Evaluation Of Female Reproductive Toxicity Of Hydroalcoholic Extract Of Cassia Occidentalis (CO-A002) In Rodents

Narinder Kumar[1*], Surjeet Singh[2]

[1*]Government Gandhi Memorial Science College Jammu, Email: narinderbhagatt@gmail.com
[2]IIIM-CSIR Jammu, Email: surjeetiiim@gmail.com

***Corresponding Author:** Narinder Kumar
*Government Gandhi Memorial Science College Jammu, Email: narinderbhagatt@gmail.com

Abstract

Objectives:

The aim of present study is to evaluate the toxicity of the hydroalcohlic extract of leaves of *Cassia occidentals*, (Fabaceae), on reproductive parameters (on theestrous cycle, reproductive performance, post-natal growth and offspring survival of rats). Here four groups of not-pregnant Wistar rats received distilled water and the doses 250, 500 and 1000 mg/kg of plant extract for thirty days, at the end of which they were examined as to the frequency of their phases in toxicity on the estrous cycle.

Methodology:

Thirty-two (32) female wistar rats with consecutive 4 to 6 days estrous cycle were given distilled water (as control) and CO-A002 at 250, 500 and 1000 mg/kg dose daily by gavaging ten days prior to mating, mating (a maximum period of ten days), gestation and lactation periods of seven days. Dams and fetuses were sacrificed on day seven postnatal. Four groups of not-pregnant Wistar rats received distilled water and the doses 250, 500 and 1000mg/kg of plant extract in toxicity on the estrous cycle at the end of which they were examined as to the frequency of their phases in toxicity on the estrous cycle.

Results:CO-A002extracts did not bring change in the estrous cycle and general health of all wistar rats. All the animals proceed towards successful mating and pregnancies. There was no significant alteration in the duration of pregnancy. Moreover, pregnant rats delivered pups normally. Statistically no test agent-related changes in the body weight of female wistar rats, number of implantations, litter size and pup body weights were observed. Other parameters measured include pup male: female ratio, live birth index, pup viability index and percentage of implantation death which also showed no significant change.

Conclusion: The present findings reveal that hydroalcohlic extract of *Cassia Occidentalis* didn't exhibit any remarkable reproductive toxicity.

Keywords: Herb, estrous cycle, pregnancy, fetuses, lactation, adverse effect

Introduction

80% of people use herbal medicine for basic holistic health care according to the World Health Organisation (WHO), [1]. Because over 25% of the current medications used in the United States are derived from plants, according to WHO, allopathic medicine must bear a sizable portion of responsibility for medicinal plants [2]. The level of sophistication of those treatments changes as technology advances in the nations that produce and use herbal therapies. Scientific research has shown that herbal remedies are either safer or less dangerous than conventional pharmaceuticals [3,4]. Brazil published 18579 plant case studies between 1999 and 2009 which offered evidence in support of this assertion (5).

The current study's goal is to assess CO-A002's potential ability to induce labour in rats as well as any potential risks to the female reproductive system. To test the efficacy and safety of this plant leaf extract, standardised hydroalcohlic extract of Cassia Occidentalis was given at dosages of 250, 500, and 1000 mg/kg/day. The identical extract has already been used in general acute and subacute (28-day) toxicity tests on rats [6,7]. The study's findings demonstrated that the extract's estimated LD50 is greater than 5000 mg/kg and that there were no negative effects to be seen [8]. The dams' litters were permitted to give birth in a normal way. Puppies were weighed, numbered, sexed, and checked for exterior defects on the first day after birth. The dams and foetuses were sacrifices.

The parameters measured are presented in Table 1. In order to analyse the data, SPSS 11.0 was used. Maternal body weight data from the entire study are shown in Table 1 and were analysed using general linear model repeated measures. One-way ANOVA was used to analyse the mean days of the estrous cycle, the length of the pregnancy (days), the

pregnancy index, the number of pups present at delivery and on day seven of lactation (litter size), the weight of the pups throughout lactation, and the number of implantation sites per litter. If differences were discovered, the Scheffe test was applied. Additionally, the live birth index, viability index, and percentage of post-implantation death were evaluated using the non-parametric Kruskal Wallis test and Mann-Whitney test, if needed.

Cassia occidentalis Linn., a widespread plant of the Caesalpiniaceae family that is also known as "Edible weeds of Agriculture" or "Famine food," is found from the foothills of the Himalayas to West Bengal, South India, Burma, and Ceylon [9]. It is a perennial or annual plant that is used in traditional medicine to treat a variety of ailments. The plant has a large number of anthraquinones glycosides. Emodin is abundant in the roots, whereas chrysarobin (sometimes referred to as 1, 8-dihydroxy-3-methyl-9-anthrone) and N-methylmorpholine are abundant in the seeds. Senna leaves and pods include sennasoides A, B, C, D, G, rhein, aloe-amine, Kaempferein, chrysophanol, emodin, aloe-emodin, and iso-rhein in both free and complex glycoside forms [10].

Native Americans frequently use stinking weed as a modern medicine and as a coffee substitute. It can be found in the multihedral Ayurvedic drug Himoliv. Several polyherbal products, such as the Liv.52 syrup and pill, which are often used to treat viral hepatitis, can also contain it [11]. More study is needed to prove the effectiveness and security of this species before it may be used as a botanical remedy in the future. The purpose of this study is to determine whether the hydroalcoholic extract of Cassia Occidental leaves could affect Wistar female rats' ability to reproduce.

Materials and methods

Extract Preparation:

Cassia occidentalis leaves were harvested in August and September from the Akhnoor neighbourhood of Jammu, Jammu & Kashmir India, dried in the shade, and powdered. The powdered leaves (measured in grammes) were extracted with 500 cc of 50% ethanol. The extract was filtered, and the filtrate was then freeze dried to produce an amorphous powder.
In this study, 32 non-pregnant females weighing 180-200 g were used. Wistar rats with successive 4 to 6 days of estrous cycle were gavaged with CO-A002 at dosages of 250, 500, and 1000 mg/kg daily, as well as distilled water as a control vehicle, ten days before mating, gestation, and lactation periods of seven days. They were housed in the CSIR-IIIM animal home in Jammu for the duration of the experiments. In a room kept at a constant temperature of 22 2 °C, the animals were housed in separate conventional cages with free access to food and water. All procedures were carried out in compliance with the guidelines of the Animal Ethical Committee.

Estrous cycle toxicity evaluation:

The experiment employed four groups of eight animals each (n = 8). CO-A002 was given orally to three groups at doses of 250, 500, and 1000 mg/kg (10 ml/kg body weight) for ten days (before to mating (a maximum of ten days), gestation, and lactation periods of seven days). As a treatment, the vehicle control group received distilled water. The technique developed by Goldman et al. was used in the estrous cycle toxicity evaluation procedure (2007). The rats were watched every day between 8 and 9 a.m. for the first seven days to see whether or not their circadian rhythms were normal. Only animals with regular cycles were used in the experiment. The vaginal smear was obtained using a plastic pipette filled with approximately 10 l of saline (NaCl 0.9%) and placed on a glass slide, where the estrous cycle was observed under light microscopy at 10 and 40X objectives. The estrous cycle phases (proestrus, estrus, metestrus, and diestrus) used in the CO-A002 effect analysis were classified based on the cellular profile of the vaginal smear visible under microscopy.
The frequency of the estrous cycle phase was determined by integrating the occurrences of the registered phases across the treatment time. By calculating the total number of treatment days by the number of days between estruses, the interestrus interval was computed. The diestrus index was determined by multiplying the total number of diestrus episodes by 100 and dividing the total number of treatment days by the number of treatment days.

Results

TThe extracts of Cassia Occidentalis had no effect on the estrous cycle or the general health of wistar rats. All of the species can reproduce and have children. There were no notable changes throughout the pregnancy. Furthermore, pregnant rats frequently gave birth. The test agent had no statistically significant impact on the weight of female Wistar rats, the number of implantations, the litter size, or the pups' body weights. Other features evaluated included the ratio of pup males to females, the nascence index, the pup viability index, and the proportion of implantation mortality, although none of them revealed any discernible changes. The interestrus interval and the frequency of the other phases were unaffected.

Table 1. Parameters measured in rats treated with Cassia occidentalis prior to mating, mating period, gestation periodand lactation period of seven days.

Maternal	Pregnancy	Fetal
1.General observation and Behavior (lethargy, sleepy, withdrawn, excitement, respiratory, abnormal posture, abnormal coat condition	1.Length of pregnancy (days)	1.Number of pups at birth (litter size)
2.Fates of females(survivability or death)	2.Pregnancy Index= No. of females delivering live youngs/No. of females with evidence of pregnancy)x100	2. Live birth Index = (No. of live offspring/ No. of offspring delivered) × 100
3.Body weight throughout study		3.Sex distribution (at birth and day 7 post - natal)
4. Estrous cycle(duration and cyclist		4. Viability index = (No. of live offspring at D7 Lactation/No. of live offspring born) × 100
5. Mating index=No. of female mated/ No. of females cohabited x100		5.Growth rate of offspring during lactation(D1-D7 postnatal)
6. Maternal visceral changes(days 7. Post-partum)		6.Body weight of male and female pups (D1-D7 postnatal)
		7.Gross malformation
		8.Pups development (movement, spontaneous righting reflexes and forelimb grasp)
		9. No. of implantation sites at autopsy
		10.Percentage of post implantation death (No. implantation – No. live fetuses/No. implantation × 100) Pregnancy and fetal parameters of rats treated withCO-A002 prior to mating, mating period, gestation and lactation periods of 7 days Maternal

Table2: Reproductive parameters evaluated in pregnant rats administered with NaCl(.9%) and hydroalcoholic extractof Cassia Occidentalis

Treatment	No.of implantation	No.of live fetuses	No.of dead fetuses	Fetuses weight(g)	Placenta weight(g)
NaCl .9%	8.60+0.80	8.60+0.80	0.10+0.02	5.22+0.32	0.59+0.02
COA002,250mg	9.36+2.24	9.22+2.06	0.16+0.24	5.28+0.38	0.69+0.08
COA002,500mg	8.40+1.34	8.22+0.98	0.12+0.14	6.02+0.20	0.56+0.10
COA002,1000mg	6.88+1.54	7.86+1.12	1.04+0.32	4.98+1.02	0.38+1.04

Table 3. Pregnancy and foetal parameters of rats treated with Cassia Occidentalis prior to mating, mating period, gestation, and Lactation period of 7 days

	Control group	CO-A002 250mg	CO-A002 500mg	CO-A002 1000mg	P-value
No. of rats examined	8	8	8	8	_
Pregnancy index (%)	100	100	100	100	n/s
Length of pregnancy (days)	22.00 ± 0.16	22.08 ± 0.19	21.80 ± 0.29	21.58 ± 0.32	n/s
No. of pups at birth / litter	7.84 ± 1.24	8.26 ± 0.60	8.60 ± 0.90	7.48 ± 0.42	n/s
Live birth index (%)	100 (7.50)	100 (9.34)	100 (0)	100 (0)	n/s
No. of pups on day 7 post-natal	7.45 ± 1.22	7.89 ± 0.28	7.78 ± 1.10	7.28 ± 0.45	n/s
Viability index (%) b	100 (10.20)	100 (16.80)	100 (0)	100 (0)	n/s
Implantation sites / litter	10.58 ± 1.12	10.44 ± 0.43	9.65 ± 0.26	9.38 ± 0.66	n/s
Post implantation death (%) b	12.76 (39.40)	10.20 (21.60)	14.24 (34.62)	18.26 (25.58)	n/s
Sex ratio M: F (at birth)	1.40: 1	1.42: 1	1.20: 1	1.46: 1	n/s
Sex ratio M: F (at D7 post-natal)	1.58: 1	1.14: 1	1.23: 1	1.25: 1	n/s
Malformations of pups	Nil	Nil	Nil	Nil	__

Diestrus index, Number of days with clear diestrus smear × 100/total duration of treatment (days). The data representthe mean ± SEM animals, n = 8. a $p < 0.05$ vs. vehicle.

Table4. Frequency of estrus cycle phases, interval between estrus and diestrus index after treatment of rats with different doses of CO-A002 in mg/kg

Frequency				
Phase	Treatment			
	Vehicle Control	CO-A002-250	CO-A002-500	COA002-1000
Proestrus	7.375 ± 0.263	7.750 ± 0.250	8.500 ± 0.423	6.750 ± 0.313
Estrus	7.375 ± 0.324	7.125 ± 0.350	6.750 ± 0.453	6.500 ± 0.500
Interestrus Interval	4.093 ± 0.19	4.423 ± 0.316	4.775 ± 0.293	5.068 ± 0.525
Met estrus	8.125 ± 0.549	8.000 ± 0.423	7.750 ± 0.366	7.375 ± 0.375
Diestrus	8.125 ± 0.295	9.125 ± 0.895	11.000 ± 0.707	11.375 ± 0.90
Diestrus index (%)	26.200	29.438	30.587	38.338

Values are mean ± SEM; median (Interquartile range); $P > 0.05$ between all groups: Statistically not significant

Discussion

Numerous potentially active components, including phenolic compounds, flavonoids, and terpenes, were found in coffee weed leaf extract [12, 13]. Other chemicals with pharmacological and biological effects are also identified in the genus Cassia in line with Ajaiyeoba, including steroids, tannins, saponins, and cardiac glycosides[14]. These compounds might very well be in charge of a therapeutic reproductive cycle [15]. Pregnancy initiates the formation of this mechanism, but puberty marks the beginning of its structural and functional maturation [16]. Sexual organs and later the central nervous system, which still do not appear to be differentiated, are regularly impacted by the substances that are present in the mother blood through the placenta during the prenatal and postnatal stages. Humans also have lower metabolic and excretory capacity as well as fewer endocrine system feedback mechanisms, making them more vulnerable to the impacts of chemicals during crucial developmental phases [17].

Because maternal toxicity may be a long-lasting or temporary alteration in the mother's physiology with the potential to have negative effects on the offspring during embryo development, research on potential effects on the maternal organism is included in the evaluation of a drug's toxic effects [18]. Through the placenta, the embryo and endometrium make physical touch, which is referred to as implantation. The synchronised development of the embryo for the blastocyst stage, and therefore the differentiation of the uterus for the receptive condition, is the key component of this process. Then the process of implantation between the activated blastocyst and the uterine epithelium begins [19].

In rodents, implantation typically occurs between the fourth and fifth day; however, any abnormalities can cause losses in the implantation of embryos [20,21]. The number of implantation sites did not change after CO-A002 administration (table 1), indicating no harm during this stage and confirming the absence of embryonic estrogenic activity.

The CO-A002 treatment of the mothers did not change the quantity of offspring in each litter, indicating that CO-A002 does not exhibit toxicity at this stage [22].Additionally, there was no change in the newborn's weight, indicating no evidence of toxicity during the development stage. Congenital defects were not discovered, proving that the extract had no teratogenic effects. This means that the extract had no significant impact on the immunological, endocrine, nutritional, or vascular aspects required for the embryo's growth and normal development[23].

Recognising the pattern of the estrous cycle's events allows for the monitoring of the most favourable moment for mating as well as the evaluation of the hormonal cycle based on anatomical, cytological, and histological changes in the genital systems. It also serves as a useful indicator of the normality of the neuroendocrine function of non-pregnant females. By tracking the changes in the typical cytological vaginal smear, the estrous cycle can be monitored in rats [24,25].

The estrous cycle evaluation's findings demonstrate the likely reproductive toxicity of CO-A002 at dosages of 1000 mg/kg since it affected the frequency of the estrous cycle's phases, prolonged the diestrus, and decreased proestrus, as suggested by Goldman et al. in 2007 [26]. The estrous stage of the estrous cycle was prolonged in rats treated for five days with an ethanol extract of the stem of Musa paradisiacal, Musaceae, at doses of 250 and 500 mgkg body weight, according to studies from Soniet al. in 2013 [27]. The presence of progestogens in Musa paradisiacal, which have the potential to inhibit the hypothalamic-pituitary axis and subsequently follicular development, may be the cause of these changes, as was suggested by Bakryet al. in 2010 in their study of mice treated with medroxyprogesterone acetate [28]. These chemicals are frequently included in the list of steroid compounds listed in the study of Ajaiyeoba's extract from a plant belonging to the same species. The chance that this effect, which is similar to progestogens, will cause anestrus in domestic animals who often consume the plant's pods must be researched, as well as its potential application in phytotherapy [29].

The female pregnant rats from the groups treated with the extract in comparison to the control group did not exhibit any symptoms of degenerative, inflammatory, or necrotic abnormalities in the pancreas, heart, liver, or kidneys. As a result, it can be concluded that the extract had no effect on the morphological structures of those organs and was not hazardous to the body as a whole.

Conclusion

The current findings show that a hydroalcoholic extract of Cassia Occidentalis did not cause any significant reproductive damage or complications during pregnancy or delivery in female rats. The extract had no estrogenic effect on the uterus and produced no histopathological remodelling in the liver, lungs, or kidneys of pregnant female rats. However, it altered the estrous cycle, shortening the duration and decreasing the amount of estrus.

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