I J A B E R, Vol. 14, No. 2 (2016): 1109-1125

THE EFFECT OF INDIAN MALLOW (ABUTILON INDICUM) HYDROETHANOLIC EXTRACT ON LEVELS OF REPRODUCTIVE HORMONES OF FEMALE ALBINO RATS

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Abstract: *Estrogen, which is one of the normal reproductive hormones in females can lead to, and/or complicate a variety of diseases such as estrogen-responsive tumors. When it gets elevated in the blood, it is also capable of proliferating and metastasizing tumors and complicating atherosclerosis, obesity, etc. Hyperestrogenemia occurs in premenopausal women but can be correlated with heart problem like thrombosis of myocardial infarction in men. This study evaluated the ability of the plant extract in lowering the serum level of estrogen, progesterone and follicle stimulating hormone. Its effect on ovary and uterus was also studied. Confirmatory tests for reported phytoconstituents ascertained the presence of alkaloids, proteins, carbohydrates, saponins and tannins. Results of acute oral toxicity test revealed that the plant extract did not elicit any observable acute toxic effects at a dose of 2000 mg/kg monitored for 14 days. Test doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were chosen for use in the study. The methods used in the evaluation of the anti-estrogenic effect of A. indicum were combined measurements of serum hormone levels and weighing of selected reproductive organs affected by levels of the hormones under investigation. Evaluation of the serum levels of the hormones using an enzyme-linked immunosorbent assay kit after 6 weeks of administration showed a significant reduction ($p<0.05$) of serum follicle stimulating hormone level from 4.51± 0.06 μI/ mL in the control to 4.21 ± 0.06 μI/ mL at a dose of 400 mg/kg; serum estradiol level from 13.40 ± 0.07 pg/mL in the control to 12.23 ±0.09 pg/mL and 11.47 ±0.43 pg/mL at doses of 200 mg/kg and 400 mg/kg respectively; and serum progesterone level from 13.40 ±0.12 ng/ mL to 12.10± 0,01 ng/ mL and 10.72±0.21 ng/mL at doses 0f 200 mg/ kg and 400 mg/kg respectively. Weight evaluation of the investigated reproductive organs also showed a significant reduction ($p<0.05$) in the weight of the uterus from 399.7±2.87 mg in the control to 378.6 ±3.42 for the 100 mg/kg dose group, 373.0±2.32 mg for the 200 mg/kg dose group, and 360.7±2.41 for the 400 mg/kg dose group; and also in the ovarian weight from 28.6±0.17 mg in the control to 27.5±0.19 mg for the 100 mg/kg, 27.0±0.20 mg from the 200 mg/kg dose group and 27.1 for the 400 mg/kg dose group. Thus, from this study, Abutilon indicum extract possesses significant and potent anti-estrogenic activity as it presented significant and positive results in both of the test methods used in the evaluation.*

Keywords*: Indian mallow, hydroethanolic extract, reproductive hormones*

INTRODUCTION

Abnormal hormone levels, as in the case of a hyperestrogenemia, is a common occurrence in premenopausal women, but it can also be observed in various disease conditions, in both genders. Correlations between hyperestrogenemia as a complication in major disease have been studied and presented such as in coronary thrombosis, in men, and thrombosis of myocardial infarction (Phillips et al., 1996), with diabetes, with obesity, atherosclerosis, and coronary artery disease (Phillips et al, 2000). In the various studies carried out for correlation of estradiol, the active form of estrogen, with these diseases, increased levels of the hormone were found in most of the subjects.

According to the World Health Organization (WHO), non-communicable diseases were responsible for 67% (36 million) of all deaths globally in 2011, up from 60% (31 million) in 2000. Cardiovascular diseases alone such as ischemic heart disease killed 7 million (11.2%) in 2011 as the leading cause of death. In the Philippines, diseases of the heart rank first as the leading cause of mortality with a total of 100,908 (104.4) deaths, based on the Department of Health statistics. In the local region, diseases of the heart were in the top four leading cause of mortality with a number of 779 (51.55) in 2011 as stated by the City Health Office of Davao City.

The fact that hyperestrogenemia in men was related to myocardial infarction but not coronary artery disease suggests that it too may underlie the thrombosis of myocardial infarction. Supporting this possibility are the relation of estrogen to hemostasis and the observation that administration of estrogen to men led to myocardial infarction and venous thrombosis. Thus, hyperestrogenemia, which appears related both to the infrequent shaving and smoking, and which may be related to cardiovascular disease by the same mechanism as smoking, could provide a common link between infrequent shaving, smoking, and cardiovascular mortality (Phillips et al., 2000). Hence, the researchers aimed to confirm the hydroethanolic extract of *Abutilon indicum* with its reported phytoconstituents; if there is exhibition of acute oral toxicity; and the dose wherein there is lowering of the levels of female reproductive hormones, follicle stimulating hormone, estrogen and progesterone.

MATERIALS AND METHODS

Research Design

The researchers utilized an experimental research design. Confirmatory test for the phytoconstituents, OECD acute oral toxicity testing and pharmacological screening

of the plant extract were conducted to identify the effect of hydroethanolic extract of *Abutilon indicum*) on the reproductive hormone of female albino rats.

The plants were collected from Bansalan, Davao del sur. The samples were taken to the chemistry laboratory of the University of the Immaculate Conception for extraction after plant authentication. The researchers conducted the experiments at Analytical Chemistry laboratory of the University of the Immaculate Conception, Davao City.

Research Instruments

The instruments that were used in confirmatory testing, toxicological and pharmacological screenings are: Extraction method through rotatory evaporator, test tube reaction method, FTIR (using SHIMADZU spectrophotometer, model FTIR-8400S), UV-VIS, organ excision, and hormonal assay using ELISA test kit.

Preparation of Extract

Preparation of the Hydroethanol Solution

The solvent was prepared as a 4:1 mixture of absolute alcohol and distilled water. The whole plants were air dried for 2 days at room temperature made into powder. About 150 g of the plant material was macerated in an Erlenmeyer flask with the hydroethanolic solvent for 48 hours after which evaporation followed using rotary evaporator and percentage yield was calculated. The collected residue was preserved in a refrigerator at 2-8°C for use in the different tests.

Confirmatory Test of the Different Phytochemical Constituents

Alkaloids

Twenty grams (20g) syrupy concentrate was collected after evaporation of stock extract over a steam bath. 5ml of 2M HCl was added, heated with stirring for 5minutes and cooled then added with 0.5g NaCl, stirred and filtered. The residue was washed with 2M HCl to obtain a volume of 5mL. A 28% ammonia was added and solution extracted three times with small portions of less than 10mL chloroform. The extract was then evaporated to dryness over a steam bath then added with 5ml of 2M HCl and stirred for 2 minutes and cooled. It was then filtered and divided into two portions. One portion was tested with Mayer's reagent and the other with Dragendorff's reagent. Results and observations were recorded as (+) or (-) for primary, secondary or tertiary alkaloids. To the two portions tested with Mayer's reagent and Dragendorff's reagent, a positive resulted indicated the presence of quaternary and or amine oxide bases (Aguinaldo, 2004).

Carbohydrates

Three (3) ml of extract was added to 2ml of Molisch's reagent, shaken and then 2ml of concentrated H_2SO_4 carefully added down the side of the test tube. A violet ring at the interphase indicated the presence of carbohydrates.

Flavonoids

Ten grams equivalent of plant material was obtained by evaporating to dryness stock solution over a steam bath and then cooled to room temperature. Defatting was done by adding 9mL hexane and water mixture (2:1) and then discarded the hexane portion. The defatted layer was then diluted with 10mL of 80% ethanol, filtered and then divided into 3 with one portion as control (Aguinaldo, 2004).

To the first portion, 0.5ml of concentrated HCl (12M) was added, color change observed and then warmed for 15 minutes. Further observations were made for an hour and then compared to the control. Absence of strong red or violet color indicated a negative result (Aguinaldo, 2004).

To the second portion, 0.5ml of concentrated HCl (12M) was added together with 4 pieces of magnesium turnings, observed for 10 minutes and compared with control. With no definite coloration appearing, equal volume of water and octyl alcohol was used for dilution, shaken and allowed to stand. Non-appearance of colors ranging from orange to red, crimson, magenta, and occasionally blue or green indicated a negative result (Aguinaldo, 2004).

Proteins

Few drops of Ninhydrin reagent was added to 1ml of extract solution in a test tube and then vortexed. The test tube was then placed in a water bath of boiling water for 5 minutes and cooled to room temperature.

Saponins

An equivalent volume of 2 g plant material was added to 10mL distilled water, stoppered, shaken vigorously for 30 seconds and allowed to stand. Observations were made for 30 minutes. Honeycomb frothing was observed. In the case or poor frothing, little sodium bicarbonate solution was added. Stable and dense froth indicated the presence of free fatty acids. A capillary tube was loaded with extract to a height of 10mm and another with distilled water. Both tubes were left in a vertical position, liquid allowed to flow out freely and then compared. As the level of plant extract in the tube was less than that in the tube with water, presence of saponins was inferred (Aguinaldo, 2004).

Steroids

Ten grams of stock extract was evaporated to dryness. Defatting was done by adding 6 mL of hexane and water (2:1) to the residue. The upper hexane layer was pipetted out. This was repeated until most pigments were removed and then hexane layer was discarded. The remaining was cooled to room temperature and divided into three portions. The steps were performed following the protocol of Aguinaldo (2004).

Tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution added. The formation of a green precipitate indicated the presence of tannins.

Fourier-Transform Infrared Spectroscopy

Prior to testing, the hydroethanolic extract of *A. indicum* was first subjected to freeze drying to produce a solid powdery sample. The freeze-dried sample was then prepared for the FTIR using a diffuse reflectoma attachment (DRS-8000 by SHIMADZU). The principle behind this spectroscopy involved molecules which vibrates at a certain frequency absorb infrared energy at these frequencies causing them to vibrate to a higher level.

Ultraviolet-Visible Spectroscopy

Ultraviolet and visible radiation interacts with matter, which causes electronic transitions (promotion of electrons from the ground state to a high energy state). The spectral region used in the analysis falls within the range of 190-750 nm. The UV-Vis measured the response of the sample to visible radiation.

Acute Oral Toxicity Testing (OECD Guidelines)

Plant extracts of *A. indicum* whole plant was studied for acute oral toxicity according to the guidelines set by Organization for Economic Co-operation and Development (OECD) guideline number 423. Female albino mice were used for this study. Since existing literature suggested evidences of significant toxicity, extract was administered at a dose 2g/kg of body weight (p.o.) and treated animals were observed for behavioral changes for the first 30 minutes after administration, hourly for first 24 hours, and daily over a period of 14 days. Animals were fasted of food overnight prior to testing.

PHARMACOLOGICAL SCREENING

Animal Testing

Nulliparous female albino rats, purchased from a reliable research laboratory and certified by a veterinarian weighing about 120-150g were used for the antifertility activity screening. They were maintained at 25 ± 2° C and relative humidity of 45% to 55% and under standard environmental conditions (12h light: 12h dark cycle). The animals had free access to food and water *ad libitum* throughout study. All the experiments were carried out between 9:00am to 4:00pm. All rats were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of 2000 mg/kg. From this data and pilot study reports; three different doses 100, 200 and 400 mg/kg were selected. The rats were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed in the animal house of University of the Immaculate Conception. Animal Ethical Committee approved the protocol.

Administration of Extract

There were 4 groups involved in the experiment and each group contained 5 rats. Group 1 (control) was administered with 0.2 mL of 100 mg/kg body weight. Group 2 received the extract in 0.9% NaCl at 100 mg/kg; group 3 had received the extract in 0.9% NaCl at a dose of 200 mg/kg and group 4 has similar percentage of extract but at a dose of 400 mg/kg body weight. All rats were fed with normal rat chow + water *ad libitum.* Before the administration of *A. indicum* extract began, the initial weights of the rats were taken (120-150g). Their daily weights were also taken before the extract was administered. This was done orally for 6 weeks

Sample Collection

At the end of the 6-week period, the animals were sacrificed following animal care and guidelines, and blood was obtained via cardiac puncture. Blood samples from each animal were put in labeled non-heparinized sample tubes which were then allowed to stand for three hours in iced water, and later centrifuged at 7000g for 10 minutes. The serum was then collected and stored at -15^{0}C. Hormonal assay was then carried out on the blood the next day.

Hormonal Assay

A. Follicle Stimulating Hormone

Immunoextraction

A 100μL of plasma from test animals was pipetted into the test tubes. The same procedures were repeated for animals from the control group. A 100μL of FSH

EIA magnetic antibody was added to the tubes and covered with foil and briefly water mixed. After mixing, the tubes were transferred to a water bath and allowed to incubate for 30mins. The temperature of the water bath is at 37°C.

To separate the hormones bound to magnetic particles from other component of plasma, the plasma samples were washed. A 500μL of diluted FSH EIA wash buffer was added to the test tubes and tubes were briefly water mixed. The rack of tubes was placed on a magnetic separator and allowed to stand for 5 minutes after which the supernatant liquid was decanted by inverting the rack and separator.

Labelled Antibody Reaction

The rack was removed from magnetic separator and 300μL of diluted FSH EIA labeled antibody to the test tubes. All test tubes were then covered and vortex mixed. After mixing, the tubes were transferred to the water bath. The test tubes were allowed to incubate for two hours. All test tubes were then washed twice as described above. The tubes were washed twice to ensure that all unbound labeled anti-body was removed.

Color Development Stage

The tubes were removed from the magnetic separator. About 500μl of substrate solution was pipetted into all test tubes plus one empty served as blank tube. The tubes were covered and vortex mixed. After mixing, the tubes were placed into a water bath left for 1 hour, after which 1mL of diluted stop buffer was added to all 16 test tubes and the test tubes briefly vortex mixed. The tubes were then placed in a magnetic separator for a minimum of 10mins.

Measurement of Optical Density

This was done using the serono seroenzyme 1 machine. This machine determined the optical density of the samples from programmed standards at 500nm and 492nm.

B. Estradiol 17-*β*

Estradiol-17β concentrations were determined using a modification (Ginther et al., 2005) of a commercially available RIA kit (Double-Antibody Estradiol Kit, Siemens Medical Solutions Diagnostic, Munich, Germany). Equine serum (1000 μl) was extracted with 5 mL of diethyl ether by shaking samples for 10 minutes. Samples were briefly centrifuged, frozen and the ether extract was decanted into glass assay tubes. Estradiol standards (3.125pg/ml - 400pg/ml) were prepared in 100% ethanol. Ether (samples) and ethanol (standards) were removed by evaporation on a hot plate at 45° C and 55°C, respectively. Dried samples were reconstituted with 250 μL assay buffer (PBSMG) and dried standards were reconstituted with

100 μL of PBSMG and vortexed. The reconstituted samples were aliquoted into glass tubes in duplicate (100 μL per assay tube). Sample and standard tubes had 30 μl of Estradiol 17-β antiserum added to all tubes except TC and NSB. Samples were vortexed and incubated at room temperature for 2 h. Next, 75 μL to 100 μL of 125I-labeled estradiol (approximately 22,000 cpm) was added to all tubes. Samples were vortexed and incubated at room temperature for 1 h. Samples (except TC) then had 1 ml of cold precipitating solution added and were vortexed and incubated at room temperature for 10 min. Samples were centrifuged (3000 x *g*) for 15 min, decanted, inverted and allowed to dry for 10 min. Samples were then placed in a gamma counter for 1 min.

C. Progesterone

Reaction of Antiserum with Serum Progesterone

The test tubes used were labeled appropriately, 4 test tubes for plasma of test animals and 4 test tubes for plasma of control animals. A 50μl of serum, 100μl of progesterone EIA blocking reagents, 100μl progesterone EIA antiserum and 100μl of progesterone EIA separation reagents were pipetted into all 8 tubes, covered and briefly vortex mixed. After which the test tubes were incubated in a water bath for 2 hours. The rack of the test tubes were then placed on the base of the magnetic separator and left for 5-10min, this was to allow all magnetic particles sediment with all tubes still in contact with the magnetic base. Tubes were removed from the magnetic base and 100μL of diluted progesterone EIA was buffer 1 added to the test tube. The tubes were briefly vortex mixed, placed on the magnetic separator for 5-10min and the supernatant liquid decanted. This was a wash step.

Reaction of Anti-Serum with Enzyme Labelled Progesterone

A 700μL of diluted testosterone EIA enzyme label was pipetted into the test tubes. Test tubes were covered and briefly vortex mixed, then placed in water bath for 15mins. After which the rack of tubes was placed on magnetic separator for 5-10mins and the supernatant liquid decanted. The content of the tubes were then washed twice following the same wash procedure above but using estrogen EIA wash buffer 2.

Color Development

The same procedure as LH but tubes were incubated in water bath for 1 hour. This was done using serono seroenzyme 1 machine

Measurement of Optical Density

This machine determined the optical density of the samples from programmed standards at 500nm and 492nm.

STATISTICAL ANALYSIS

The result obtained from this study was analyzed using Statistical Package for Social Sciences (SPSS) version 18.0 for windows. One way Analysis of Variance (ANOVA) was used to compare means followed by Dunnett-test. Tukey's multiple range test was used to detect differences among groups. The occurrence of deviation between groups was compared using a chi square test. Values were compared at $p<0.05$. All the data were expressed as Mean $\pm$ Standard Error of Mean (SEM).

RESULTS AND DISCUSSIONS

Table 1
Preliminary Qualitative Test of Abutilon indicum: Test Tube Method

Reagent	*Test for*	*Inference*
Dragendorff's test	Alkaloids	+
5%KOH	Flavonoids	-
Salkowski reaction	Sterol/Steroid	-
5% $FeCl_3$	Tannins	+
Millon's Test	Proteins	+
Saponin glycosides	Saponins	+
Molisch test	Carbohydrates	+

Legend: + positive; - negative

Figure 1: FTIR Spectra of *Abutilon indicum* Extract

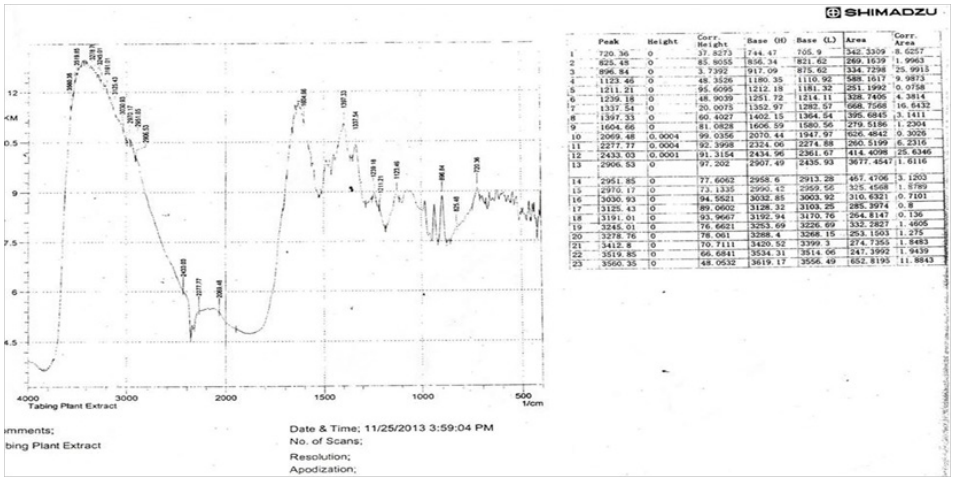

Table 2
Interpretation of FTIR Spectra of Abutilon indicum

Experiment frequency (cm^{-1}) Obtained	*Reference frequency (cm^{-1})*	*Bond present*	*Functional group*
720.36 – 896.84	675 – 1000	C-H	alkenes, aromatic rings
1123.46 – 1239.18	1000 – 1260	C-O	alcohols, esters, ethers, carboxylic acids
1337.54 – 1397.33	1350 – 1470	C-H	alkanes
1604.66	1580 – 1650	N-H	amines
3245.01 – 3560.35	3200 – 3600	O-H	alcohol, carboxylic acids, phenol
3412.8	3300 – 3500	N-H	amine
2906.53 – 2970.17	2500 – 3000	O-H	carboxylic acid
3030.93	3000 – 3100	C-H	Aromatic ring, alkene
3278.76	3267 – 3333	C-H	alkyne

Figure 2: UV-VIS Spectra of *Abutilon indicum* Extract using Distilled Water

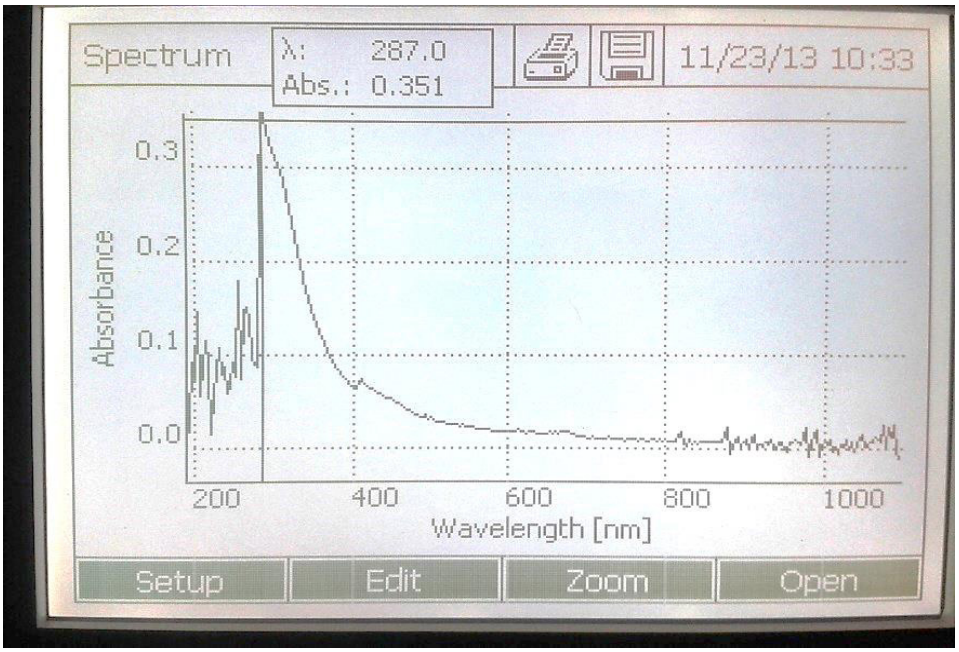

Figure 3: UV-VIS Spectra of *Abutilon indicum* Extract using Absolute Ethanol

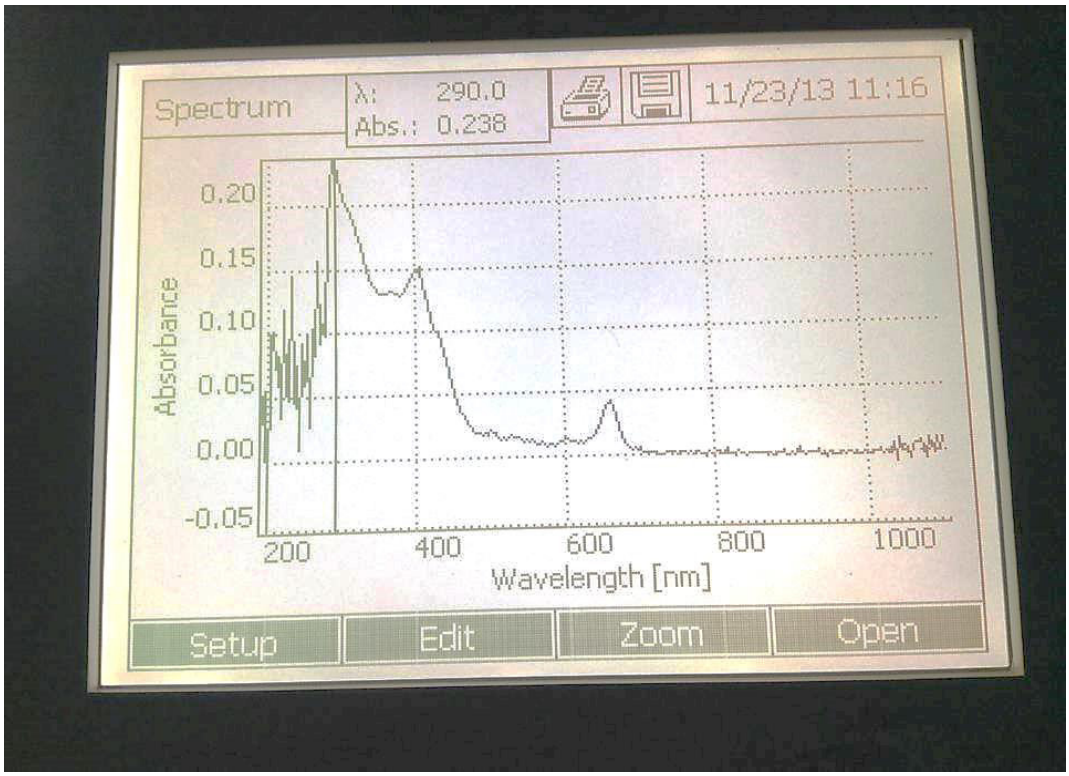

Table 3
Interpretation of the UV-VIS Reading of *Abutilon indicum* Extract in Distilled Water and Ethanol

Test Materials	*Maximum Absorbance*	*Wavelength*	*Chromophore*
Distilled water	0.3351	287.0	–C-O bond
Absolute alcohol	0.238	290.0	
Plant Extract			

As indicated in the table, –CO bond was predominant in the test sample. This may indicate carbonyl or alcohol compounds which might be present in the test samples.

Table 4
Cage-side Observations for All Animals

Sr. No.	*Parameters*	*Cage Side Observations*
1.	Condition of the fur	Normal
2.	Skin	Normal
3.	Subcutaneous swellings	Nil
4.	Abdominal distention	Nil
5.	Eyes (Dullness)	Nil
6.	Eyes (opacities)	Nil
7.	Pupil diameter	Normal
8	Ptosis	Nil
9.	Color & consistency of the faeces	Normal
10.	Wetness or soiling of the perineum	Nil
11.	Condition of the teeth	Normal
12.	Breathing abnormalities	Nil
13.	Gait	Normal

Legend: Normal-normal; Nil-nonexistent

Table 5
Effect of *Abutilon indicum* whole plant extract on body and selected organs weight

Parameters	*Group I Control*	*Group II 100mg*	*Group III 200mg*	*Group IV 400 mg*
Liver (g)	3.42±0.00	3.43±0.00	3.42±0.00	3.42± 0.00
Kidney (mg)	0.230±0.00	0.23±0.00	0.24±0.00	0.24±0.00
Uterus (mg)	399.7±2.87	378.6±3.42	373.0±2.32	360.7±2.41
Ovary (mg)	28.6±0.17	27.5±0.19	27.0±0.20	27.1±0.16*
Body weight (g)	164.6±8.67	169.5±9.74	157.6±8.93	169.2±10.2

N=5. Data represents mean ± SE. *$p<0.05$

Table 6
Effect of *Abutilon indicum* on Reproductive Hormone Levels in Female Albino Rats

Groups	*FSH (μl/ml)*	*Progesterone (ng/ml)*	*Estradiol (pg/ml)*
Control	4.51±0.06	13.40±0.12	13.40±0.07
100mg/kg	4.41±0.04	12.90±0.08	13.06±0.18
200mg/kg	4.33±0.06	12.10±0.01*	12.23±0.09*
400mg/kg	4.21±0.06	10.72±0.21*	11.47±0.43*

n = 5. Values are expressed as mean ± SEM. *p<0.05

DISCUSSIONS

Confirmatory Test

Phytochemical screening of the extract revealed the presence of alkaloids, tannins, proteins, saponins and carbohydrates. On the other hand, flavonoids and sterol/steroid showed negative results signifying the absence of the constituents (Table 1). Bianca et al. (2006) stated that alkaloids and flavonoids are responsible for reducing the hormonal levels of luteinizing hormone, estradiol and follicle stimulating hormone. However, as flavonoids were not detected during the confirmatory tests performed, the alkaloidal component of the extract is believed to be responsible for its anti-estrogenic effect.

Fourier Transform Infrared Spectroscopy Reading

In this study the researchers used infrared spectroscopy, specifically Fourier Transform Infrared, to determine qualitatively the functional groups present in *Abutilon indicum*. This was done by analyzing the infrared spectra of the extract, which represents a fingerprint of the sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. The sample was prepared using the KBr Disc Method. Based on the IR spectra of the extract, it was clear that the following functional groups are present: alcohol, carboxylic acids, alkanes, amines, alkynes, esters, ethers, alkenes and aromatic rings.

UV-VIS Reading

This is to detect the presence of color-absorbing carbon-carbon, carbon-oxygen, or carbon-nitrogen double bonds, as well as carbon-halogen single bonds. An absorbance-based analytical technique measures and identifies chemicals that

absorb in the contiguous ultraviolet and visible regions of the electromagnetic spectrum. UV wavelengths range from 10 to 400 nanometers, while the visible spectrum—colors visible to the human eye—lie from approximately 400 to 800 nanometers.

Toxicity

Toxicity is one of the most important aspects of any medication to govern the extent of therapeutic utility. Since preliminary phytochemical results gave positive indications to encourage further pharmacological screening, it became mandatory to evaluate the extract for its toxicity profile to confirm its safety (Rang et al., 2003). As per the principles of pharmacology, any drug shall not only be pharmacologically effective but also free of toxicity to an acceptable extent. The maintenance of desirable risk and benefit ratio is prerequisite to label any compound as a drug (Gad et al., 2002; Tajuddin et al., 2004). The acute oral toxicity studies of *A. indicum* extract was found to be safe, up to the dose of 2000mg/kg, consistent with findings of Pingale and Virkar (2011) that *A. indicum* extract were negative for toxicity for single administration of the extract up to a dose of 10,000mg/kg. From these findings, three different doses: 100, 200 and 400 mg/kg were selected for the study on the extract's anti-estrogenic effect.

Table 4 shows the physical characteristics of the female albino rats prior to, and during testing. This includes the condition of the fur and skin, pupil diameter, color and consistency of the feces, condition of the teeth, and gait which were all normal; subcutaneous swellings, abdominal distention, eye dullness, eye opacities, ptosis, wetness or soiling of the perineum, and breathing abnormalities were also checked and were not observed on any of the animals during the physical examination.

Shivalingapa et al (2002) described the ovary as an aggregate of three endocrine tissues; the stroma, follicles and corpus luteum, where the weight of the ovary is the combined weight of these three tissues. However, since growth of these tissues depend on the influence of gonadotropic and steroidal hormones (estrogen & progesterone), a decrease in the weight of the ovary generally represents a decrease in the activity of the three ovarian tissues, caused by decrease or non-availability of gonadotropic or steroidal hormones, or both. Thus the significant reduction in the weight of the ovary at all the doses: 100, 200, and 400 mg/kg is an indication of the steroid hormone depletion effect of the extract. Also, the weight of the uterine muscles (endometrium) is regulated directly by interactions between the levels of progesterone and estradiol. Estradiol normally is responsible for boosting the weight of the endometrium, however, progesterone increases its weight whenever there is low levels of serum estradiol. Thus, the decrease in the uterine weight is indicative of a depletion of both estrogen and progesterone. The decrease in

the ovarian and uterine weight may be taken as indicative of the antiestrogenic nature of *A. indicum* since antiestrogenic substances decreases the wet weight of the uterus.

Behavioral Profile, Body and Organs Weight

There was no treatment related changes in the behavioral profile at all the tested dose levels. All animals, both in control and extract treated groups appeared healthy, alert, and were responding to pain and touch. Vocalization, restlessness and irritability in animals were also not observed (Table 4). The animals responded to loud noise, indicating CNS excitation. No significant difference was observed in the body weights of the extract treated and control groups (Table 5). A significant reduction ($P<0.05$) in ovarian weight at 400 mg/kg; uterine weight at 200 and 400 mg/kg dose level was noticed when compared to their respective control groups (Table 5).

The effects of administration of aqueous whole plant extract of *Abutilon indicum* at 100, 200 and 400 mg/kg body weight for 6 weeks on the concentration of serum reproductive hormones in the female rats are depicted in table 6. The administration of the extract did not produce any significant effect on serum hormone levels. The concentrations of follicle stimulating hormone (FSH), estradiol and progesterone in the serum were reduced by the extract (table 6). While the concentrations of FSH, estradiol and progesterone were reduced in all the groups, the least dose (100 mg/kg body weight) did not produce any significant change ($p>0.05$) in the concentration of the hormones (table 6). Except for the 400mg/kg of extract, the other doses did not produce a significant decrease in the levels of FSH, whereas both the 200 and 400mg/kg body weight of the extract produced reduction in the serum estradiol and progesterone concentrations compared to the control group. By the end of the experimental period, only the 400mg/kg body weight dose had significantly reduced all the investigated serum hormones significantly ($p<0.05$) compared to the control group (table 6)

CONCLUSIONS

The phytochemical composition of the extract confirmed the presence of alkaloids, saponins, tannins, carbohydrates and proteins. However, the tests were negative for the presence of steroids and flavonoids. Using Fourier-Transform infrared (FTIR) and ultraviolet-visible light (UV-VIS) spectroscopy, the results showed the functional groups -C-H, -C-O, -N-H and -O-H at specific wavelengths which suggested the presence of such chemical classes as alcohol, carboxylic acids, alkanes, amines, alkynes, esters, ethers, alkenes and aromatic rings. The *A. indicum* extract did not elicit any observable acute toxic effects in the test animals when

administered at a dose of 2000mg/kg of body weight and monitored for a period of 14 days. The administration of the extract at a dose of 100 mg/kg body did not produce any significant change ($p>0.05$) in the concentration of the three hormones (follicle stimulating hormone, estrogen and progesterone). However, with administration of the extract at a dose of 200mg/kg of body weight, the concentrations of estradiol and progesterone were reduced significantly, while for administration at a dose of 400 mg/kg, there was a significant reduction in the levels of the three hormones under investigation. There was a significant difference between the effects of the negative control and the extract on the levels of FSH, estrogen and progesterone) as the negative control showed no pharmacological effect whereas the extract significantly lowered the levels of the hormones in a dose dependent manner.

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