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EVALUATION OF TERATOGENIC EFFECTS OF DIFFERENT DOSES OF HERBAL FORMULATION (i-Coffee+) IN WISTAR RAT'S FETUS OR EMBRYO BY NO-OBSERVED-ADVERSE-EFFECT LEVEL (NOAEL) METHOD: AN *IN VIVO* STUDY

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ABSTRACT
iCoffee+ is a novel herbal formulation that effectively treats diabetes mellitus; the unique composition has been formulated with herbs, which do not have any teratogenic potential. The purpose of this study was to evaluate the teratogenic potential of the final formulation iCoffee+ on embryos and foetuses as a result of repeated oral administration during pregnancy in Wistar rats by the No-Observed-Adverse-Effect Level (NOAEL) method. Wistar albino rats were used as the experimental animals in four groups, with each group containing twenty pregnant rats. Group I served as a normal control group and received 0.5% CMC vehicle orally, whereas groups II, III, and IV received iCoffee+ at doses of 1.55 g/kg, 3.1 g/kg, and 6.2 g/kg of body weight, respectively. Up to the end of the investigation, all rat groups were evaluated twice daily for mortality, morbidity, and clinical signs and symptoms. Body weight and food consumption were measured twice a week throughout the treatment period, and then lastly on the day of sacrifice. The data was reported as three cumulative values. The fetus was then separated and weighed. The body weight of the fetus was recorded in the raw data. The complete fetuses were checked for skeletal and soft tissue abnormalities. The dams' uteruses were weighed and recorded. The entire before-and-after treatment period did not show any clinical symptoms. With these evidences, we conclude that, consumption of iCoffee+ in large doses of during pregnancy does not possess any health risk.

KEYWORDS: iCoffee+, Wistar rats, Teratogenicity.

INTRODUCTION
Teratology, which is the study of aberrant prenatal development and congenital abnormalities brought on by exogenous chemical or physical factors, is still a rapidly developing field of medicine in the fight to eliminate avoidable birth defects. Given the wide range of medications and chemicals that people are exposed to on a daily basis, it is critical to identify those with the potential to cause teratogenic effects.

Natural phytochemicals found in plants that have medicinal and pharmacological effects are a source of Nutraceutical formulations. They have a key role in the treatment of serious and mild ailments and are commonly employed in the development of pharmaceutical medications.[1-3] Despite their value, some of these natural chemicals contain toxic substances that are harmful to human health.[4-6] The toxicity of medicinal herbs on human organs such as the kidneys, liver, and heart has been extensively studied[7-9], but there are few studies describing the toxicity and teratogenicity of such plants on developing embryos. Teratogenicity is the third-ranking issue with drug preparations, behind cardiovascular and hepatic toxicities.[10] Before being used in humans, all drugs must now undergo pharmacological testing for the possibility of teratogenicity.[11] As a result, one of the most ostentatious studies ever conducted on herbs was the teratogenicity study.

According to the World Health Organization (WHO), almost 80% of the global population depends on the consumption of medicinal plants.[12] The preclinical stage of the research of plants for medicine involves a number of procedures, including the choice of the plants to be examined, accurate botanical identification, phytochemical characteristics, and pharmacological and toxicological studies.[13] Genotoxic compounds can alter an organism's genetic makeup[14], which can result in teratogenic effects and germ cell mutations, affect aging processes, and cause somatic cell mutations that can result in the development of cancer.[15-18] For these

reasons, tests for the detection of genotoxic compounds are crucial. Teratogens are substances that can affect genetic material and result in mutations in somatic and germ cells when the damage is caused during pregnancy. Any stage of the fetus' development can result in physiological or biochemical harm at delivery from a variety of chemical substances, which can result in uterine death, abortion, premature birth, or neonatal poisoning.[19-21]

The formation of the micronucleus is related to the teratogenic potential.[22,23] A substance is regarded as a potential teratogen if it can pass the placental barrier and cause micronucleated erythrocytes in the developing embryo. Numerous studies show that the growing fetus is more vulnerable to estrogenic substances than the adult. As a result, concern has grown over the teratogenic impact of herbal extract formulations for exposure during the developmental stages of fetuses, but there has been no conclusive research yet. The teratogenic implications of using herbal formulations, however, have not yet been published.

Animal model studies, however, are now crucial for determining if environmental chemicals have the ability to cause teratogenic effects. Early teratologists subjected pregnant animals to environmental contaminants while checking the fetuses for severe visceral and skeletal deformities. The study of teratology is going to a more molecular level and looking for the mechanism(s) of action by which these agents function, even though this is still a part of the teratological evaluation methods nowadays. Additionally, pregnancy registries are sizable prospective studies that track the exposures women receive during their pregnancies as well as the results of their births. These studies offer details on potential hazards associated with drug intake or other exposures during human pregnancies. Understanding how a teratogen works is essential for preventing congenital malformations as well as for the development of novel therapeutics that are safe for use during pregnancy.[24]

Therefore, this study was done to see if the herbal formulation iCoffee+ has any teratogenic effects on Wistar pregnant rats. The teratogenic test outcomes confirmed that this is safe for rats to consume while pregnant.

MATERIALS AND METHODS

Preparation of iCoffee+

I-COFFEE^{+} is manufactured and registered by Indusviva Health Sciences Pvt Ltd., Jayamahal Extension, Bangalore, Karnataka, India.

Experimental animals

Radiant Research Services Pvt. Ltd. in Karnataka, India, provided the male Wistar rats, which were ten weeks old and weighed 200 to 220 gm. The CPCSEA Registration Number 1803/PO/RcBi/S/2015/CPCSEA was used to supervise and regulate animal research studies. Picric acid has been used to identify each animal, and each has been given a unique number. The animal was housed in a standard polypropylene cage with a stainless steel top grill, common food, and water in bottles accessible to it. The bedding material used was sterilized paddy husk (source: Shree Balaji Rice Industries, Bangalore). The Aqua Guard service allowed free access to the water. Animals have always had access to clean, uncontaminated water for drinking. They were kept in these cages in standard laboratory conditions, which included a 12-hour light/dark cycle, a temperature of 22±3°C, and a relative humidity range of 30–70%. Under the guidance of skilled professionals, every procedure involving animals was carried out in an ethical manner. Radiant Research Services Pvt. Ltd.'s Institutional Animal Ethical Committee (IAEC) examined and approved the research protocol before the study's start.

Pain or Distress category

The study director and veterinarian were alerted right away in the event that adverse reactions showed signs of pain or distress. The animals were treated or euthanized according to the professional judgment of the veterinarian, in consultation with the study director when possible. Depending on the situation and in accordance with the CPCSEA Pain, Distress, and Protocol Guidance, treatment or euthanasia was selected.

Feed and water

All of the animals were provided with a standard chow diet (Amrut Feeds, Krishna Valley Agrotech LLP, Prashanth Enterprises, Pune) throughout the trial. There was unlimited access to fresh water. Animals were allowed to access clean, potable water that was not contaminated. Water microbiological contamination was periodically monitored. To ensure good operation, drinking water bottles and their tubes were periodically inspected.

Randomization

Picric acid was used to mark each animal, and each animal was given a unique number to help identify the group. Based on their body weight, animals was randomly assigned. A Microsoft Excel worksheet was used for the randomization.

Justification for selection of vehicle

Carboxymethyl cellulose sodium was a widely recognized and frequently used oral route for animal research. An internal test showed that the iCoffee+ dissolves uniformly in 0.5% carboxymethyl cellulose sodium. Hence, for test formulations, 0.5% of carboxymethyl cellulose sodium was used as a vehicle control.

Experimental procedure

The purpose of this study was to evaluate the teratogenicity of iCoffee+ at the No-Observed-Adverse-Effect Level (NOAEL) and the toxicity of repeated oral gavage administration of iCoffee+ to pregnant female

Wister rats during their gestation. Eighty female Wistar rats were divided into four groups. Group I served as the normal control group and received 0.5% CMC vehicle orally, whereas group II, group III, and group IV received iCoffee+ at doses of 1.55 g/kg, 3.1 g/kg, and 6.2 g/kg of body weight. Until the end of the study, the condition of all rat groups was assessed twice a day for mortality and morbidity. Everyday clinical signs and symptoms were recorded in all the animals. The animals' body weights in each group were weighed once before the start of dosage, twice weekly throughout the course of treatment, and finally on the day of sacrifice. Food intake was measured twice a week, and the data was presented as a 3-day cumulative value.

Isoflurane, an anesthetic for inhalation, was used to kill all the animals. The fetuses were shown being surgically removed from the mothers. The fetus was separated and weighed. In the raw data, the fetus' body weights were recorded. Then, the complete fetuses were examined for abnormalities in the soft tissue and skeleton. The dams' uteruses were weighed and recorded. The total number of corpora lutea, living and dead fetuses, implantation sites, and fetus body weights were all observed and recorded.

Formulation

Indusviva Health Sciences Pvt Ltd., Jayamahal Extension, Bangalore, Karnataka, India, manufactured the iCoffee+. Every day, the iCoffee+ formulation for the experiment was prepared. iCoffee+ was measured out, thoroughly ground in a mortar and pestle, and then transferred into a selected beaker. The iCoffee+ formulations were continuously stirred with a magnetic stirrer during dosage administration to keep them homogeneous. The volume of the formulations and the amount of iCoffee+ prepared varied depending on the requirements and animal body weight. The exact volume of iCoffee+, the volume of the developed formulation, and the volume of administration were all recorded in the raw data.

Dose administration

The iCoffee+ formulations were given orally to rats in each group once daily at doses of 1.55 g/kg, 3.1 g/kg, and 6.2 g/kg of body weight for a minimum of 18 consecutive days. At exactly the same time each day for the control group, each rat received 10 ml/kg/day of the dose volume for a minimum of 18 consecutive days. On the first day of treatment, the dose volume was calculated for each individual animal, and it was then updated in accordance with the most recent body weights reported throughout the trial. The study records represented daily individual dose volumes.

OBSERVATIONS

Mortality and Morbidity

Throughout the trial, at least twice-daily cage-side inspections were done to look for dead or moribund animals as well as abnormal animal behavior and appearance.

Clinical signs

Throughout the course of the trial, a daily cage-side assessment for audible clinical symptoms was carried out. Before starting treatment, thorough clinical examinations were performed twice (once during acclimatization and once after randomization), and then once every week (on the day of weekly body weights) after that, except the days of necropsy. Comprehensive examinations evaluated changes in the skin, fur, eyes, and mucous membranes, as well as the respiratory, circulatory, and behavioral aspects. Tremors, convulsions, salivation, diarrhea, lethargic behavior, and coma were also taken into consideration.

Body weight

Each individual's body weight was recorded at the time of delivery, on the day of randomization, on Day 1 (the first treatment day), before administration, on the following treatment days (every two weeks after that), and on the last treatment day. On the day of the scheduled necropsy, final body weights were recorded. The body weight data was calculated, and the changes in body weight for each animal were reported.

Feed weight

From the first day of treatment until the sacrifice, food intake was measured twice weekly (on the days that body weights were taken). Food input and waste were assessed twice a week.

Necropsy and Gross pathology

All of the animals were sacrificed under isoflurane on Day 18 of the treatment period, and they were then all subjected to a gross pathological investigation.

Organ collection, Weighing and Preservation

The uterus was removed during necropsies and stored in 10% neutral buffered formalin.

Gross pathological evaluation

All study animals underwent necropsy and gross pathology, which were both performed on them. The animals were exsanguinated, weighed, and slaughtered at the point of death while being sedated with isoflurane, and the gross pathological findings were recorded. The uterus was collected and weighed. All the pups were removed and separated. The number of corpora lutea, the size of the pups, and the total number of implantation sites were determined. The uterus that was collected was kept in a buffer solution with 10% neutral formalin.

Statistical analysis

All data, including body weight, feed intake, pup weights, corpora lutea count, and uterus weights, were statistically evaluated using Graph Pad Prism software, version 5.01. All values were given as mean ± SD. A one-way ANOVA combined with Dennett's test was used to determine the significant difference between the treatment and control groups. Separate tables were created to list each result of the statistical analysis. In

each case, P<0.05 was used to determine whether the data were statistically significant.

RESULTS AND DISCUSSION

Mortality

The vehicle control and iCoffee+ treatment groups (1.55, 3.1, and 6.2 g/kg b.w.) showed no signs of mortality or morbidity over the course of the experiment. None of the treatment groups, including the vehicle control, experienced any clinical symptoms associated with the treatments (Table 1).

Table 1: Effect ofiCoffee+ on Mortality and Morbidity Data.

Mortality and Morbidity Data																		
Groups	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**D15**	**D16**	**D17**	**D18**
Group I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*D Indicates Day, 0 Indicates No mortality and +1 indicates Moribund																		

Clinical signs

All of the animals in the vehicle control and iCoffee+ treatment groups (1.55, 3.1, and 6.2 g/kg b.w.) were observed to be normal throughout the experiment (Table 2).

Table 2: Effect ofiCoffee+ on Clinical symptoms of female dams.

Clinical observations of dams																		
Groups	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**D15**	**D16**	**D17**	**D18**
Group I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GroupIV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*D Indicates Day, 0 Indicates No mortality and +1 indicates Moribund																		

Body weight and Body weight changes

Throughout the entire study period, it was noticed that the body weight and body weight variations of the female treated groups at all dose levels (1.55, 3.1, and 6.2 g/kg b.w.) were equivalent to vehicle control (Table 3 and Figure 1).

Table 3: Effect ofiCoffee+ on female gestation body weights.

Mean body weight of females in gestation						
Groups	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 15**	**Day 18**
Group I	198.70±8.59	209.19±8.66	219.55±8.28	231.49±8.47	246.61±8.47	262.76±8.53
Group II	199.61±6.88	210.07±6.95	219.66±6.98	231.70±7.04	247.66±7.03	264.16±7.27
Group III	199.71±6.83	209.72±7.02	220.45±7.06	231.94±7.97	247.26±7.84	263.59±7.80
Group IV	199.28±8.70	209.22±8.06	219.63±6.91	231.38±8.04	246.63±7.96	262.74±7.54

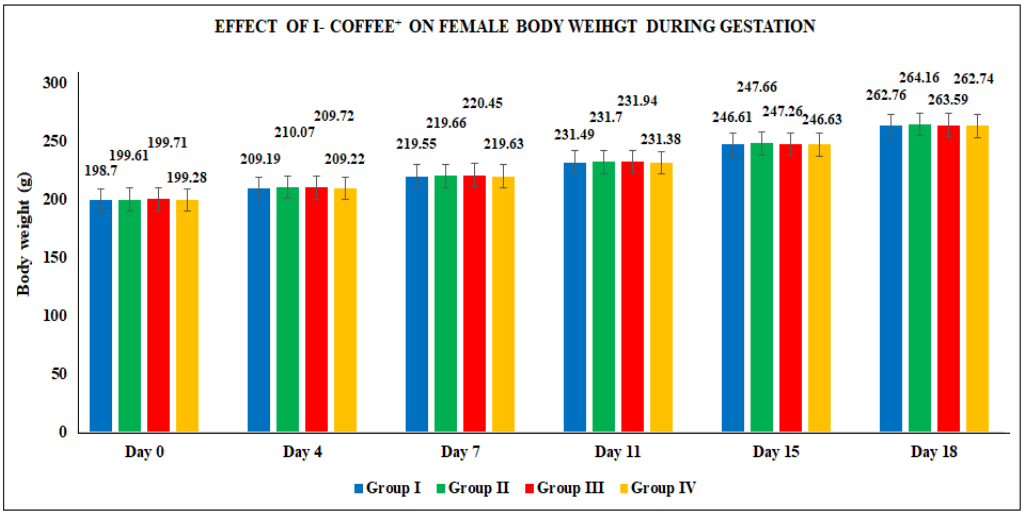

Fig. 1: Effect of iCoffee+ on female body weight during gestation.

Feed consumption

Throughout the entire gestation, it was found that the food consumption (g/rat/day) data of the female treatment (gestation) groups were comparable to those of the vehicle control group. According to the comparison to the control group, there was no evidence of reduced feed consumption during the gestation period in the treatment groups (Table 4 and Figure 2).

Table 4: Effect ofiCoffee+ on feed Intake of Females during gestation period.

Mean feed consumption values females					
Groups	**Day 0-4**	**Day 4-7**	**Day 7-11**	**Day 11-15**	**Day 15-18**
Group I	18.45±0.83	20.50±0.80	20.55±0.89	20.40±0.83	21.75±1.62
Group II	18.25±0.87	20.70±1.01	20.55±1.20	20.30±1.15	21.50±1.65
Group III	18.45±0.98	19.60±1.10	20.35±1.03	20.30±0.91	21.95±1.36
Group IV	18.35±0.89	20.20±1.32	20.20±0.90	20.20±0.63	21.60±1.15

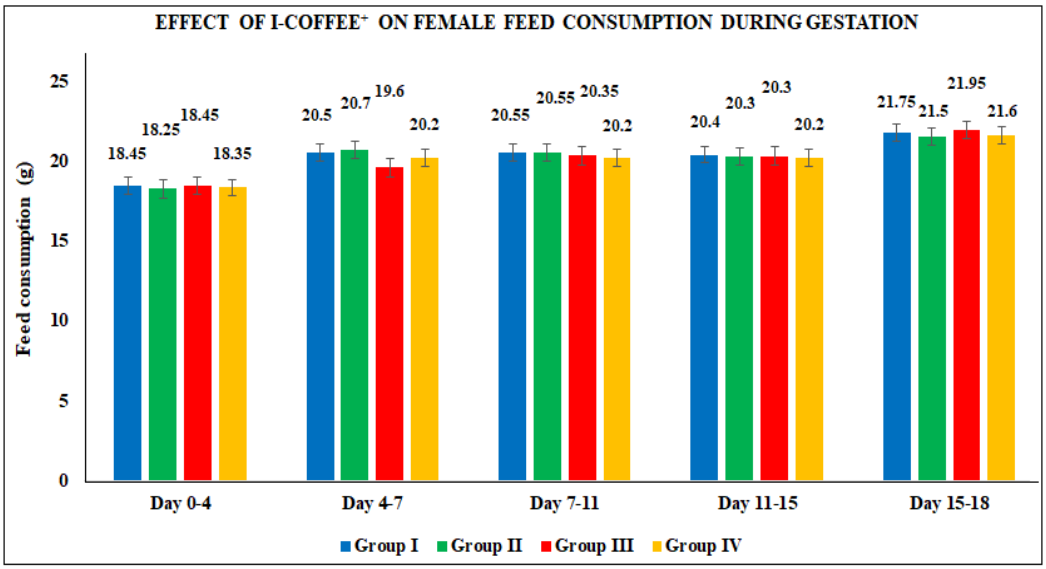

Fig. 2: Effect of iCoffee+ on female feed consumption during gestation.

Gross pathology examination

The female dams were slaughtered one day before the anticipated delivery day at the conclusion of the experiment. The period of necropsy showed macroscopically visible dams as well as structural and pathological alterations. The investigations of dams' uteruses were observed (Table 5-8).

Table 5: Effect of gross pathological analysis of iCoffee+ on Fetus or Embryo (Group I: Normal Control).

Maternal data								
Group	**Dam No.**	**Pregnancy status**	**Implantations**	**Live fetuses**	**Dead fetuses**	**Early resorptions**	**Late resorptions**	**No. of Corpora lutea**
G I	RA 01	P	10	10	0	0	0	12
	RA 02	P	12	12	0	1	1	11
	RA 03	P	10	10	0	0	0	12
	RA 04	P	8	8	0	0	0	10
	RA 05	P	9	9	0	0	0	13
	RA 06	P	11	11	0	0	0	10
	RA 07	P	12	12	0	0	0	9
	RA 08	P	10	10	0	0	0	10
	RA 09	P	9	9	0	0	0	9
	RA 10	P	11	11	0	0	0	10
	RA 11	P	10	10	0	1	1	10
	RA 12	P	12	12	0	0	0	11
	RA 13	P	10	10	0	0	0	10
	RA 14	P	11	11	0	0	0	9
	RA 15	P	9	9	0	1	1	13
	RA 16	P	10	10	0	0	0	12
	RA 17	P	12	12	0	0	0	13

	RA 18	P	10	10	0	0	0	11
	RA 19	P	11	11	0	0	0	13
	RA 20	P	12	12	0	0	0	11
P- Pregnant								

Table 6: Effect of gross pathological analysis of iCoffee+ on Fetus or Embryo (Group II: iCoffee+ Low Dose (1.55 g/kg b.w.)).

Maternal data								
Group	**Dam No.**	**Pregnancy status**	**Implantations**	**Live fetuses**	**Dead fetuses**	**Early resorptions**	**Late resorptions**	**No. of Corpora lutea**
G II	RA 21	P	10	10	0	0	0	12
	RA 22	P	12	12	0	0	0	13
	RA 23	P	9	9	0	1	1	11
	RA 24	P	10	10	0	0	0	10
	RA 25	P	10	10	0	0	0	10
	RA 26	P	9	11	0	0	0	13
	RA 27	P	12	9	0	0	0	14
	RA 28	P	10	12	0	1	1	12
	RA 29	P	9	10	0	0	0	11
	RA 30	P	11	10	0	0	0	13
	RA 31	P	10	11	0	0	0	11
	RA 32	P	11	9	0	0	0	11
	RA 33	P	9	10	0	0	0	12
	RA 34	P	10	10	0	0	0	11
	RA 35	P	9	9	0	1	1	10
	RA 36	P	12	12	0	0	0	12
	RA 37	P	10	10	0	0	0	10
	RA 38	P	11	11	0	0	0	11
	RA 39	P	12	12	0	0	0	12
	RA 40	P	10	10	0	0	0	10
P- Pregnant								

Table 7: Effect of gross pathological analysis of iCoffee+ on Fetus or Embryo (Group III:iCoffee+ Mid Dose (3.1 g/kg b.w.)).

Maternal data								
Group	**Dam No.**	**Pregnancy status**	**Implantations**	**Live fetuses**	**Dead fetuses**	**Early resorptions**	**Late resorptions**	**No. of Corpora lutea**
G III	RA 41	P	12	12	0	0	0	10
	RA 42	P	9	9	0	1	1	13
	RA 43	P	10	10	0	0	0	11
	RA 44	P	12	12	0	0	0	11
	RA 45	P	10	10	0	0	0	12
	RA 46	P	11	11	0	0	0	11
	RA 47	P	10	10	0	0	0	10
	RA 48	P	10	10	0	0	0	12
	RA 49	P	11	11	0	0	0	13
	RA 50	P	9	9	0	0	0	12
	RA 51	P	10	10	0	0	0	10
	RA 52	P	9	9	0	0	0	10
	RA 53	P	10	10	0	0	0	11
	RA 54	P	9	9	0	0	0	11
	RA 55	P	12	12	0	0	0	12
	RA 56	P	11	11	0	0	0	12
	RA 57	P	10	10	0	0	0	11
	RA 58	P	12	12	0	0	0	12
	RA 59	P	11	11	0	0	0	14
	RA 60	P	10	10	0	0	0	12
P- Pregnant								

Table 8: Effect of gross pathological analysis of iCoffee+ on Fetus or Embryo (Group IV: iCoffee+ High Dose (6.2 g/kg b.w.)).

Maternal data								
Group	**Dam No.**	**Pregnancy status**	**Implantations**	**Live fetuses**	**Dead fetuses**	**Early resorptions**	**Late resorptions**	**No. of Corpora lutea**
G IV	RA 61	P	12	12	0	0	0	11
	RA 62	P	10	10	0	0	0	13
	RA 63	P	11	11	0	0	0	10
	RA 64	P	10	10	0	1	1	12
	RA 65	P	11	11	0	0	0	13
	RA 66	P	9	9	0	0	0	10
	RA 67	P	12	12	0	0	0	11
	RA 68	P	10	10	0	0	0	11
	RA 69	P	9	9	0	0	0	14
	RA 70	P	10	10	0	0	0	11
	RA 71	P	10	10	0	0	0	12
	RA 72	P	11	11	0	0	0	11
	RA 73	P	9	9	0	0	0	11
	RA 74	P	10	10	0	0	0	12
	RA 75	P	12	12	0	0	0	12
	RA 76	P	11	11	0	0	0	13
	RA 77	P	9	9	0	0	0	12
	RA 78	P	9	9	0	0	0	11
	RA 79	P	11	11	0	1	1	11
	RA 80	P	10	10	0	0	0	12
P- Pregnant								

Uterine Examination

The uterus was taken out, collected, and weighed immediately following the study's conclusion. The number of corpora lutea has been determined and recorded. The uterine continents were examined, and the number of fetuses was reported (Table 9-10 and Figure 3).

Table 9: Effect of iCoffee+ on Uteri (Groups I and II: Normal Control and Low Dose).

Group	Dam No.	Uterus weight	Observations	Group	Dam No.	Uterus weight	Observations
G I	RA 01	3.6	NAD	**G II**	RA 21	3.6	NAD
	RA 02	3.3	NAD		RA 22	3.3	NAD
	RA 03	3.9	NAD		RA 23	3.4	NAD
	RA 04	3.1	NAD		RA 24	3.2	NAD
	RA 05	3.7	NAD		RA 25	3.9	NAD
	RA 06	3.2	NAD		RA 26	3.9	NAD
	RA 07	3.4	NAD		RA 27	3.2	NAD
	RA 08	3.6	NAD		RA 28	3.5	NAD
	RA 09	3.8	NAD		RA 29	3.3	NAD
	RA 10	3.3	NAD		RA 30	3.4	NAD
	RA 11	3.2	NAD		RA 31	3.5	NAD
	RA 12	3.9	NAD		RA 32	3.8	NAD
	RA 13	3.4	NAD		RA 33	3.4	NAD
	RA 14	3.6	NAD		RA 34	3.6	NAD
	RA 15	3.8	NAD		RA 35	3.2	NAD
	RA 16	3.9	NAD		RA 36	3.3	NAD
	RA 17	3.3	NAD		RA 37	3.6	NAD
	RA 18	3.6	NAD		RA 38	3.8	NAD
	RA 19	3.2	NAD		RA 39	3.4	NAD
	RA 20	3.4	NAD		RA 40	3.8	NAD
***NAD: No observed defects**							

Table 10: Effect of iCoffee+ on Uteri (Groups III and IV: iCoffee+ Mid dose and High Dose).

Group	Dam No.	Uterus weight	Observations	Group	Dam No.	Uterus weight	Observations
G I	RA 41	3.8	NAD	G II	RA 61	3.3	NAD
	RA 42	3.4	NAD		RA 62	3.9	NAD
	RA 43	3.3	NAD		RA 63	3.3	NAD
	RA 44	3.2	NAD		RA 64	3.6	NAD
	RA 45	3.6	NAD		RA 65	3.8	NAD
	RA 46	3.6	NAD		RA 66	3.5	NAD
	RA 47	3.9	NAD		RA 67	3.9	NAD
	RA 48	3.1	NAD		RA 68	3.5	NAD
	RA 49	3.3	NAD		RA 69	3.6	NAD
	RA 50	3.9	NAD		RA 70	3.4	NAD
	RA 51	3.8	NAD		RA 71	3.2	NAD
	RA 52	3.2	NAD		RA 72	3.3	NAD
	RA 53	3.3	NAD		RA 73	3.7	NAD
	RA 54	3.6	NAD		RA 74	3.9	NAD
	RA 55	3.8	NAD		RA 75	3.4	NAD
	RA 56	3.9	NAD		RA 76	3.2	NAD
	RA 57	3.7	NAD		RA 77	3.8	NAD
	RA 58	3.6	NAD		RA 78	3.7	NAD
	RA 59	3.7	NAD		RA 79	3.3	NAD
	RA 60	3.9	NAD		RA 80	3.4	NAD
*NAD: No observed defects							

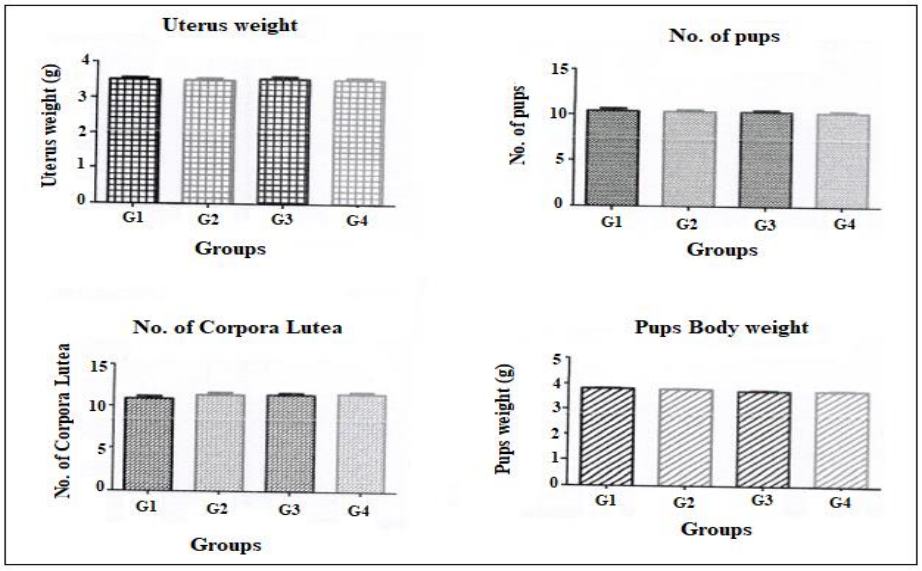

Fig. 3: Effect of iCoffee+ on the Uterus, Fetus, or Embryo.

Examination of Fetus

The female dams were immediately sedated to death using inhalation anesthesia, and all the dams underwent caesarean sections, with the puppies being taken and separated from the dam. The body weights of all the puppies were measured and recorded. All of the pups from each dam were examined for skeletal and soft tissue changes after the body weight measurements. Malformations and changes to the soft tissue were examined in each pup separately. The pups were sliced in half, the skin and internal organs were removed, and skeletal and soft tissue assessments were taken (Table 11-14 and Figure 4).

Table 11: Effect of iCoffee+ on fetus growth.

Groups	Pregnant rats	Total fetus	Fetuses observed	Remarks
Group I	20	209	100	NAD
Group II	20	208	112	NAD
Group III	20	208	102	NAD
Group IV	20	206	103	NAD

Table 12: Effect of iCoffee+ on Fetus Body Weight.

Fetus Mean Body Weight Record						
Groups	Number of female (dam) rats	Number of fetus	No. of pups		Body Weight (g)	
			Mean	SD	Mean	SD
Group I	20	209	10.00	1.19	3.80	0.07
Group II	20	208	10.00	1.05	3.80	0.08
Group III	20	208	10.00	1.05	3.80	0.09
Group IV	20	206	10.00	1.03	3.80	0.08

Table 13: Effect of iCoffee+ on Fetus Skeletal growth.

Groups	Pregnant rats	Total fetus	Fetuses observed	Malformation Occurrence (%)			
				Cervical	Thorax	Lumbar	Sacrocaudal
Group I	20	209	100	0	0	0	0
Group II	20	208	112	0	0	0	0
Group III	20	208	102	0	0	0	0
Group IV	20	206	103	0	0	0	0

Table 14: Effect of iCoffee+ on fetal soft tissue alternations.

	Percentage of fetuses with malformed organ (%)							
Groups	Pregnant rats	Total fetus	Brain	Eyes	Heart	Liver	Kidney	Testis/Ovaries
Group I	20	209	0	0	0	0	0	0
Group II	20	208	0	0	0	0	0	0
Group III	20	208	0	0	0	0	0	0
Group IV	20	206	0	0	0	0	0	0

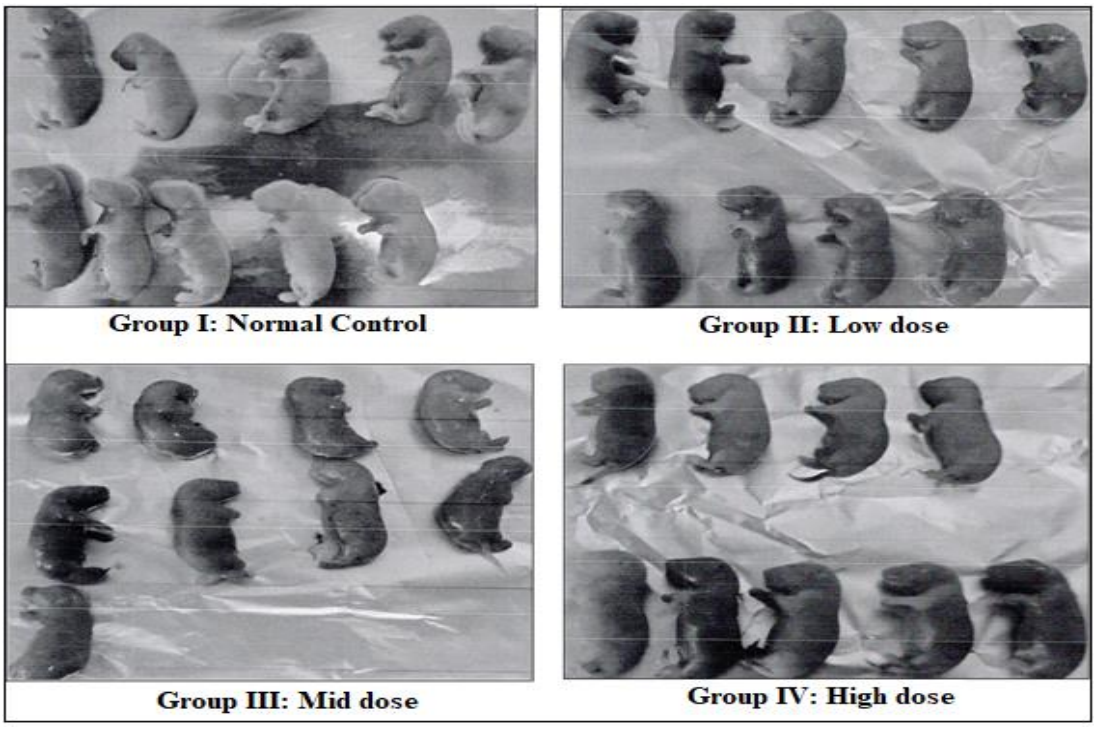

Fig. 4: Visual representations of fetus.

Fetal development is a highly structured process in which several complicated changes are coordinated sequentially in time and adjustments at the cellular and molecular levels are integrated to allow the expression of a specific phenotype in the entire organism. Since numerous products derived from herbal plants that claim to have pharmacological effects are becoming more and more popular in the global health market without information on their toxicological profile, it is essential to evaluate the embryotoxic and teratogenic toxicity of therapeutic plants on the development of the fetus. Despite the information on their toxicity, a number of plant items that are said to have therapeutic value are finding popularity in the worldwide health sector.[25] However, a number of the phytochemical components found in therapeutic plants have the potential to induce teratogenic effects. The majority of these are often employed in daily life.[26] The embryo or fetus can be

affected by toxic chemicals directly or indirectly. The developing embryonic or fetal tissue is immediately harmed when hazardous chemicals pass through the placental membrane. Embryos and fetuses can be indirectly hampered in their development by toxins that damage the placental tissue and impede placental function.[27,28]

Given that coffee use is rising globally, there is an increasing interest in elucidating the effects of coffee toxicity on fetal development. Research is necessary to increase our understanding of coffee's effects on fetal development because we currently have inadequate knowledge in this area. Additionally, there has not been a published assessment of the teratogenic potential of any herbal formulations. Therefore, the purpose of the current investigation was to examine the teratogenic potential of the herbal formulation iCoffee+ in rats. The development of the skeletal system and soft tissue in rat embryos and fetuses was used as the basis for evaluating the teratogenic potential of iCoffee+. No animals perished during the course of the study. When compared to the mean body weights of the control group, there is no appreciable drop in the gestation mean body weights of high dose during the gestation period. Throughout the whole course of treatment, both before and after, there were no clinical symptoms observed. When compared to the normal control group during the gestation period, there is no difference in feed consumption in any of the treatment groups.

The weight of the uterus was determined to be similar to that of the vehicle control group without any discernible change. In the development of pups and their skeletal growth, there were not any abnormalities to be detected. When compared to the vehicle control group, there were no differences in the soft tissue of any of the treated groups.

CONCLUSION

Information and trustworthy data on the safety of nutraceutical products during pregnancy are sparse and frequently inconsistent. This is why it is important to carry out research like the one in the present work, which provides a summary of the literature that is currently available. In this context, it is essential to continue the development of studies on the efficacy, effectiveness, and safety of natural products and herbal medications in order to ascertain their appropriate use. Therefore, it is essential today to evaluate the herbal formulation's teratogenic potential. No mortality or undesirable clinical symptoms were observed in the iCoffee+ at treated dose levels of 1.55, 3.1, and 6.2 g/kg b.w./day. iCoffee+ did not exhibit any treatment-related impacts on behavioral observations, body weight, body weight changes, or feed consumption at the treated dose levels. The uterus, fetus, and pup body weight did not undergo any treatment-related alterations. None of the treatment groups' fetuses showed any skeletal abnormalities. None of the therapy groups showed any signs of soft tissue alterations. As a result, iCoffee+ was determined to be safe during pregnancy.

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