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Evaluation of Anti-abortifacient Activity from Methanolic Extract of *Withania somnifera* in Misoprostol Induced Female Wistar Rats

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Abstract:

Abortion is the termination of a pregnancy by removal or expatriation of an embryo or fetus. According to WHO, revocation is a common health intervention. But currently, it has come a major issue that substantially women suffer confinement during early or late pregnancy. It has been suggested that some women who misfire may not make enough progesterone in the early part of pregnancy. Supplementing these women with specifics that act like progesterone (these are called progestogens) has been suggested as a possible way to help intermittent confinement. Misoprostol which is chemically known as 7-((1R, 2R, 3R)-3-hydroxy-2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl) heptanoic acid methyl ester is a prostanoid, which induces labor, beget an abortion. For abortions it's used by itself and with mifepristone or methotrexate. By itself, effectiveness for abortion is between 66 and 90. For labor induction or abortion, it's taken by mouth. *Withania somnifera* (Family Solanaceae), generally known as ashwagandha (winter berry) have been reported to be useful in abortion worldwide and but at a great extent can be used as anti-abortifacient and colorful other remedies. *W. somnifera* is also rich in multitudinous valued secondary metabolites similar as steroids, alkaloids, flavonoids, phenolic, saponins, and glycosides. A wide range of preclinical trials similar as cardio protective, anticancer, antioxidant, antibacterial, antifungal, anti-inflammatory etc.

Keyword: Abortion, gestation, mifepristone, methotrexate, *Withania somnifera*, Solanaceae.

Introduction:

Abortion is a widespread health intervention, according to WHO. When performed by a skilled individual using a technique suited for the stage of pregnancy, it is safe. All unplanned pregnancies result in an induced abortion in six out of ten cases. [1, 2, 3] 97% of unsafe abortions—or about 45% of all abortions—occur in underdeveloped nations. Around 73 million induced abortions are carried out year all throughout the world. The WHO will produce a list of essential health services in 2020 that includes comprehensive abortion treatment. Misoprostol is classified as a prostanoid and has the molecular name 7-[(1R, 2R, 3R)-3-hydroxy-2-(4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl] heptanoic acid methyl ester. [4, 5, 6]

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It is a synthetic prostaglandin used to induce labour, produce an abortion, treat postpartum haemorrhage brought on by insufficient uterine contraction, and prevent and treat stomach and duodenal ulcers. It is used both alone and in conjunction with methotrexate or mifepristone for abortions.[7, 8, 9, 10] The effectiveness of abortion on its own ranges from 66% to 90%. It is ingested, dissolved in the mouth, or injected into the vagina to induce labour or end an abortion. Misoprostol, which has the chemical formula $C_{22}H_{38}O_5$ and a relative molecular mass, is used to cause abortion in female rats.[11, 12]

Withania somnifera, also referred to as "winter cherry," is an evergreen shrub that grows in India, the Middle East, and some regions of Africa. It is a member of the nightshade family, or Solanaceae. There is little scientific proof that this plant, [12, 13, 14] despite being used as a medicine in Ayurveda and being sold as a dietary supplement in many nations, is safe or effective for treating any ailment or, to a considerable extent, preventing miscarriage.[15, 16, 17]

W. somnifera is also abundant in a variety of highly valuable secondary metabolites, including glycosides, alkaloids, flavonoids, phenolic, and steroids. Different elements of the plant have been linked to a variety of preclinical experiments, including cardio protective, anticancer, antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, anti-depressant, and hypoglycemic effects.[18, 9, 20, 21]

Withania somnifera root extract was administered orally to the test group of pregnant rats as part of a study to examine its potential anti-abortifacient effects.

Materials and Methods:

Collection of Plant:

The studies have been carried out the anti-abortifacient properties of methanolic extract of *Withania somnifera* roots, sample were collected from rural belt of Purba Medinipur, West Bengal, India in the month of November 2022. After the collection of Plants, It authentified by Botanical Survey of India, Botanical Garden, Howrah, West Bengal, India.

Plant extraction:

Decoction is a method of extraction is used for extraction by boiling herbal or plant material (roots), after this evaporate the solvent and then collect the crude drug. [22,23,24]

Experimental animals:

Albino Wistar rats (female) weighing 125-150g, maintained under controlled conditions of temperature (23±2°c), humidity (50±5%) and a 12h light-dark cycle, will be used for the experiment. They were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water ad libitum. For one week, the animals were allowed to adjust to the laboratory environment. All experimental methods were carried out in accordance with guidelines established by the Institutional Animals Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), both of the Government of India's Ministry of Social Justice and Empowerment.[25, 26, 27]

Acute oral toxicity:

The acute oral toxicity study was conducted according to Organization for Economic and Cultural Development for testing of chemicals (OECD, 2001, 423 guideline) and up and down method was used for the study. 3 female rats were taken for toxicity testing. The Wistar rats were weighed (125-150gm), and were treated with oral dose i.e (1st dose- 400mg/kg, 2nd

dose- 2000mg/kg) of methanolic extract of *Withania somnifera* roots respectively for 2 different days.

1. Firstly, 400mg/kg drug was administered, after the administration of the 1st dose of the *Withania somnifera* root extract and food was withheld for a further 3-4 hrs. At least once within the first 30 minutes following dosing, as well as on occasion at intervals of 8, 14, and 48 hours, the animals were observed individually.
2. After passing of the 1st dose and observing the survival of the rats, 2nd dose 2000mg/kg of the *Withania somnifera* root extract was administered and food was withheld for a further 3-4 hrs. At least once within the first 30 minutes following dosing, as well as on occasion at intervals of 8, 14, and 48 hours, the animals were observed individually.
3. Since 2000mg/kg drug seems to be passed on the rats, therefore this dose is considered as the safe dose (LD 50).

The animals were observed for signs of drowsiness, hair loss, loss of appetite, salivation, tremors, convulsion and bulging of the eyes. The animals were thereafter observed for a period of 14 days for any signs of delayed toxicity and mortality.[28, 29, 30]

Experimental design:

The albino (Wistar) rats used in this study were collected. They were transported in plastic ventilated cages. The rats, kept under room temperature, with 12- hour light and 12- hour dark cycle and were allowed to acclimatize for two (2) weeks before the commencement of the experiment. The animals were provided commercial feed and were allowed access to water ad libitum. Protocols for this experiment was in accordance with the guidelines on the care and wellbeing of research animals.[31, 32]

Twenty white albino rats of both sexes weighing between 125 to 190 gm were used for the studies. The female rats were paired overnight in the evening with sexually active males in the ratio of 2:1. The presence of a vaginal plug and/or sperm cells in the vaginal smear the next morning between 9.00 and 10:00 am served as proof that the mating had been successful. The day sperm cells are found in the vaginal smear was considered as day 1 of pregnancy. Thereafter, six female rats and two male rats are randomly separated and divided into four groups each and were treated as follows:[33, 34,]

1. Group I (NORMAL GROUP)- the pregnant rat was treated with vehicle alone, normal saline to have healthy and normal pregnancy.
2. Group II (CONTROL GROUP)- the pregnant was treated with Misoprostol (500mg/kg p.o) on the 3rd day of the pregnancy.
3. Group III (STANDARD GROUP)- the pregnant rat was treated with Progesterone Susten SR 2OO tablets (50mg/kg orally p.o) for 10 days consecutively and Misoprostol (500mg/kg p.o) on the 3rd day of pregnancy.
4. Group IV (TEST GROUP)- the pregnant rat was treated with *Withania somnifera* root extract (1000mg/kg p.o) for consecutively 10 days and Misoprostol (500mg/kg p.o) on the 3rd day of pregnancy.

Treatment was given orally using an intra-gastric tube once daily for 10 days continuously from the 1st day of pregnancy. On the 15th day of pregnancy, the rats from each Group (i, ii, iii, iv) were taken and they were arrested under hyper anesthesia for various biochemical estimations. The animals were sacrificed and the uterus along with the umbilical cord (Placenta) was dissected out for the further observations of the condition of the foetus and was immediately washed in normal saline to observe and stored for further biochemical estimations.[35, 36]

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Result:

Phytochemical investigation:

Fig 1: Dissection of animal

Table 1: Phytochemical investigation of methanolic extract of *Withania somnifera*.

Phytochemical Test	Result
Alkaloids	Positive+
Steroids	Positive+
Terpenoids	Positive+
Starch	Positive+
Reducing sugar	Positive+
Glycosides	Positive+
Amino acids	Positive+
Tannins	Positive+

Testing of various chemical compounds within the extract, represents the preliminary phytochemical studies. Little amount of methanolic extracts of *Withania somnifera* was subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals like alkaloids, Glycosides, Steroid, Terpenoids, tannins, Starch, amino acids, reducing sugar.

In vivo activity:

Table 2: The effect of the administration of the methanolic extract of the *Withania somnifera* roots in Misoprostol induced female Wistar rats.

Groups	Treatment	No. of foetus
I	Normal	6.5 ± 0.2236
II	Control	$5 \pm 0.3651^{\#\#}$
III	Standard	6 ± 0.3651
IV	Test	6.5 ± 0.2236

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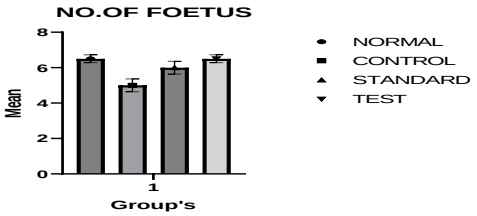


Fig 2: Foetus

Values are expressed as Mean ± SEM, n=6; ## P<0.01 considered statistically significant as compare to normal control group; ***P<0.001, ** P<0.01 and * P<0.05 considered statistically significant.

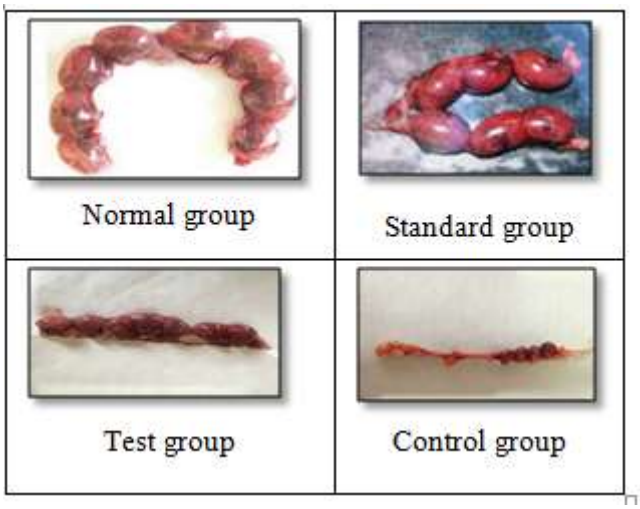
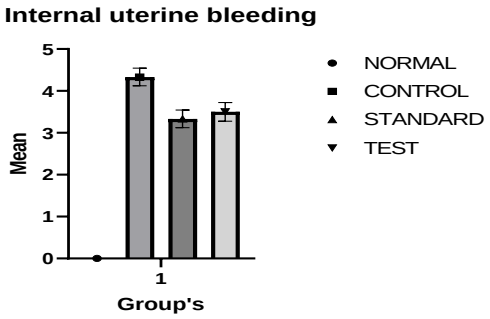


Table 3: The effect of the administration of the methanolic extract of the *Withania somnifera* roots in Misoprostol induced female Wistar rats.

Groups	Treatment	Internal bleeding of foetus
I	Normal	0 ± 0.000
II	Control	4.33 ± 0.2108##
III	Standard	3.33 ± 0.2108
IV	Test	3.5 ± 0.2236

Values are expressed as Mean ± SEM, n=6; ## P<0.01 considered statistically significant as compare to normal control group; ***P<0.001, ** P<0.01 and * P<0.05 considered statistically significant.



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Discussion:

As per the study of the reproductive information about the albino Wister rats, during menstruation period they releases 9-10 ovum from their ovary. Thereby, during mating by the male rats they cause breeding of 9-10 ovum. All the ovum fertilizes to form multigravida and as per the formation of 8-9 ovum, total of 6 – 8 babies are found into the uterine cavity.

Normally, (Normal) during gestational period the foetus, umbilical cord, placenta starts developing in the uterus with a normal diffusion and supply of o2, proteins, nutrients from the mother to the foetus.

But during (Induced control) Misoprostol induction it was found causing barrier in the supply and diffusion of the O_2, nutrients from the mother to foetus, resulting in Gestational retardation (G.D) i.e., no development of the foetus, causing foetal injury and also internal bleeding occurs.

However, during (Standard) Progesterone induction there was a bit increase in the diffusion and supply of the sufficient o2, nutrients from the mother to foetus. Therefore, due to the secretion of progesterone hormone it causes less internal bleeding, G. D is less and coming to the normal development of the foetus.

During (Test) *Withania somnifera* root extract induction the presence of the active constituents- With anolides, steroidal lactones increase and boosts the progesterone hormone secretion and other essential sex hormones, we expected and found causing less internal bleeding and G. D also decreases. Thus, present research provides with the result that

Normal- all the 8 babies were okay and normal. Control- 4 babies were found with internal bleeding and G. D. Standard- 6 babies were found with very less abnormalities and internal bleeding. Test- 7 babies were found with very less internal bleeding, abnormalities and G. D was decreased than control and standard.

Conclusion:

The present study reveals that the Methanolic extract of *Withania somnifera* significantly improved the abortifacient activity in Misoprostol induced female albino Wistar rats. In addition, the extract significantly increased the progesterone levels and other sex hormones. The internal bleeding, Gestational retardation and other abnormalities was lowered by the extract in rats. The observation of the no. of foetus, less internal and uterine bleeding, decreased G. D showed as a mark of improvement in structural features in *Withania somnifera* extract treated groups. Thus, from this present study it can be concluded that the Methanolic extract of roots of *Withania somnifera* has potential antiabortifacient activity against Misoprostol induced female albino Wistar rats.

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