

**Neurotoxicological study about a medicinal plant of *Cissus sulcicaulis*
Baker in pregnancy rats****Estudo neurotoxicológico de uma planta medicinal de *Cissus sulcicaulis*
Baker em ratas prenhes**

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ABSTRACT

The *Cissus sulcicaulis* Baker species belongs to the Vitaceae family is popularly used for its anti-inflammatory activity. This study investigated for the first time the reproductive capacity of pregnant rats exposed daily to *Cissus sulcicaulis* Baker, the fetal organogenesis (prenatal assay), and the development of the pups (postnatal assay). Pregnant rats were exposed for the entire gestational period to a saline solution (control), *Cissus sulcicaulis* Baker (0.2 or 0.4 g/kg/day) and diclofenac potassium (positive control - 1.0 mg/kg/day). Fertility and pregnant weight gain were monitored. Pups were monitored for body weight, offspring vitality, morphology, and physical and neurobehavioral development. *Cissus sulcicaulis* Baker at a dose of 0.4 g/kg/day promotes alterations in reproductive performance of pregnant rats ($p < 0.05$); at doses of 0.2 and 0.4 g/kg/day promoted morphologic changes in the offspring parameters ($p < 0.05$); and in the dosage of 0.2 mg/kg/day significantly promotes neurotoxicity effects in the offspring ($p < 0.05$). In conclusion, these results indicate that the exposure of rats, during pregnancy period, to the *Cissus sulcicaulis* Baker proved not to be safe.

Key words: *Cissus sulcicaulis*, neurobehavioral development, pregnancy rats, physical development, teratogenicity.

RESUMO

A espécie *Cissus sulcicaulis* Baker pertence à família Vitaceae e é popularmente usada por sua atividade anti-inflamatória. Este estudo investigou pela primeira vez a capacidade reprodutiva de ratos grávidas expostas diariamente a *Cissus sulcicaulis* Baker, a organogênese fetal (ensaio pré-natal) e o desenvolvimento dos filhotes (ensaio pós-natal). As ratas prenhes foram expostas durante todo o período gestacional a uma solução salina (controle), *Cissus sulcicaulis* Baker (0,2 ou 0,4 g / kg / dia) e diclofenaco de potássio (controle positivo - 1,0 mg / kg / dia). Fertilidade e ganho de peso da gestante foram monitorados. Os filhotes foram monitorados quanto ao peso corporal, vitalidade da prole, morfologia e desenvolvimento físico e neurocomportamental. *Cissus sulcicaulis* Baker, na dose de 0,4 g / kg / dia, promove alterações no desempenho reprodutivo de ratos prenhes ($p < 0.05$); nas doses de 0,2 e 0,4 g / kg / dia promoveu alterações morfológicas nos parâmetros da prole ($p < 0.05$); e na dosagem de 0,2 mg / kg / dia promove significativamente efeitos de neurotoxicidade na prole ($p < 0.05$). Em conclusão, esses resultados indicam que a exposição de ratos, durante o período de gravidez, ao *Cissus sulcicaulis* Baker mostrou-se não segura.

Palavras-chave: *Cissus sulcicaulis*, desenvolvimento neurocomportamental, ratos prenhes, desenvolvimento físico, teratogenicidade.

1 INTRODUCTION

The *Cissus* genus, extensive from Vitaceae family, is constituted of 350 up to 400 species of creepers and a pantropical distribution. The *Cissus sulcicaulis* Baker species belongs to the Vitaceae family and is known in Brasil as “chupão,” “cipó água,” “cipó mãe boa” and “parreira brava” (Correa, 1926; Rodrigues *et al.*, 2014). In South America, it is the

only natural occurrence gender of Vitaceae family. The *Cissus sulcicaulis* Baker has the following scientific synonymies: *Cissus gongyloides*, *Vitis pterophora* Baker and *Vitis sulcicaulis* Baker (Tropicos, 2017). It is a kind of creeper with big leaves, composed of three leaflets with trichomes in their inferior portion. The fruit is a prolate blackberry, eatable although very acid, containing only one seed. After being cut, *Cissus sulcicaulis* Baker presents a drinkable sap that “relieves thirst of a man” (Correa, 1926). Several studies have been made founding some pharmacological activities especially anti-inflammatory (Santos *et al.*, 2007). Its dye is commercialized for oral and topical treatments of inflammatory processes.

The cooked leaves of *Cissus sulcicaulis* Baker are used in the treatment of edema and chronic rheumatism. The chemical composition of the South American species of *Cissus* is not very known. Some phytochemical studies indicate the presence of proanthocyanins and cyanogenic species, as well as the occurrence of phytoalexins and triterpenes with antioxidant activity (Cronquist, 1981). In previous studies with dichloromethane extract successively fractionated on a silica gel 60 column eluting with n-hexane mixtures of dichloromethane and ethyl acetate of increasing polarity in proportions, were isolated compounds with lower polarity, such as fat, terpene chains, followed by β -sitosterol (Lopes *et al.*, 2002). Until the present, few toxicological studies were realized with *Cissus sulcicaulis* Baker. However, for oral administration, one can state that the LD₅₀ is over 10.000 g/kg, demonstrating a high margin of safety in rats. For intraperitoneal administration, the LD₅₀ is of 3.730 g/kg, resulting in a slightly toxic composite (Lopes *et al.*, 2002).

Toxicological assays that guarantee safety in the utilization of vegetable drugs are highly considered, while they can contribute to studies on vegetal drugs with proved therapeutic indication. In this way, the present study aims to investigate the effects and the safety of *Cissus sulcicaulis* Baker intake by rats in the following assays.

i) Prenatal assays, exploring the reproductive capacity of female rats and the teratogenicity in pups. ii) Postnatal assays, verifying *Cissus sulcicaulis*'s effects on the physical and neurobehavioral development of the litter.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND EXTRACTION

The *Cissus sulcicaulis* Baker specimens were collected in Piracicaba, State of São Paulo, Brasil. The species identification was made by the USF Herbarium - Bragança Paulista. A voucher specimen has been deposited in the Herbarium Frei Velloso at the University of

São Francisco (USF) under the no. VELL 835. Fresh leaves of *Cissus sulcicaulis* Baker were dehydrated into a Fanen® air circulation stove at 40°C during 48 hours then pulverized in a Marconi® knives and hammers mill. The hydroalcoholic extract was prepared by maceration using 92.8° GL ethyl alcohol as extracting liquid. The drug was left in ethanol for three weeks regularly agitated and weekly washed, while the extracting liquid was renewed. The obtained extract was concentrated on a rotating evaporator device and then lyophilized.

Animals

Adult Wistar rats weighing 160 g to 200 g, of both genders, were obtained from Anilab - Laboratory Animals Conception and Market, São Paulo State. The animals were kept in the Laboratory of Toxicological Research (Lapetox-Uniso) facility. All the procedures by the “Guide for the Care and Use of Laboratory Animals” (National Research Council, 2011) and the Organization for Economic Co-operation & Development guidance document (2000) approved in Committee on the Care and Use of Experimental Animals. Registered under protocol no. 2010-15P, Brazilian Lutheran University (ULBRA). All animals were maintained in groups of 5 rats per cage. Food and water were provided *ad libitum*, except during the neurotoxicological assays for screening doses, where the animals were kept fasting for four hours before each experiment. Twelve-hour light/dark cycle and constant temperature (23 ± 1°C) were maintained. The animals were previously adapted to laboratory conditions one week before the experiments.

The *Cissus sulcicaulis* Baker hydroalcoholic extract was administered by oral gavage in all assays. The chosen doses LD₅₀ (Lopes *et al.*, 2007), for the acute neurotoxicity tests were based on the, and their results defined the doses of the prenatal assays that, in turn, defined the doses for the studies of postnatal neurotoxicity.. For all assays to assess perinatal toxicity, diclofenac potassium (1.0 mg/kg/day) was used as a positive control .

2.2 ACUTE NEUROTOXICITY ASSAYS

For *Cissus sulcicaulis* Baker acute neurotoxicity assays (general physical activity in open field, exploratory activity in plus-maze, and social interaction) (File *et al.*, 1993; Habr *et al.*, 2014), ninety male rats were distributed into three groups, one control and two experimental. The first experimental group received 0.2 g/kg of *Cissus sulcicaulis* Baker (Cs200) reconstitute hydroalcoholic extract and the second received a dosage of 0.4 g/kg (Cs400). Control group received the saline solution. Both, saline solution and *Cissus*

sulcicaulis Baker administration pretreatment time were 30 minutes for each assay. For evaluation of the toxic dosages in the general activity, plus maze assays, and social interaction activity, the control, *Cs200* and *Cs400* groups, were divided into three subgroups, each one submitted to only one of the assays. Before the introduction of the animal in open field or plus-maze, they were cleaned with ethanol 5% in order to avoid possible odors. Control and experimental rats were intermixed and the observations were made between 2 pm and 4 pm.

2.3 REPRODUCTIVE ASSAYS

A hundred sexually-naive female rats were mated to males, two females and one male per cage. Pregnancy was confirmed through the presence of spermatozoids in vaginal-washing rubbing observed by microscopy analysis (Bennet and Vickery, 1970). The presence of spermatozoids was considered as the first day of pregnancy. Pregnant females were distributed in two groups to evaluate prenatal and postnatal effects, as below.

Prenatal Assays

For the prenatal evaluation, 40 pregnant rats were shared in four groups (control, *Cs200*, *Cs400* and positive control); all administration were done by oral gavage using water as vehicle. The animals were treated during the 21 days of the gestational period and weighted daily. On the 21st day, the pregnant rats were anesthetized with ketamine (100 mg/Kg) and xylazine hydrochloride (6 mg/Kg), ovaries and uterus were removed via cesarean section (followed by euthanasia). Corpus luteum were counted, fetuses and placenta were taken off, and offspring vitality was observed (Damasceno *et al.*, 2008; Randazzo-Moura *et al.*, 2011).

Fetuses, anesthetized (halothane) and euthanized, were randomly divided into two groups to external and internal morphologic parameters observations. To the macroscopic external analyses, the fetuses were fixed in Bouin's solution for 24-48 h, replaced by a 70% hydroalcoholic solution to be measured (in cm). Internal morphologic parameters were assessed after an evisceration and diaphanization process, fetuses were fixed in ethanol, then cleared in KOH and stained with Alizarin red-S. Morphometric analysis was done using a stereomicroscope.

Postnatal Assays

For postnatal evaluation, 30 pregnant rats were divided in three groups (control, *Cs200* and positive control) and the administration were done by oral gavage, using water as vehicle.

Only term pregnancies were considered to evaluate the female reproductive performance and postnatal development of the offspring (Schwarz *et al.*, 2003; Cope *et al.*, 2015). The females were exposed to treatment throughout the 21 gestation days.

The following parameters were evaluated to observe the reproductive performance of rats: I) number of gestations carried out considering the litters size; II) pregnant weight gain; III) number of offspring; IV) number of dead and alive-born offspring. The weight of each offspring was daily recorded during all lactation period, for 21 days. Number of offsprings was standardized, each female was allowed to kept only eight pups, and the others were discarded. The number of male and female offsprings was equally distributed for each rat dam (Couderc *et al.*, 2014).

2.4 PHYSICAL DEVELOPMENT OF THE OFFSPRING

The parturition day was defined as the litter first day of life. On this first day, the offspring was macroscopic examined externally and sexed. Same male and female pups were used for the physical and developmental tests. Were observed the following physical development parameters: fluff and hair appearing; ear unstitching and opening; incisor teeth eruption; eyes opening; testis descent; and vagina opening (Gerenutti *et al.*, 2014).

Neurobehavioral Development of the Offspring

Latency for uprightness

In the first day, each pup successfully turned prone over their four paws in less than 10 seconds when placed in supine position. It was recorded as the “latency for uprightness”. This parameter was evaluated from the second to the seventh day after birth. Pups were observed daily in the morning, between 8 am and 11 am, separated from their mother for no more than 3 minutes, and immediately taken back to their cages after the test (Gerenutti *et al.*, 2014).

Walking adult

In this test, according Farkas *et al.* (2009), each animal was placed in the center of the open-field observing whether the animal walks on four legs, without touching the ventral region on the apparatus floor.

Negative geotaxis

This assay was performed to test vestibular and postural reflexes requiring motor coordination for a successful completion. From the seventh to the twelfth day after birth, each pup was placed in the prone position with its head down on a 45° inclined ramp with abrasive surface. It was recorded the time that pups required to turn their head 180° and take the first step in this new direction - within a not exceeding 30 seconds period. Also in this test, pups were observed daily, between 8 am and 11 am. They were separated from their mother during this assay, no more than a three minutes period, and immediately returned to the cages (Li *et al.*, 2014).

2.5 STATISTICAL ANALYSIS

Bartlett test was employed to evaluate the outcomes homogeneity. Mothers and litters body weight, placenta weight, external morphologic dimensions, general activity and social interaction, were reported as mean \pm standard deviation (SD). The litters were considered units of the statistical analysis. Differences among each treatment were evaluated using nonparametric one-way ANOVA, followed by Tukey-Kramer's multiple range tests. Parameters (offspring vitality, pre- and post-implantation losses, internal morphologic parameters, physical, neurobehavioral developments and plus-maze assay), expressed in percentage, were evaluated by Chi-square test. P values < 0.05 were deliberated as significant. Data were analyzed in GraphPad Prism 5 (GraphPad Software -USA).

3 RESULTS

3.1 NEUROTOXICITY ASSAYS

The result in Table 1 indicate that the administration of *Cs200* and *Cs400* in the open field test showed decrease in general activity, not altered entries in open arms, although had considerably decreased time into open arms and crossings and reduced social interaction of animals.

3.2 REPRODUCTIVE ABILITY EVALUATION AND OFFSPRING SURVIVAL

Table 2 shows the mean (\pm SD) of the pregnant rat weight gain in grams during the gestation period. Despite the statistically significant differences between the three groups in many periods; *Cs200* and *Cs400* reduced the weight gain of females during pregnancy. This weight reduction was not accompanied by fur bristling, shiver, cyanosis or any other signal of sub-chronic toxicity. The *Cs400* affects the reproductive capacity of female rats, increasing

post-implantation loss, reduced the vitality of offspring and the fetal weight, and increased the placental weight. In postnatal assay observed there was a reduction in the number of live births in pups *Cs200* group compared to control group, however, there was an increase in alive-born pups weight and pups weight gain in this experimental group compared to the control group.

3.3 FETAL DEVELOPMENT

Completing the prenatal effects of *Cissus sulcicaulis* on the offspring, the morphologic external measures, skeletal and visceral changes parameters are reported in Table 3. No changes were observed in measures of the skull antero-posterior, thorax latero-lateral and cranio-caudal in the experimental groups *Cs200* and *Cs400* when compared to the control group. Fetal alterations were observed among the experimental groups compared to control group in the skull circumference. In *Cs400* group were observed increased skull latero-lateral, skull circumference, thorax anterior-posterior when compared to the *Cs200* group.

Skeletal analysis of fetuses showed that *Cissus sulcicaulis* promoted changes in all evaluated parameters. In addition, visceral abnormalities were observed in both doses studied. However, syndactyly, cleft palate, abnormal eyes/ears, bipartite centrum, and supernumerary ribs were not observed.

3.4 POSTNATAL DEVELOPMENT

Physical development parameters

Figure 2 shows that maternal exposure to *Cissus sulcicaulis* compared to the control group and positive control group, promoted a delay in physical development parameters of the pups, considering the effective time in days required for startup of each parameter.

Neurobehavioral development assay

Figure 3 shows the percentage of pups able to perform the latency for uprightness and negative geotaxis, beyond age to display the walking floor. Our findings indicate that *Cissus sulcicaulis* promoted a delay in the development of the three evaluated parameters.

4 DISCUSSION

Until present time, the biological essays realized with the extract of *Cissus sulcicaulis* Baker leaves mainly refer to anti-inflammatory effects. However, studies about its effects on the Central Nervous System (CNS) and on the reproductive performance are still rare. This

way, our studies are new and demonstrate that *Cissus sulcicaulis* Baker presents effects over CNS, toxicological effects on fetuses and rats pups.

Ours results indicate that the administration of *Cissus sulcicaulis* Baker in the open-field test decreased locomotion frequency and increased the time of immobility, suggesting a reduction in general activity. Reduction in the locomotion as well as immobility increase, seem not to be associated with the stomach fullness, once the observation in the open field occurred 30 minutes after administration of vegetal extract in animals under complete fasting. In addition, as far as the observed decrease in rearing behavior is concerned, it can be explained thinking that *Cissus sulcicaulis* Baker would reduce the rat's habituation process (Perez-Guerrero *et al.*, 2001; Wilson, 2001). Considering that Barros *et al.* (1994) and Knox *et al.* (2014) observed that GABAergic antagonists reduce grooming of rats in the open-field, suggesting the usefulness of this parameter to detect the effects of GABAergic drugs and anxiolytic effects, and considering also that open-field grooming duration was decreased in our results, it demonstrates the anxiolytic effects of the *Cissus sulcicaulis* Baker extract administration. The result in elevated plus-maze and social interaction activity reinforce that the administration of *Cissus sulcicaulis* Baker extract in rats (200 and 400 mg/kg) reduced general activity. However, more evident toxic effects were not observed. We have used these results to define a less toxic dosage to be employed in reproductive assays studies.

Many of the changes observed during birth, growth and development are due specially to the exposure of the mother to chemical agents (Shintaku *et al.*, 2012). Mammals reproduction is a long-lasting process and involves several stages, increasing their susceptibility to chemicals (Ozyurt *et al.*, 2011).

The protocol for the reproduction toxicology depends on the therapeutic indication of the drug. The protocols are described by regulatory agencies, as the Organization for Economic Co-operation and Development (OECD) and the Environmental Protection Agency (EPA) In Brazil, the regulatory body for herbal medicines is National Health Surveillance Agency (ANVISA). Phytotherapy is a treatment method based upon the employment of fresh herbs or vegetal drugs, or even vegetal extracts prepared with those two types of raw-materials (Asif *et al.*, 2015). The use of medicinal plants, as a therapeutic resource, returns to traditional medicine and has reached a wider public, which demands a position of the scientific community (Ameni *et al.*, 2015). In this regard, prenatal assays with *Cissus* at doses of 0.2 and 0.4 g/ kg /day, showed that it is not safe during pregnancy because of the alterations in the reproductive capacity of females and fetal morphological changes promoted.

The methodology used in the present study was similar to the other studies that observed the acute toxicity (Perez-Guerrero *et al.*, 2001), the reproductive ability (Schwarz *et al.*, 2003; Giriko *et al.*, 2013) the physical development of rat offspring (Gerenutti *et al.*, 2005) the neurobehavioral development, which included rearing (Barros *et al.*, 1994) and negative geotaxis (Gerenutti *et al.* 2005; Bezpalko *et al.*, 2015).

The genus *Cissus* has been widely used in traditional herbal medicine for the treatment of hemorrhoids, gastric ulcers and bone healing. Miida *et al.* (2008) after a single administration of diclofenac, on observed the area under plasma concentration-time curve (AUC) based on free diclofenac in pregnant rats was 3.9 times higher than that in non-pregnant rats. This difference is due to a lower concentration of serum albumin and a higher concentration of non-esterified fatty acid (NEFA) that inhibits drug binding to albumin, in pregnant rats.

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) in full-term pregnant women leads to fetal or neonatal toxicity, such as constriction of the ductus arteriosus (DA) and persistent pulmonary hypertension in the newborn. The aim of this study was to predict quantitatively the fetal toxicity of and diclofenac using the transplacental pharmacokinetic parameters obtained from our previous placental perfusion studies. Shintaku *et al.* (2012) claim that in human placental perfusion study and pharmacokinetic/pharmacodynamic analysis may provide basic data for predicting human fetal toxicity of drugs. Ozyurt *et al.* (2011) suggest that development of neurons and volume of cervical spinal cord are affected in prenatal animals after administration of diclofenac sodium, when 1 mg/kg daily diclofenac was injected into the pregnant rats beginning from the 5th day after mating to the 20th day of the pregnancy. Comparisons of stereological estimations among these three groups showed that axon number and mean axon cross-sectional area, but not average myelin sheet thickness, were significantly decreased in rats that were exposed to diclofenac; the electron microscope analysis revealed, in treated groups, deterioration of myelin sheaths that was more pronounced in rats that were exposed to diclofenac sodium; suggesting that this potential teratogenic impairs peripheral nervous system development Canan *et al.*(2008).

The weight gain of the animals, which are submitted to any chemical agent, during a specific period, is one of the most used parameters to show toxic effects (Gerenutti *et al.*, 2008; Paronis *et al.*, 2015). No differences (t-test, $p > 0.05$) considering water ingestion and food consumption (data not shown) were observed between dams of groups control, positive control, *Cs200* and *Cs400*. This weight reduction was not accompanied by fur bristling, shiver,

cyanosis or any other signal of sub chronic toxicity. However, there is a significant difference between the number of alive-born pups in the groups. On the other hand, exposure to *Cs200* and *Cs400* promoted a reduction in the number of pups per group of mated females, indicating a possible action embryolethal (Leonard, 1982; Keller, 2001). All alive-born pups remained alive until the end of assays and in general *Cissus sulcicaulis* hydro-alcoholic extract did not affect the weight gain of offspring. The results of physical development parameters indicated that *Cs200* interfere with physical development of both male and female pups.

Both, alteration on physical parameters of litters and the number of postnatal deaths are important indexes of perinatal toxicity (Leonard, 1982). The reproductive ability of females was not affected by *Cissus sulcicaulis* hydroalcoholic extract. All live-born pups of both groups remained alive and, in general, the weight gain of pups, irrespectively of genders, were not affected by *Cs* hydroalcoholic extract.

The exposure to chemical agents during the pregnancy is one of the main factors that could interfere in the offspring physical development and cause behavioral abnormalities (Lazarini *et al.*, 2004; Ognio *et al.*, 2006). The *Cs* hydroalcoholic extract did interfere with some of the physical and behavioral development parameters of both male and female pups observed in the present study; the effects observed include a delay in physical development of offspring. In addition, these results together suggest a reduced development of the neuromuscular function in the *Cs* group.

The benzodiazepine-like activity could explain the CNS depression, which could be characterized by the increased latency for uprightness and negative geotaxis, observed in the pups of the present study. However, diazepam causes the immediate-evasion reflex missing in rats, but it had no effect on the litter size and in the negative geotaxis in three-week pups, whose mothers received 10 mg/kg/day diazepam from day 7 to 21 of pregnancy (Laitinen *et al.*, 1986). Similarly, as observed in the present study, these authors classified the changes caused by diazepam in the motor development of pups (aging 2-to-3-weeks) as transient.

Considering all results together, the use of hydroalcoholic extract of *Cissus sulcicaulis* Baker during the pregnancy slightly interfered in neurobehavioral development of the offspring and in the physical parameters of their mothers. These results reinforce the importance of studies in this area, to clear possible neurotoxic effects of this commonly used medicinal plant (Xu *et al.*, 2010; Estrada-Reyes *et al.*, 2014).

In conclusion, the above data suggest that the acute administration of *Cissus sulcicaulis* Baker hydroalcoholic extract has promoted general alterations in behavior models, while its perinatal administration promotes neurotoxicity effects on the fetuses. One may also suggest that the perinatal toxic effects of *Cissus sulcicaulis* Baker are more expressive than those of the diclofenac potassium.

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ANNEXES

Table 1. Effects of *Cissus sulcicaulis* Baker hidroalcoholic extract administration in acute toxicity assays.

Parameters	Control	General activity	
		Cs200	Cs400
Locomotion frequency	66.40 ± 5.59	62.2 ± 11.21	42.8 ± 9.20*
Rearing frequency	29.4 ± 2.51	21.60 ± 2.45*	17.0 ± 1.78*
Grooming duration	21.0 ± 4.0	18.0 ± 2.0	14.0 ± 2.0*
Immobility duration	8.0 ± 2.0	23.0 ± 4.0*	50.0 ± 4.0*
Parameter	Control	Social interaction	
		Cs200	Cs400
Social interaction duration	64 ± 4.8	57 ± 4.0	36 ± 2.6*
Parameters	Control	Plus-maze assay	
		Cs200	Cs400
% Entries into open arms	35	33	30
% Time into open arms	18.03	10.55*	10.50*
% Central time	21.00	10.33*	11.50*

Groups: Control: saline solution; Cs200: *Cissus sulcicaulis* Baker. 0.2/kg; Cs400: *Cissus sulcicaulis* Baker. 0.4 g/kg/. n: 10 rats per group. Data are reported in general activity and social interaction: mean ± S.D, *p<0,05 in comparison to Control group, nonparametric one-way ANOVA, followed by Tukey-Kramer's; Data are reported in in plus-maze assay: percentage, *p<0,05, Chi-square Test.

Table 2. Effects of *Cissus sulcicaulis* Baker on reproductive performance of pregnant rats.

Weight gain (in grams) of the pregnant rat (21 days)				
Control (n: 20)	Positive Control (n: 10)	Cs200 (n: 20)	Cs400 (n: 10)	
100.04 ± 3.92	89.28 ± 3.58	88.60 ± 4.82*	87.34 ± 5.47*	
PRENATAL ASSAY				
Parameters	Control (n: 10)	Positive Control (n: 10)	Cs200 (n: 10)	Cs400 (n: 10)
Implantation losses Post (%)	2.19	4.41*	1.50	9.87*
Offspring vitality (%)	97.80	95.58	98.48	90.12*
Weight of fetus (in grams)	4.07 ± 0.63 (n: 89)	4.00 ± 0.32 (n: 65)	3.79 ± 0.60 (n: 65)	3.64 ± 0.33* (n: 73)
Weight of placenta (in grams)	0.53 ± 0.09	0.58 ± 0.08*	0.52 ± 0.07	0.57 ± 0.10*
POSTNATAL ASSAYS				
Parameters	Control (n: 10)	Positive Control (n: 10)	Cs200 (n: 10)	
Total number of alive-born pups	9.8 ± 0.2	8.7 ± 0.9	8.4 ± 0.3*	
Weight alive-born pups (in grams)	6.41 ± 0.74	5.82 ± 0.86*	7.07 ± 1.00*	
Weight gain (in grams) of the litter rat (21 days)	39.10 ± 0.44	34.30 ± 1.72*	39.58 ± 1.12	

Groups: Control: saline solution; Positive control: diclofenac potassium (1.0 mg/kg); Cs200: *Cissus sulcicaulis* Baker. 0.2g/kg. Cs400: *Cissus sulcicaulis* Baker. 0.4g/kg Data are reported in mean ± S.D, *p<0,05 in comparison to Control group., nonparametric one-way ANOVA, followed by Tukey-Kramer's; Data are reported in percentage, *p<0,05, Chi-square Test.

Table 3. Prenatal effects of *Cissus sulcicaulis* Baker on the offspring morphologic parameters.

Parameters	Control (n: 45)	Positive Control (n: 30)	Cs200 (n:32)	Cs400 (n:36)
Morphological Changes (cm)				
Skull antero-posterior	1.31 ± 0.15	1.44 ± 0.08	1.19 ± 0.08	1.49 ± 0.09
Skull latero-lateral	0.96 ± 0.08	1.0 ± 0.05	0.95 ± 0.06	0.99 ± 0.04
Skull circumference	2.81 ± 0.31	3.15 ± 0.25	2.25 ± 0.18*	2.53 ± 0.16*
Thorax antero-posterior	1.12 ± 0.14	1.15 ± 0.10	1.08 ± 0.08	1.16 ± 0.06
Thorax latero-lateral	1.17 ± 0.18	1.22 ± 0.11	1.11 ± 0.10	1.18 ± 0.09
Cranio-caudal	3.58 ± 0.41	3.54 ± 0.22	3.55 ± 0.23	3.52 ± 0.16
Skeletal Changes (% in the fetus)				
Sternebrae agenesis	0	21.42*	6.25*	8.33*
Esternebrious incomplete ossification	0	32.14*	25.00*	30.55*
Abnormal esternebrious	0	21.43*	56.25*	47.22*
Rib agenesis	0	10.71*	0	5.55*
Structural rib anomalies	0	-	3.12	8.33*
Visceral Changes (% in the fetus)				
Dilated hepatic vessels	0	71.42*	62.50*	52.63*
Abnormal pigmentation	0	28.57*	37.50*	10.52*
Unilateral Renal Atrophy	0	7.14*	0	5.55*
Pigmentation of the thoracic and abdominal organs	0	35.71*	17.64*	27.77*

Groups: Control: saline solution; Positive control: diclofenac potassium (1mg/kg); Cs200: *Cissus sulcicaulis* Baker. 0.2g/kg. Cs400: *Cissus sulcicaulis* Baker. 0.4g/kg Data are reported in mean ± S.D, *p<0,05 in comparison to Control group,, nonparametric one-way ANOVA, followed by Tukey-Kramer's; Data are reported in percentage, *p<0,05, Chi-square Test

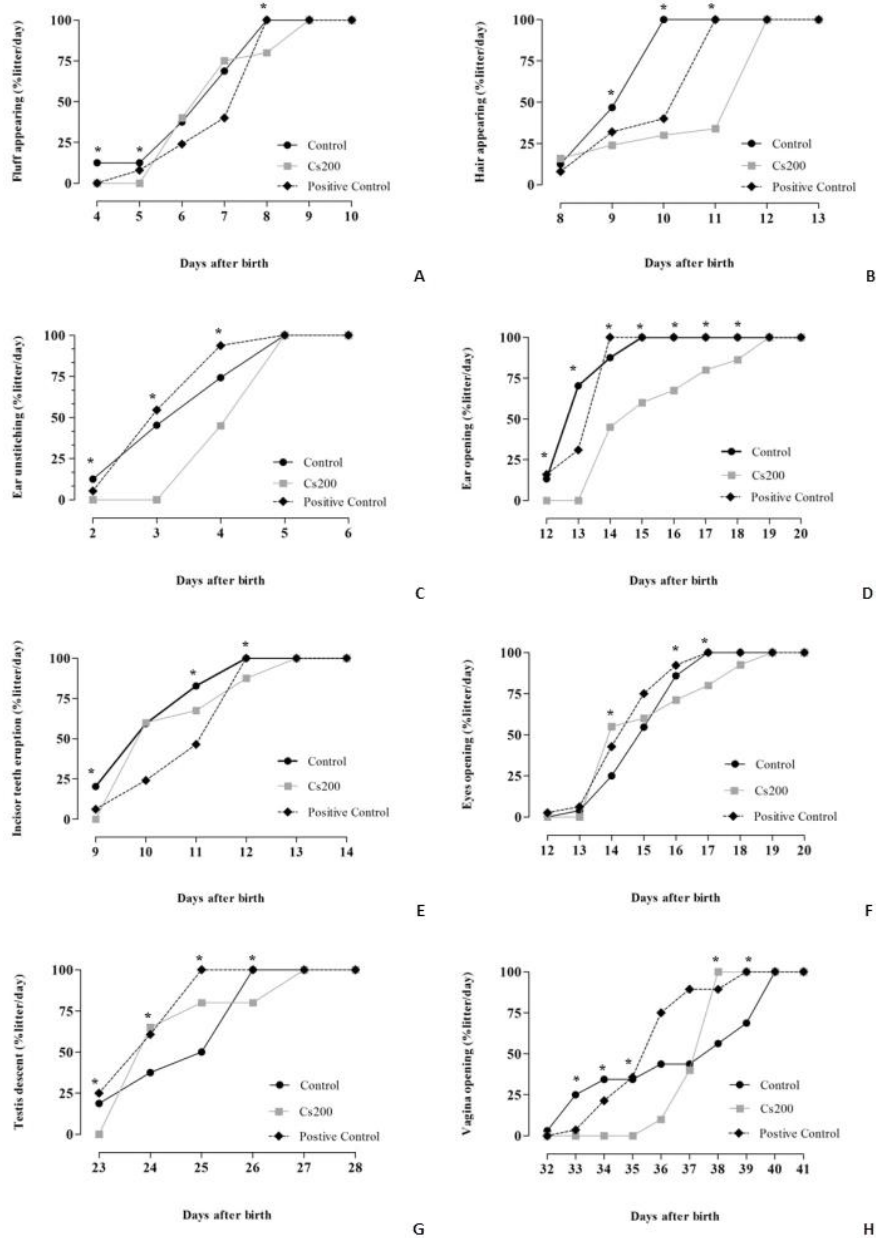
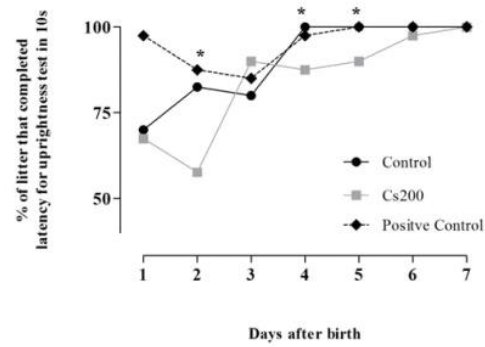
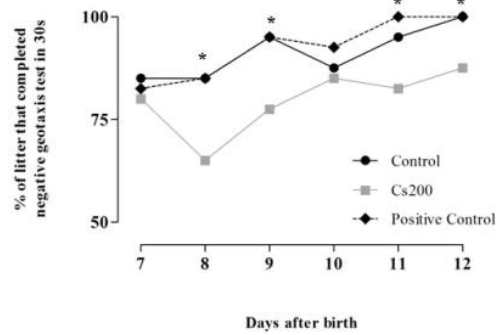


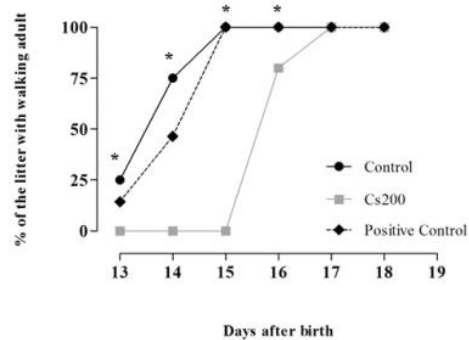
Figure 1. Physical development and puberty onset of the offspring after pregnancy exposure to *Cissus sulcicaulis* Baker. A) Fluff appearing; B) Hair appearing; C) Ears unsticking; D) Ears opening; E) Incisor teeth eruption; F) Eyes opening; G) Testis descent; and H) Vagina opening. Data are expressed as percentage of pups in each litter that developed the parameter per day. (n: 8 animals, *p<0.05, Chi-square Test).



A



B



C

Figure 2. Neurological development of the offspring after pregnancy exposure to *Cissus sulcicaulis* Baker. (A) Latency for uprightiness, (B) negative geotaxis and (C) walking adult. Data were expressed in percentage of pups in each litter that successfully completed the tests at each postnatal day test. (n: 8 animals, *p<0.05, Chi-square Test).