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Original Article

Safety Profile of a Polyherbal Formulation (*Gynocare* capsules) in Female Rats by Subchronic Oral Toxicity Study

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ABSTRACT

Gynocare capsules, is a polyherbal formulation, are used as uterine tonic and for treating gynaecological ailments like infertility, leucorrhea, and menstrual disorders. The formulation contains ingredients of herbal origin, such as, extracts of *Ashoka, Vasaka, Durva, Chandan, Musk*, and so on. It was evaluated for its safety at the therapeutic dose level by a repeated dose oral toxicity study in albino Wistar rats. The herbal formulation was administered orally at a therapeutic dose of 100 mg/kg/day, for 90 days. All animals were monitored daily for their health status and signs of abnormalities. The body weight, water consumption, and food intake were measured once weekly. At the end of the experimental period, various hematological and biochemical parameters were estimated and histopathologies of selected organs were conducted. The study resulted from the long-term oral administration of *Gynocare* capsules (100 mg/kg), did not cause any relevant signs of toxicity nor significant changes in the physical, hematological and biochemical parameters. However, statistically significant differences were seen in the relative organ weights of adrenal gland, ovary, and serum creatinine levels. The reduction in ovary weight revealed the possibility of the drug targeting the ovary. Moreover, no pathological features were identified in the treated group as monitored by the histopathological analysis of the internal organs. The study established that *Gynocare* capsules at the dose given (100 mg/kg) did not induce any remarkable or significant toxic effects, indicating that it was safe in rats following oral administration for 90 consecutive days.

Key words: Polyherbal formulation, repeated dose oral toxicity, sub-chronic oral toxicity

INTRODUCTION

Herbal formulations have attained wide recognition in comparison to crude plant materials and extracts, due to reduction in dose, convenience, and ease of administration. These formulations are popular worldwide as therapeutic agents, in various ailments that impact the quality of life. In developing countries, most of the population rely on traditional medicines, considering their affordability, traditional background knowledge on medicinal plants, and a belief that they are harmless.[1] Many synthetic drugs are known to act on a single molecular target and provide symptomatic relief. The multitarget responses of herbal drugs are proven to be beneficial in chronic conditions such as diabetes, cancer, and so forth, and also in restoring the health status.[2] Although many natural plant extracts used traditionally have passed the test of time, in terms of toxicity and adverse effects, the safety of the active phytochemicals from these plants must precede their pharmaceutical use. There is a need to assure the safety of herbal formulations in order to acquire their maximum benefits even though

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these have been proven to be efficacious in pharmacological studies or by clinical evaluation.[3] Toxicity studies are considered necessary, especially on drugs that are to be used in chronic conditions.

Gynocare capsule, a polyherbal formulation, contains crude extracts of *Ashoka, Vasaka, Durva, Chandan, Musk, Lodhra, Anantmul, Gokhru, Punarnava, Nagkeshar, Shalmali*, *Rubia cordifolia,* and *A. muricatum*. These plants have been used in traditional medicine and act as a uterine tonic for treating many gynaecological ailments. The safety studies of *Gynocare* capsules have not been established so far. In the present study, the safety profile of the *Gynocare* capsule has been investigated at the therapeutic dose level by a sub-chronic oral toxicity study in female Wistar albino rats, in order to optimize its safe use. The experiment was conducted as recommended by the *Organization for Economic Cooperation and Development* (OECD) guideline 408.[4]

MATERIALS AND METHODS

Drugs and biochemistry kits

Gynocare capsules were supplied by the manufacturer. Clinical chemistry kits for serum biochemistry parameters, namely, alkaline phosphatase (ALP), alanine amino-tranferase (ALT), asparatae amino-tranferase (AST), total protein, albumin, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, and triglycerides were purchased from Span Diagnostics, Mumbai, India.

Animals and housing

Female Albino Wistar rats were procured from the Haffkine's Research Centre, Mumbai, and housed in the animal house of C.U. Shah College of Pharmacy, SNDT Women's University, Mumbai, India. The animals were conditioned to room temperature and natural photoperiods. Each group of rats was separately housed in standard cages and had free access to water and standard pellet diet. The study protocol was approved by the Institutional Animal Ethical Committee of the C.U. Shah College of Pharmacy (CUSCP/IAEC/10/09-10).

Selection of dose

The human clinical dose of *Gynocare* capsule was three capsules/day. The rat therapeutic dose of *Gynocare* capsules was selected as 100 mg/kg by calculating from the human clinical dose (1050 mg/day/70 kg). It was calculated based on the total body surface area of the rat, using 0.018 as the conversion factor 0.018.[5] The animals were divided into two groups, each having five animals, while the control group received 0.05% suspension of CMC and the second group received 100 mg/kg/day of *Gynocare* capsules. The drug suspended in 0.05% CMC and the vehicle 0.05% CMC were administered in a volume of 5 ml/kg.

Repeated dose 90 days oral toxicity

For the 13-week, sub-chronic toxicity study, five female albino Wistar rats were randomly selected in each group. The control animals received 0.05% CMC and the test animals received contents of the *Gynocare* capsules suspended in 0.05% CMC administered orally for 90 days at the calculated therapeutic dose of 100 mg/kg/day. All animals were monitored daily for their health status and signs of any abnormalities. The animals were weighed once weekly. The water consumption and food intake of all the rats were measured once every week.

At the end of the experimental period, the rats were fasted overnight and blood samples were withdrawn from the retro-orbital plexus. Blood and serum samples were used for various hematological and biochemical estimations. The haematological parameters were analyzed by a fully automated blood cell counter, on ERMA PCE-210. The serum was separated from the blood by centrifugation and stored at –20°C for analysis. The biochemical parameters on the serum samples were analyzed using a semi automatic clinical chemistry analyzer, AGD 400. A complete necropsy, including selected organ weights, was conducted on all rats. Histopathology was performed on selected organs, which were preserved in 10% formalin solution. Tissues were processed, sectioned, and stained, with hematoxylin and eosin, and were examined by a pathologist.[6-9]

Statistical analysis

All statistical analyses were made using the software InStat for windows. All results were expressed as mean±SEM. Student's *t*-test was applied to determine statistical significance. The values were considered statistically significant when $P<0.05$.

RESULTS

Effects of *Gynocare* capsules on body weight, food intake, and water consumption

Body weight, food intake, and water consumption of all the animals were measured every week throughout the study [Table 1]. The difference in the body weights of the rats treated with the therapeutic dose of capsules and control rats was not significant. Overall, the changes in body weight among the groups were statistically not significant at $P<0.05$. The food intake and water consumption of the female rats treated with *Gynocare* capsules was found to be identical to that of the control and the differences if any, were statistically insignificant.

Effects of *Gynocare* capsules on the gross morphology

At the end of the observation period all the rats were sacrificed. All major organs including heart, brain, lung,

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Table 1: Effect of *Gynocare* capsules on body weight, food, and water intake on rats treated for 90 days

Study week	Body weight (g)		Food intake (g)		Water intake (ml)	
	Control	*Gynocare*[a]	Control	*Gynocare*[a]	Control	*Gynocare*[a]
1	248.60 ± 12.98	248.20 ± 11.34	13.1 ± 0.3	13.1 ± 0.6	30.2 ± 0.7	30.4 ± 0.4
2	247.40 ± 10.89	243.00 ± 9.78	13.1 ± 0.3	12.7 ± 0.6	29.3 ± 0.7	30.2 ± 0.5
3	250.60 ± 11.35	248.40 ± 7.90	13.4 ± 0.5	12.6 ± 0.5	29.4 ± 0.7	28.6 ± 0.4
4	250.20 ± 11.11	246.40 ± 7.06	13.6 ± 0.4	12.5 ± 0.6	30.0 ± 0.8	29.2 ± 0.8
5	248.80 ± 10.74	241.60 ± 6.36	12.7 ± 0.6	10.9 ± 1.1	32.0 ± 1.2	29.6 ± 1.6
6	253.00 ± 10.73	245.00 ± 6.41	14.2 ± 1.0	13.5 ± 1.0	27.4 ± 2.0	26.4 ± 1.6
7	252.20 ± 12.36	244.20 ± 5.89	14.5 ± 1.0	15.4 ± 1.4	29.4 ± 1.2	29.8 ± 1.6
8	252.40 ± 11.15	242.80 ± 6.13	15.1 ± 0.5	13.5 ± 1.5	28.2 ± 1.1	25.6 ± 0.9
9	254.00 ± 12.42	245.60 ± 6.42	14.3 ± 0.5	13.3 ± 1.2	29.2 ± 1.0	26.8 ± 0.9
10	255.00 ± 12.69	244.60 ± 6.98	14.1 ± 0.8	12.4 ± 0.8	29.2 ± 0.4	27.4 ± 0.7
11	258.00 ± 13.54	250.40 ± 7.95	13.2 ± 1.3	13.2 ± 0.6	28.6 ± 1.3	29.6 ± 0.7
12	256.20 ± 13.47	248.80 ± 8.40	13.4 ± 1.2	13.2 ± 0.9	27.8 ± 1.2	29.8 ± 0.2
13	253.00 ± 13.85	247.00 ± 6.13	13.2 ± 0.9	12.7 ± 0.7	28.2 ± 1.0	27.8 ± 1.2

Values are expressed as mean ± SEM, *n*=5. [a]Group was administered orally with 100 mg/kg *Gynocare* capsules daily for 90 days

kidney, liver, spleen, ovary, thymus, and pancreas were examined grossly. There were no detectable abnormalities in the treated groups, which indicated that there were no remarkable gross differences among the treatment group when compared with the control.

Effects of *Gynocare* capsules on relative organ weight

In the relative organ weights, no statistically significant changes were seen in the treatment group when compared with the control [Table 2]. However, the relative ovary weight was significantly ($P<0.05$) reduced in the group treated with *Gynocare* capsules as compared to the control group. Similarly the relative adrenal gland weight significantly reduced ($P<0.05$) in the treatment group. Treatment with capsules resulted in atrophy of the adrenal gland and ovary. These internal organs did not exhibit any gross morphological lesions or histopathological features. Therefore, the results obtained suggest that *Gynocare* capsules are safe and nontoxic.

Table 2: Effect of *Gynocare* capsules on relative organ weights in rats treated for 90 days

Organ	Relative organ weight (kg)	
	Control	*Gynocare*[a]
Liver	25.19 ± 1.43	23.83 ± 0.56
Kidney	6.37 ± 0.37	5.96 ± 0.28
Brain	6.24 ± 0.59	6.31 ± 0.24
Ovary	1.09 ± 0.18	0.43 ± 0.07*
Uterus	0.33 ± 0.05	0.17 ± 0.05
Heart	4.23 ± 0.44	4.75 ± 0.33
Lung	7.90 ± 0.86	7.94 ± 0.70
Adrenal glands	0.72 ± 0.12	0.44 ± 0.04*
Spleen	3.25 ± 0.29	3.52 ± 0.36

*Significantly different from Control, $P<0.05$. Values are expressed as mean ± SEM, *n*=5. [a]Group was administered orally with 100 mg/kg *Gynocare* capsules daily for 90 days

Effects of *Gynocare* capsules on hematological parameters

The data show that there was no statistically significant difference in levels of RBCs, WBCs, platelets, lymphocytes, monocytes, granulocytes and haemoglobin levels of control groups and groups treated with *Gynocare* capsules during the period of study [Table 3]. The formulation was considered safe at the studied dose level of 100 mg/kg.

Table 3: Effect of *Gynocare* capsules on the hematological parameters in rats treated for 90 days

Parameter	Control	*Gynocare*[a]
RBC (10×12/L)	8.01 ± 0.40	8.35 ± 0.35
WBC (10×9/L)	9.32 ± 1.48	11.90 ± 0.99
PLT (10×9/L)	1043.6 ± 146.61	1177.8 ± 264.39
LYM %	74.3 ± 9.5	81.6 ± 6.7
MON %	0.71 ± 0.14	1.86 ± 0.63
GRA %	24.6 ± 9.6	16.5 ± 7.2
HGB (g/dl)	13.62 ± 0.66	13.52 ± 0.13
HCT %	53.34 ± 2.70	52.66 ± 1.43
MCV (fl)	67.0 ± 3.7	63.2 ± 1.6
Clotting time (seconds)	84.0 ± 11.2	72.0 ± 7.3

Values are expressed as mean ± SEM, *n*=5. [a]Group was administered orally with 100 mg/kg *Gynocare* capsules daily for 90 days

Effects of *Gynocare* capsules on the biochemical parameters

The repeated oral dose treatment for 90 days did not cause significant changes in hepatic functional transaminases ALT, AST and ALP levels and other biochemical parameters like albumin, glucose, cholesterol and triglyceride levels among the control and treated groups [Table 4]. However, a statistically significant ($P<0.05$) decline in serum creatinine

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Table 4: Effect of *Gynocare* capsules on the biochemical parameters in rats treated for 90 days

Parameter	Control	*Gynocare*[a]
SGPT/ALT (IU/L)	49.9 ± 5.7	45.6 ± 3.3
SGOT/AST (IU/L)	102.56 ± 6.10	101.80 ± 8.34
ALP (IU/L)	49.6 ± 10.9	34.6 ± 5.2
Total protein (g/dl)	9.9 ± 0.5	9.7 ± 0.9
Albumin (g/dl)	6.0 ± 0.2	5.8 ± 0.5
BUN (mg/dl)	14.1 ± 0.9	14.8 ± 2.0
Creatinine (mg/dl)	0.47 ± 0.02	0.37 ± 0.02*
Glucose (mg/dl)	53.94 ± 7.31	55.37 ± 7.64
Cholesterol (mg/dl)	82.86 ± 6.61	77.23 ± 6.71
Triglycerides (mg/dl)	68.25 ± 10.47	75.83 ± 10.14

Values are expressed as mean ± SEM, n=5. [a]Group was administered orally with 100 mg/kg of *Gynocare* capsules daily for 90 days. *Significantly different from Control, P<0.05

level was observed in animals treated with *Gynocare* capsules (100 mg/kg). The renal function was evaluated mainly by urea and creatinine serum levels. Nevertheless, these changes in serum creatinine were considered to be minimal, and were statistically identified, as the reference interval range of creatinine was 0.2 – 0.8 mg/dl.[10] The level of creatinine for both control (0.47±0.02 mg/dl) and treated groups (0.37±0.02 mg/dl) lay within the reference interval range and the changes were considered to be of little significance. Moreover, a decline in the serum creatinine levels was not accompanied by a decrease in blood urea nitrogen levels and there were no gross or histopathological changes in the kidney related to the treatment. The results were hence considered to be normal.

Effects of *Gynocare* capsules on the histopathology of the internal organs

The histopathological findings of the vital and reproductive organs of the control and treated groups are presented in Figures 1 and 2. Histopathological examination did reveal some non-significant histopathological lesions in a few internal organs of the female rats in the control group as well as in rats treated with *Gynocare* capsules (100 mg/kg). These pathological findings might have been spontaneous, functional, or incidental in Wistar rats. Overall, the histopathological observations were nonsignificant and not related to the test substance administered.

DISCUSSION

Medicinal plants are increasingly sought by patients as a source of prescription drugs, in the form of active principles, in developed and developing countries. They have been proven with undeniable and tangible therapeutic benefits, with limited toxicity, because of its long-term use as folklore medicines. The World Health Organization (WHO) insists that the safety of herbal medicines is a critical component in the quality control of healthcare products. The absolute safety of herbal formulations cannot be established from traditional knowledge for approval at the present time, even though the side effects are minimal.[3]

Numerous herbal preparations have been shown to benefit in treating various gynaecological aliments through various mechanisms, but the toxicity-related data for many of these has not been established. *Gynocare* capsules contain many ingredients of herbal origin, which have been used traditionally as uterine tonics in treating many gynaecological disorders like infertility, leucorrhea, and menstrual disorders like menorrhogia and dysmenorrhoea. Herbal formulations are multicomponent in nature, and provide synergistic effects and there is an increasing demand for these medicines among the public, for chronic use.

In the present study, the results obtained from the safety profile of orally administered *Gynocare* capsules, at a therapeutic dose of 100 mg/kg were elucidated. The treated groups were compared with the control group for body weight gain, food intake, water consumption, and relative organ weight, as also the morphological, hematological, biochemical, and histopathological parameters. No mortality or abnormal behavior was seen in the animals treated with *Gynocare* capsules, at a dose of 100 mg/kg. The formulation did not have a significant impact on the body weight, food intake and water consumption, which signified that treatment with *Gynocare* capsules did not affect the normal health status of the animals. The differences in the relative organ weights of ovaries and adrenal glands of the treatment and control groups were statistically significant. This may indicate the possibility of *Gynocare* in targeting the ovary. *Gynocare* treatment resulted in atrophy of the adrenal gland and ovary, however, these internal organs did not exhibit any gross morphological lesions or histopathological features.

The haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal.[11] The treatment with *Gynocare* capsules did not have significant impact on the haematological study. The levels of glucose, cholesterol, and triglyceride were unaffected, which indicated that the formulation did not interfere with the carbohydrate and lipid metabolism in rats.[12] Treatment with *Gynocare* capsules in rats did not alter the hepatic and renal function, as identified from the hepatic enzyme AST and ALT levels and renal serum biomarkers of creatinine. It further confirmed the normal functioning of hapatocytes and nephrons during treatment period. Despite some minor alterations, the histopathological studies of vital and reproductive organs further confirmed the safety of the formulation at the administered therapeutic dose level in rats. Based on the experimental result, no observed adverse effect level (NOAEL) of *Gynocare* capsules was greater than 100 mg/kg.

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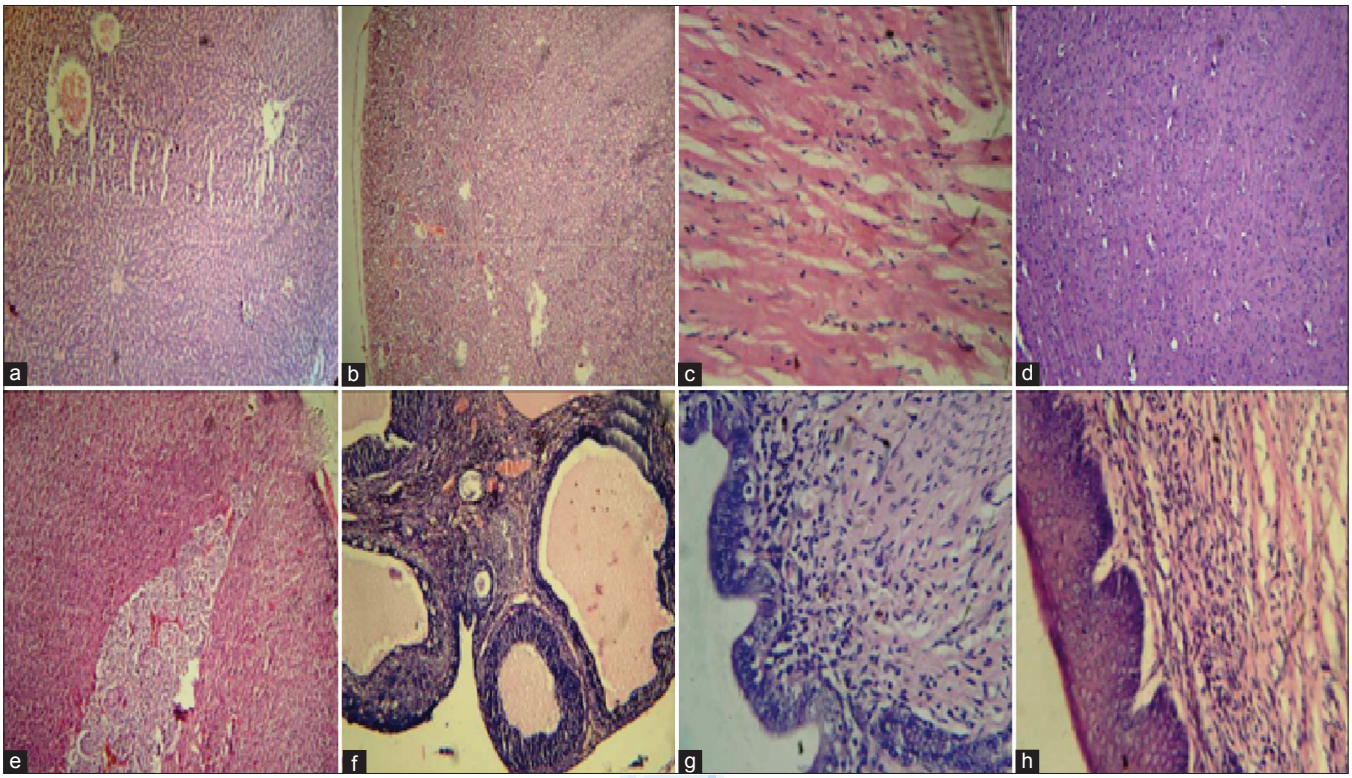


Figure 1: Representative microscopic findings of the female rats treated orally with 0.05% suspension of CMC for 90 days. (a) Liver, (b) Kidney, (c) Heart, (d) Brain, (e) Adrenal gland, (f) Ovary, (g) Uterus, and (h) Cervix and vagina (Hematoxylin-Eosin stain, ×400)

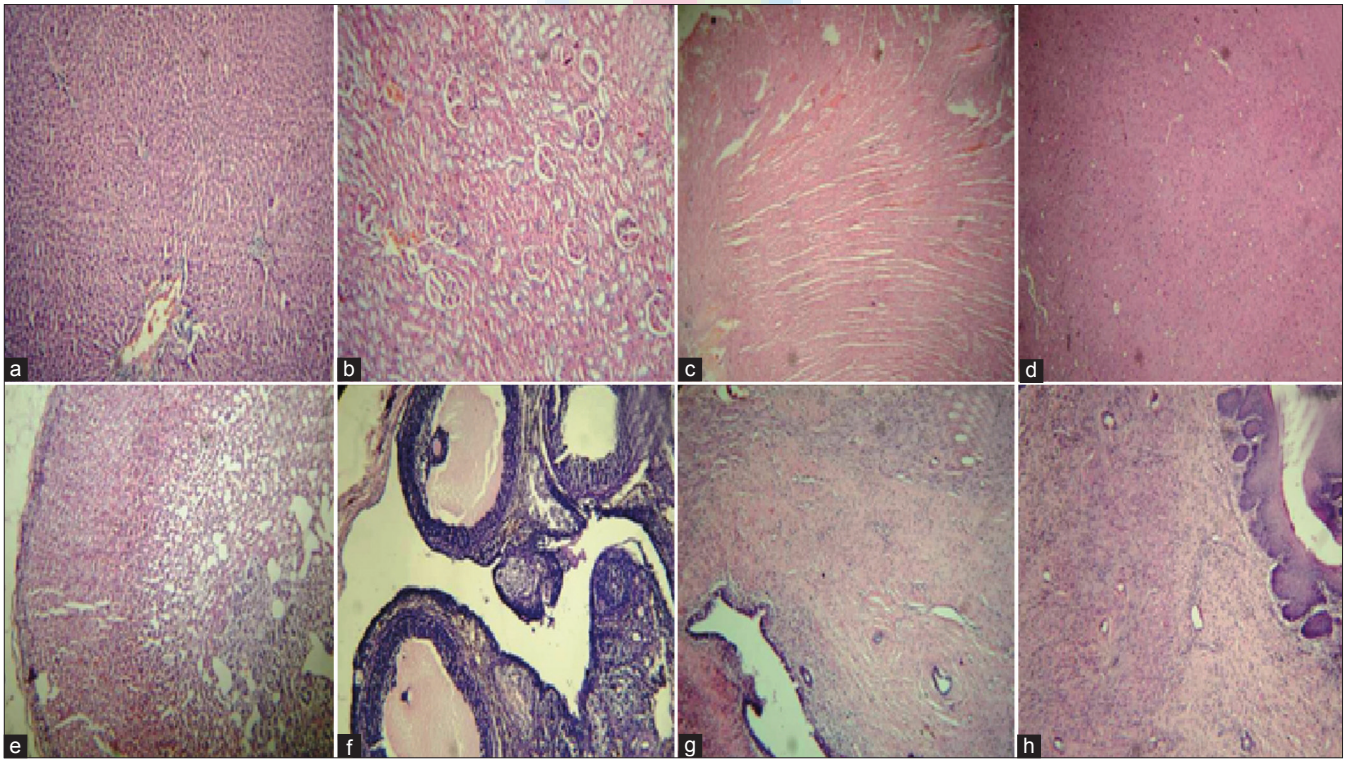


Figure 2: Representative microscopic findings of the female rats treated orally with *Gynocare* capsules (100 mg/kg) for 90 days. (a) Liver, (b) Kidney, (c) Heart, (d) Brain, (e) Adrenal gland, (f) Ovary, (g) Uterus, and (h) Cervix and vagina (Hematoxylin-Eosin stain, ×400)

In conclusion, there was no relevance of serious signs and significant changes in the physical, hematological, biochemical, and histopathological parameters that resulted from the 90-day administration of *Gynocare*

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capsules (100 mg/kg). Moreover, no pathological features were identified in both the control and treated groups, as monitored by the histopathological analysis of the internal organs. Therefore, in the present study, *Gynocare* capsules at the given dose, did not induce any remarkable toxic effects in the female rats treated for 90 consecutive days and it was found to be safe. Further studies at higher dose levels are necessary to ascertain the safety of *Gynocare* capsules.

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