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Research Article

Evaluation of Aphrodisiac Activity of *Agave americana* Leaves in Male Wistar Rats

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ABSTRACT

Agave americana has been known as century plant or American aloe. This plant consists of alkaloids, flavonoids, steroidal saponins, proteins, and saponins, indicating that the plant may have aphrodisiac properties. Although traditionally, it has been used to treat inflammation, aphrodisiac nature is not evaluated. The current study was designed to evaluate the aphrodisiac activity of *A. americana* on male albino wistar rats. The presence of different plant constituents in the plant extract was determined by the preliminary phytochemical screening methods. For the experimental procedure, the female rats were allowed to be ovariectomized for 1 month before the behavioral activity. Hydroalcoholic extracts of *A. americana* at doses of 100, 200, and 400 mg /kg were administrated for 21 days. The female rats involved in mating were made receptive by hormonal treatments. The common matting behaviors, libido, potency, mounting frequency, intromission frequency and orientation activity were studied. Results revealed there was a significant raise in the mounting frequency, intromission frequency, libido activity, erection, quick flip, long flip activities. Additionally, a significant increase in the serum testosterone hormone levels in male rat was found. Current study concludes the hydroalcoholic extract of *A. americana* shows aphrodisiac activity at a dose of 400 mg/kg body weight.

Introduction

"Herbal "plant is valued for the variety of natural compounds it contains, some of which have physiological and pharmacological effects on humans.[1] It is one of the fundamental aspects of modern society. It has significantly contributed to preserving and enhancing human health and quality of life. These plants' chemical components modify the pathophysiological features of disease in the human body. Plant research has increased substantially as a result of plants' enormous potential to treat many different ailments globally.[2]

In spite of extensive study on numerous herbal plants, many plant species remain untapped. *A. americana* was thus considered for the investigation because of its significant therapeutic qualities. It has also been referred to as American aloe or century plant.[3] It has long been recognized that various *Agave americana* parts have been traditionally utilized to treat a wide range of disorders and symptoms. The leaves include elements, including steroidal saponins, isoflavones, and coumarins that have been reported to be beneficial for treating diarrhoea, dysentery, and other gastrointestinal conditions as well as for wound healing.[4-8] Because the leaves of *A. americana* contain antibacterial qualities, they are utilised to treat digestive ailments, mucosal irritation, and plaque bases.[8] The plant is also utilized in Ayurveda to treat sciatica and rheumatoid arthritis,[9] anti-inflammatory[10] and wound healing.[11] Current drug therapy available are usually includes phosphodiesterase type 5 inhibitors, which include sildenafil (Viagra), tadalafil, vardenafil, and avanafil, which are used as aphrodisiac agents but are not devoid of side effects.[12]

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The review of the literature suggests that the plant *A. americana* possesses a variety of significant pharmacological properties. In order to demonstrate its aphrodisiac activity, the hydroalcoholic extract of *A. americana* leaves was assessed in male wistar rats.

Materials And Methods

Collection of Plant Materials

The *A. americana* leaves were collected from Bharat Nursery Field, Valsad district (Gujarat), and were authenticated by a botanist in the month of January 2021.

Procurement and Rearing of Experimental Animals

Healthy wistar strain male albino rats aged about 8 to 12 w and weighing 200–300 g were procured from ROFEL Shri G. M. Bilakhia college of pharmacy. The rats were housed in polypropylene cages and maintained in an environmentally controlled room provided with a 12:12 h light and dark cycle at 25°C. They were fed and given tap water *ad libitum*. The rats were allowed to acclimatise to the laboratory environment for 15 days before the experiments.

All experimental protocols were subjected to approval by the Institutional Animal Ethics Committee [Registration number: CPCSEA 1914/PO/Re/S/16/2016 (IAEC/2021/0005)].

Preparation of the Extract

The leaves were shade-dried, powdered, and stored in an airtight container. The powder was macerated for 24 hours in 50% v/v methanol. The hydroalcoholic extract was prepared by the percolation method using 50% v/v methanol as a solvent. Then it was dried and scraped to obtain the powder form of the extract. Before each experiment, the fresh solution of *A. americana* leaf extract was prepared by dissolving it in distilled water and then giving it orally.[11,13,14]

Phytochemical Screening

The preliminary phytochemical screening methods determined the presence of different plant constituents in the plant extract.[15]

Procedure for Ovariectomized Rats

For the experimental procedure, the female rats were allowed to be ovariectomized for 1 month before the behavioral activity. The female rats were anesthetized with ether. Then the abdominal fur was shaved, and a small incision was made in the abdomen into the peritoneal cavity. Then both ovaries were removed from the abdomen portion. Then, after the removal of the ovaries, the abdominal wall and incision were sutured, and povidone iodine was also applied to the part of the sutured. Then the animals were allowed to recover from the surgery for at least 2 weeks. Then, female rats were injected with 100 µg/animal of ethinylestradiol *via* a subcutaneous route 48 hours before the experiments and 1-mg/animal of progesterone *via* a subcutaneous route 4 hours before the experiments. For the experiments of sexual behavior, each male rat was introduced into the cage, acclimatized for 5 minutes, and then transfer to the cage for behavioral activity. Then the behavioral activity was observed for 30 minutes.[14-16]

Mating Behavior Test

The study used healthy and sexually male albino wistar rats (200–300 g). They were divided into 5 groups, and each groups had 6 animals in separate cages during the experiment. A first group is a control group in which the vehicle was given for 21 days by oral route. A second group is a standard group in which 5 mg/kg of sildenafil citrate was dissolved in distilled water and given to rats for 21 days by oral route. The third group is a low-dose treatment group in which 100 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fourth group is a medium dose of treatment group in which 200 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fifth group is a high-dose treatment group in which 400 mg/kg of hydroalcoholic extract of *A. americana* was given to the rats for 21 days by oral route.

The female rats were artificially brought into the estrus phase and the female rats allowed matting only during the estrus phase. They were administered suspension of ethinylestradiol orally at a dose of 100 µg/animal 48 hours prior to the experiments and progesterone injected subcutaneously at a dose of 1 mg/animal 6 hours before the experiments. The receptive female animal was introduced into the male animal cage with a ratio of 1:1 and parameters of mounting frequency and intromission frequency were observed.

Mount frequency may be defined as the number of times the male rats climb on the receptive female rats from when the female rat is introduced into the cage containing the male rats to the end of the observation periods. Intromission frequency may be defined as the number of times the male rat inserts its penis into the vagina of the female rat, from the time the female rat is introduced into the cage that contains the male rat to the end of the observation period.[15-17]

Libido Test

The study used healthy and sexually male albino wistar rats (200–300 g). They were divided into 5 groups, and each groups had 6 animals in separate cages during the experiment. A first group is a control group in which the vehicle was given for 21 days by oral route. A second group is a standard group in which 5 mg/kg of sildenafil citrate was dissolved in distilled water and given to rats for 21 days by oral route. Third group is a low-dose treatment group in which 100 mg/kg of hydroalcoholic extract of

A. americana was given to rats for 21 days by oral route. The fourth group is a medium dose of treatment group in which 200 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fifth group is a high-dose treatment group in which 400 mg/kg of hydroalcoholic extract of *A. americana* was given to the rats for 21 days by oral route. On the 21st day, penis was exposed by retracting the sheath and 5% xylocaine ointment was applied 5, 15, and 30 min before starting observations. The animals were placed in individual cages, receptive females were placed in the same cages, and parameters like mount frequency and intromission frequency were observed.[16,17]

Potency Test

The study used healthy and sexually male albino wistar rats (200–300 g). They were divided into 5 groups; each group had 6 animals in separate cages during the experiment. A first group is a control group in which the vehicle was given for 21 days by oral route. A second group is a standard group in which 5 mg/kg of sildenafil citrate was dissolved in distilled water and given to rats for 21 days by oral route. The third group is a low-dose treatment group in which 100 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fourth group is a medium dose of treatment group in which 200 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fifth group is a high-dose of treatment group in which 400 mg/kg of hydroalcoholic extract of *A. americana* was given to the rats for 21 days by oral route. On the 21st, the test for penile reflexes was carried out by placing the rat on its back in a glass cylinder for partial restraint. The preputial sheath was pushed behind the glans by means of thumb and index finger and held in this manner for a period of 15 min, and parameters like quick flip, long flip, and erection were observed.[18]

The quick flip may be defined as rapid movements of the penis towards the abdominal body wall with a rapid return to a resting state. A long flip may be defined as a more gradual and continuous flexion (bend) of the penis. The long flips are not, or only rarely, accompanied by pelvic ventroflexion in intact animals.

Orientation Activity Test

The study used healthy and sexually male albino wistar rats (200–300 g). They were divided into 5 groups; each group had 6 animals in separate cages during the experiment. The first group is a control group, in which the vehicle was given orally for 21 d. The second group is a standard group in which 5 mg/kg of sildenafil citrate was dissolved in distilled water and given to rats for 21 days by oral route. The third group is a low-dose treatment group in which 100 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fourth group is a medium dose of treatment group in which 200 mg/kg of hydroalcoholic extract of *A. americana* was given to rats for 21 days by oral route. The fifth group is a high-dose treatment group in which 400 mg/kg of hydroalcoholic extract of *A. americana* was given to the rats for 21 days by oral route.

The following scoring technique was used to determine male rats orientation behavior: A preference for females (licking), a self-oriented orientation (genital grooming), and an environmental orientation (Exploration, Rearing, and Climbing). After 21 days of treatment, the cumulative score for each action in the half-hour was calculated.[18]

Sexual and Vital Organ Weight

After the completion of an experiment, all the experimental groups of animals were evaluated for body weight. The animals were anesthetized with ether and all the experimental animals were sacrificed. The organs like testis, seminal vesicles, epididymis, and vas-deference were removed and they were weighed using an electronic balance.[19]

Serum Testosterone Hormone Levels

Serum levels of testosterone ng/l were determined using an ELISA kit. The blood samples were collected in Eppendorf tubes at the end of the study. The samples could coagulate at room temperature for 90 minutes, followed by centrifugation at 2000 rpm for a period of 20 minutes in a cooling centrifuge maintained at 2~4°C. The procedure was repeated for another 10 minutes if the separation was unsatisfactory. The obtained supernatant solution was transferred to fresh sterilised Eppendorf tubes and kept at 20°C until used.[19]

Statistical Analysis

All continuous data was analysed using two-way ANOVA using graph pad prism followed by Tukey's test and a value of $p < 0.05$ was considered as significant.

Results

Phytochemical Screening

In preliminary phytochemical screening, the presence of chemical constituents like alkaloids, flavonoids, steroidal saponins, proteins, and saponins were detected Table. 1.

Effect of the Extract on Mating Behavior

The administration of *A. americana* leaf extract for 21 days to male rats resulted in an intensification of sexual activity in male rats. The results of the mating behavior test show that the extract of *A. americana* at the doses of 100, 200, and 400 mg/kg body weight significantly raises mounting frequency ($p < 0.05$) and intromission frequency ($p < 0.05$) as compared to the control groups (Figs 1 and 2). The standard drug also increases the mounting and intromission frequency compared to control groups.

Table 1: Phytochemical screening of *A. americana* leaves hydroalcoholic extract.

Test	*Inferences*
Salkowski Reaction	The presence of steroids was indicated.
Liebermann- Burchard Reaction	The presence of steroids was indicated.
Foam Test	The presence of saponin glycosides was indicated.
Ferric chloride Test	The presence of flavonoids was indicated.
Hager's Test	The presence of alkaloids was indicated.
Froth Test	The presence of saponins was indicated.
Alkaline reagent Test	The presence of flavonoids was indicated.
Millon's Test	The presence of proteins was indicated.
Benedict's Test	The presence of sugars was indicated.

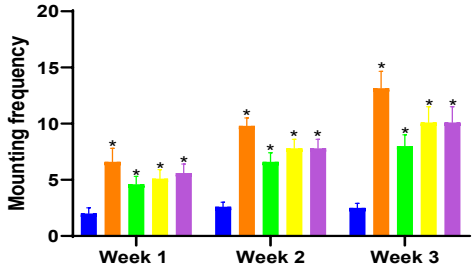

Fig. 1: Effect of aphrodisiac activity of *A. americana* leaves extract on mounting frequency activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose.

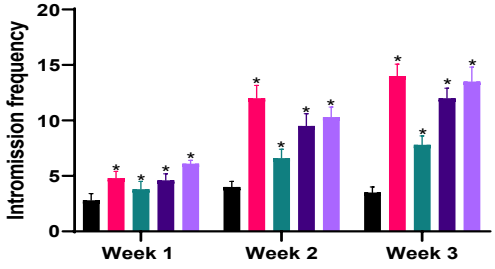

Fig. 2: Effect of aphrodisiac activity of *A. americana* leaves extract on intromission frequency activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose.

Effect of the Extract on the Libido Test

In the libido test, the result shows that the hydroalcoholic extract of *A. americana* leaves at doses of 100, 200, and 400 mg/kg significantly raises the mounting frequency ($p < 0.05$) and intromission frequency ($p < 0.05$) as it compared to control groups (Figs 1 and 2). However, in the standard group, the activity of libido also intensifies.

Effect of the Extract on Potency Test

In potency tests, the hydroalcoholic extract of *A. americana* leaves at doses of 100, 200, and 400 mg/kg significantly raised the quick flip ($p < 0.05$), long flip ($p < 0.05$), and erection ($p < 0.05$) as it compared to control groups (Figs 3-5).

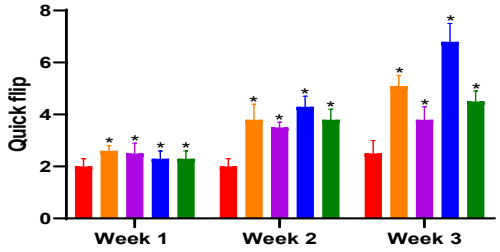

Fig. 3: Effect of aphrodisiac activity of *A. americana* leaves extract on quick flip activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose.

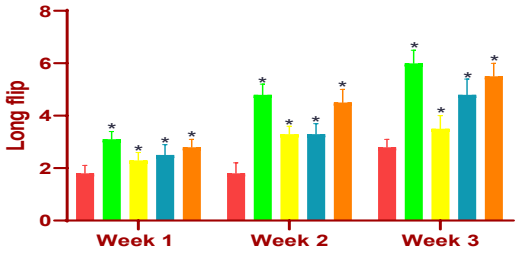

Fig. 4: Effect of aphrodisiac activity of *A. americana* leaves extract on long flip activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose.

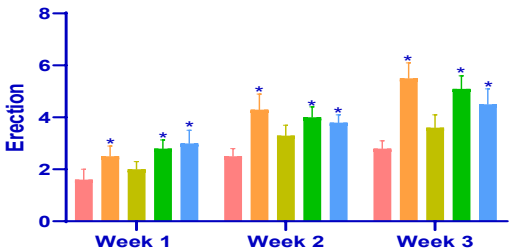

Fig. 5: Effect of aphrodisiac activity of *A. americana* leaves extract on erection activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose

When compared to control groups, standard drug treatment increases long-term flip and erection activity.

Effect of the Extract on Orientation Activity

In orientation activity, the hydroalcoholic extract of *A. americana* at doses of 100, 200, and 400 mg/kg body weight significantly raises the number of climbs and genital grooming (Figs 6 and 7). When compared to control groups, animals given standard drugs perform more lickings, exploration and rearing activities (Fig. 8-10).

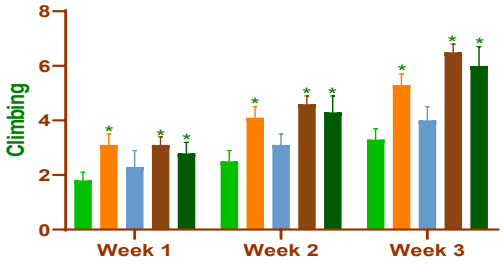

Fig. 6: Effect of aphrodisiac activity of *A. americana* leaves extract on climbing activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared to the control group. Control; Standard; Low dose; Mid dose; High dose

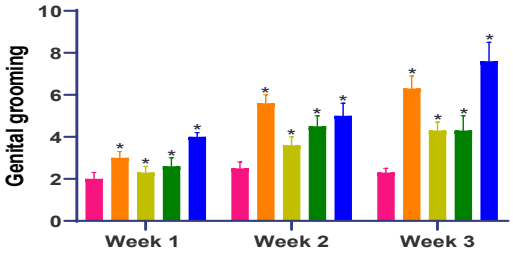

Fig. 7: Effect of aphrodisiac activity of *A. americana* leaves extract on genital grooming activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. *$p < 0.05$ when treatment group are compared with the control group. Control; Standard; Low dose; Mid dose; High dose

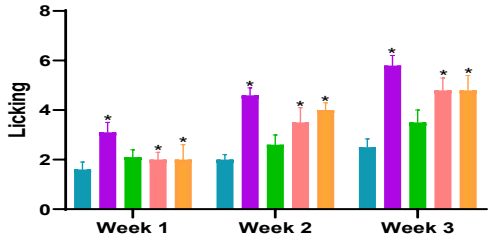

Fig. 8: Effect of aphrodisiac activity of *A. americana* leaves extract on licking activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment groups compared to control group. Control; Standard; Low dose; Mid dose; High dose

Effect of the Extract on Sexual and Vital Ogan Weight

The hydroalcoholic extract of *A. americana* leaves at doses of 100, 200, and 400 mg/kg and the standard drug sildenafil citrate 5 mg/kg body weight significantly raised the sexual organ weight as compared to the control group (Table 2).

Effect of the Extract on Serum Testosterone Hormone Level

The hydroalcoholic extract of *A. americana* leaves at doses of 100, 200, and 400 mg/kg body weight significantly raised the serum testosterone level in the high dose group as compared to the control group (Fig. 11).

Discussion

The initial screening of a test drug's aphrodisiac potential using an animal model is a recognised model. This mouse model is rapid and easy to employ[19] and can also be used

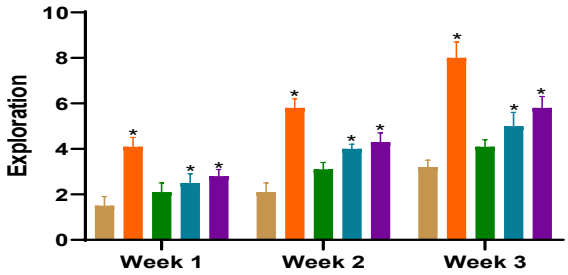

Fig. 9: Effect of aphrodisiac activity of *A. americana* leaves extract on exploration activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment groups are compared to control group. Control; Standard; Low dose; Mid dose; High dose

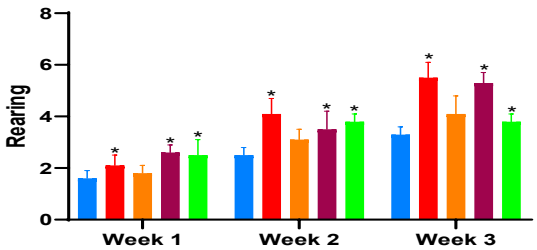

Fig. 10: Effect of aphrodisiac activity of *A. americana* leaves extract on rearing activity in male rats.
All data are expressed as mean ± SEM (n = 6 in each group) and analysed by two-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ when treatment group are compared to the control group. Control; Standard; Low dose; Mid dose; High dose

Table 2: Effects of *A. americana* leaves hydroalcoholic extract on male reproductive epididymis, testis, seminal vesicle and prostate organ weight.

Group	*Drug administration*	*Epididymis weight (g)*	*Testis (g)*	*Seminal vesicles (g)*	*Prostate (g)*
Control	Vehicle (water)	0.5 ± 0.1	1.8 ± 0.15	1.75 ± 0.24	0.83 ± 0.06
Standard	Sildenafil citrate 5 mg/kg	0.7 ± 0.06*	2.01 ± 0.22*	1.72 ± 0.54	0.79 ± 0.05
Low dose	*A. americana* extract (100 mg/kg)	0.65 ± 0.03*	2.0 ± 0.11*	1.86 ± 0.11*	0.6 ± 0.04
Mid dose	*A. americana* extract (200 mg/kg)	0.66 ± 0.05*	1.87 ± 0.12	1.3 ± 0.33	0.7 ± 0.1
High dose	*A. americana* extract (400 mg/kg)	0.69 ± 0.03*	1.9 ± 0.14	1.65 ± 0.12	0.8 ± 0.23*

All data are expressed as mean ± SEM (n = 6 in each group). *$p < 0.05$ when treatment groups are compared with the control group.

to assess the effects of aphrodisiacs and stimulants on penile erection in the context of erectile dysfunction.[20] It has been reported[21] that the steroids and saponin constituents found in these plants also intensify the aphrodisiac activity and are also used in the treatment of erectile dysfunction. In the present study, toxicity symptoms like salivation, changes in the appearance of hair, and the mortality rate were not found during the experiments.

Sexual behavioral parameters such as mount and intromission frequencies are indices of sexual vigour, libido and potency were assessed of the hydroalcoholic extract of *A. americana*. Results showed significant rise in intromission frequency and mounting frequency as it was compared with the control groups. Yakubu and Afolayan[22,23] were they observed the same reverse inhibition at the highest dose of 3000 mg/kg of *Terminalia catappa* seeds and 100 mg/kg of *Bulbine natalensis* stem in their respective studies.

Androgens play a significant role in regulating male sexual behavior, including erection and libido. These androgens may affect both central and peripheral neural systems.[24,25] One of the main androgens produced by the interstitial Leydig cells of the testis is testosterone, which is found in the male gonads. Administration of testosterone has been shown to improve libido and sexual performance. Additionally, it increased the power of ejaculations and orgasms.[26] A moderate but comparable rise in sexual desire or libido has been associated with an increase in testosterone.[27]

Based on above basis, our study animals showed significant intensified activities of the quick flip, long flip, and erection as compared to the control groups. The orientation activity study showed increase in licking, climbing, genital grooming, and rearing as compared to the control group. The hydroalcoholic extract of *A. americana* leaves at doses of 100, 200 and 400 mg/kg body weight significantly raised the serum testosterone level in the high dose group as compared to the control group. In the study, the weight of organs like testis, vas deference, seminal vesicles, and prostate also increased in the treatment group as it was compared to the control groups (Table 2).

All animal in all the groups, showed no significant adverse acute toxicological effect that can be attributed to the acute administration of the ethanol extract of *A. americana*. Also, adverse changes in behavior were not observed, indicating that physical clinical signs were unremarkable. Food and water intake were normal, suggesting that the animals had a normal appetite. No mortality was noticed during the entire period of the study. Toxicological results were similar to earlier reported study.[28-30]

In this study, the hydroalcoholic extract of *A. americana* at a dose of up to 400 mg/kg body weight., showed significant aphrodisiac activity in experimental rats. The steroidal saponins of *A. americana* may be responsible for such property. It can be inferred that higher doses of *A. americana* leaves can be employed for additional investigations to gain a better knowledge of the mechanism underlying their benefits in treating a variety of illnesses.

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