexoses (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic Acid (µg/ml)
1.	Normal	1 ml	-	3.600 ± 0.010	3.566 ± 0.136	420.66 ± 0.350	5.523 ± 0.008	48.83 ± 0.300	53.59 ± 0.420	1.642 ± 0.005	0.201 ± 0.005	308.21 ± 0.320	280.76 ± 0.523	46.10 ± 0.172	37.33 ± 0.909
2.	Control	1 ml	3.33 ± 0.12	2.618 ± 0.004	5.216 ± 0.147	662.91 ± 0.755	11.26 ± 0.014	85.66 ± 0.333	103.0 ± 0.894	1.053 ± 0.003	0.427 ± 0.006	280.33 ± 0.393	253.33 ± 0.714	27.20 ± 0.662	28.50 ± 0.763
3.	Std	30	1.43*** ± 0.04	3.361** ± 0.010	3.600** ± 0.136	424.99** ± 0.366	5.523* ± 0.008	46.83** ± 0.307	54.50** ± 0.428	1.643** ± 0.005	0.212** ± 0.007	315.27** ± 0.325	284.66** ± 0.557	47.12** ± 0.480	37.00* ± 0.683
4.	*Madhu ca longifolia* 50% alcohol	100	1.65***± 0.04	4.156*** ± 0.037	4.333** ± 0.155	583.98* ± 0.728	7.083* ± 0.124	52.0** ± 0.365	67.50* ± 0.846	1.613*** ± 0.003	0.246*** ± 0.003	292.77* ± 0.268	261.49* ± 0.341	40.52* ± 0.563	40.83** ± 1.013
5.	*Madhuca longifolia* 50% alcohol	200	1.48***± 0.03	4.563*** ± 0.020	3.150** ± 0.142	488.13* ± 0.601	6.011** ± 0.015	49.83*** ± 0.477	63.83** ± 0.477	1.851*** ± 0.003	0.258*** ± 0.004	332.99*** ± 0.242	298.82**0.400	49.92** ± 0.655	47.33** ± 0.666
6.	*Madhuca lngifolia* 50% alcohol	300	1.33*** ± 0.21	5.811*** ± 0.004	2.283*** ± 0.094	477.02* ± 0.711	5.536*** ± 0.107	47.83*** ± 0.477	58.50*** ± 0.763	1.943*** ± 0.003	0.268*** ± 0.004	360.36*** ± 0.161	308.60*** ± 0.466	56.98*** ± 0.449	53.33*** ± 0.881

Values are expressed in terms of Mean ± SEM.
*P < 0.05; **P <0.01; ***P <0.001 compared with control group.