Original Article

Effects of Kamdhenu Ark and Active Immunization by Gonadotropin Releasing Hormone Conjugate (GnRH-BSA) on Gonadosomatic Indices (GSI) and Sperm Parameters in Male *Mus musculus*

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Abstract

Background: Active immunization against GnRH decreases the secretion of gonadotropins and causes cessation of gonadal function, thereby, inducing infertility. Based on the immunoenhancing activity of Kamdhenu ark (distilled cow urine), this study was performed to evaluate its effects on the gonadosomatic indices (GSI) and sperm parameters in male mice receiving a GnRH contraceptive vaccine.

Methods: Sixty adult male mice of Parke's strain were divided into three groups of twenty. Group I served as the controls, while group II was immunized by GnRH-BSA conjugate (50/0.2/35 *µg/ml/g* BW) by four intraperitoneal injections at different intervals on days 1, 30, 60 and 90. However, group III was supplemented daily by oral Kamdhenu ark (100 *ppm*) along with GnRH-BSA immunizations. The animals were sacrificed after 30, 60, 90 and 120 days and their testis and epididymis were dissected out weighed and semen analysis was performed.

Results: GSI values, sperm motility, sperm count and sperm morphology in male *Mus musculus* were decreased significantly in all the experimental groups as compared to the control group ($p<0.01$). Kamdhenu ark significantly enhanced the effect of GnRH vaccine on the aforesaid parameters especially in 90 and 120 days treated groups ($p<0.05$).

Conclusion: The changes witnessed in sperm parameters suggested that the GnRH-BSA immunization suppressed the activities of gonadotropins and testosterone directly through hypothalamo-hypophysial-gonadal axis and indirectly by acting on the testes which may modulate the sperm morphology, sperm count and motility. However, Kamdhenu ark seems to have enhanced these effects because of its immunemodulatory properties too.

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Background

Gonadotropin-releasing hormone (GnRH) controls the production of gonadotropins, thereby having an orchestrating effect on the reproductive hormone cascade and spermatogenesis (1). Active immunization against GnRH has successfully suppressed the secretion of gonadotropins and decreased sperm production, follicular development, ovulation and conception in male and female mammals (2, 3). Vaccination against GnRH blocks the hypothalamic-pituitary- gonadal axis. Therefore, it can be used as an alternative for castration and fertility control in farm animals, companion animals and wildlife species (4-6). Application of GnRH vaccination in humans has been suggested for controlling fertility-related endocrine disorders and gonadal steroid-dependent diseases (7). Active immunization of adult animals against GnRH causes the loss of synthesis and secretion of gonadotropins and cessation of gonadal function as long as the antibody titers

remain elevated (8).

It has been reported that cow urine contains all beneficial elements such as chemical properties, potentialities and constituents that are capable of removing all the ill effects and imbalances of body caused by various infectious agents and toxicants. In this way, it ensures a protection against various ailments including the most dreaded diseases like cancer, diabetes, hepatitis etc. (9). Kamdhenu ark (distilled cow urine) has been reported as a strong immunomodulator and bioenhancer by various researchers (10, 11). Experimental studies of Rangasamy and Kaliappan revealed the protective effects of cow urine on haematological, serum biochemical parameters and immune status of broilers (12).

The present study attempts to evaluate the effects of GnRH-BSA immunization on gonadosomatic indices (GSI) and sperm parameters in male mice and to examine the modulatory role of Kamdhenu ark following the immunization.

Methods

Sixty adult male mice, *Mus musculus,* of Parke's strain (P), weighing 30±5 *g* were used in the study. The animals were divided into three groups of twenty. The mice in Group I served as the controls, receiving intraperitoneal Phosphate Buffered Saline (PBS) injections (100 *µl*) on the 1st, 30th, 60th and 90th days, while the mice in group II were immunized by GnRH-BSA conjugate (50/0.2/35 *µg/ml/g* BW) (Sigma-Aldrich, USA) dissolved in 100 µl of phosphate buffered solution (0.01 N) emulsified with an equal volume (100 *µl*) of Freund's adjuvant (Sigma Aldrich, USA). GnRH-BSA injections were given intraperitoneally at different intervals, i.e. on days 1st, 30th, 60th and 90th. However, the mice in group III were supplemented with daily Kamdhenu ark (100 *ppm*) (Gaytri Shakti Peeth, India) orally along with the intraperitoneal injections of GnRH-BSA. Five animals from each group were sacrificed in monthly intervals, i.e. on days 30, 60, 90 and 120 and their testes and epididymides were quickly dissected. The testes were weighed for observing gonadosomatic indices [gonad weight/ 100 *g* BW], while the epididymides were processed for semen analysis, *i.e.* sperm motility, sperm count and morphology by Prasad method (13). Cauda epididymides were dissected out to release sperms in normal saline (100 *mg* tissue/ 2 *ml* N.S.) for sperm suspension. For studying sperm morphology, Leishman`s stain was used and the slides were finally observed at 400× magnification (14).

Statistical Analysis: The collected data were analyzed through one way ANOVA and post-hoc methods using EZANOVA software. P-values <0.05 or <0.01 were considered significant while values <0.001 were considered as highly significant.

Results

GSI values decreased in all the experimental groups compared to the control group. However, more significant decrease in GSI was observed in the group treated by Kamdhenu ark along with GnRH-BSA, especially in the later part of the experiment (p<0.01) (Table 1). Moreover, sperm motility and sperm count significantly decreased throughout the investigation in all the treated groups compared to the control group (p<0.01) (Table 2). However, some mice immunized by GnRH-BSA + Kamdhenu ark also showed de-

Table 1. Gonadosomatic indices (GSI) in the experimental and control groups of male mice, *Mus musculus,* after different intervals

Group	GSI (gonad weight/100 *g* BW)			
	Duration			
	30 days	60 days	90 days	120 days
Control	0.40 ± 0.02	0.43 ± 0.05	0.50 ± 0.01	0.54 ± 0.04
GnRH-BSA	0.36 ± 0.06	0.30 ± 0.02 [a]*	0.23 ± 0.00 [a]	0.16 ± 0.00 [a]
GnRH-BSA + KA	0.32±0.03	0.26± 0.01 [a]	0.19±0.01 [ab]	0.12±0.00 [ab]

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)
[a] = Significant difference with the controls in the same column (p< 0.01)
[b] = Significant difference with GnRH-BSA groups in the same column (p< 0.01)
* = Significant differences (p< 0.05)

Table 2. Sperm motility and sperm count in the experimental and control groups of male mice, *Mus musculus,* after different intervals

Parameters	Group	Duration			
		30 days	60 days	90 days	120 days
Sperm Motility (%)	Control	59.00±4.33	63.76±2.77	65.05±2.31	69.60±3.19
	GnRH-BSA	39.40±3.81[a]	21.56±1.36[a]	13.00±2.11[a]	9.40±1.14[a]
	GnRH-BSA+ KA	33.80±1.72[a]	17.06±1.00[ab]*	10.46±1.65[a]	7.16±0.95[a]
Sperm Count (*million/ml*)	Control	62.00±3.18	68.60±2.10	75.50±3.76	78.00±2.11
	GnRH-BSA	49.10±2.65[a]	35.22±3.21[a]	21.00±2.20[a]	16.42±1.78[a]
	GnRH-BSA + KA	44.18±2.82[a]	26.12±2.00[ab]*	18.30±1.90[a]	12.34±1.10[ab]*

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)
[a] = Significant difference with the controls in the same column ($p< 0.01$)
[b] = Significant difference with the GnRH-BSA groups in the same column ($p< 0.01$)
* = Significant differences ($p< 0.05$)

creased values for sperm motility and count than the GnRH-BSA immunized groups ($p<0.05$). The percentage of morphologically normal sperm decreased significantly with increased percentage of abnormal forms of sperms, i.e. pin head, large head, oval head, double head, head less, bent neck, looping mid piece, coiled-tail, double-tailed, tailless in all the experimental groups as compared to the control group ($p<0.01$) (Table 3, Figure 1). Moreover, some significant alterations in normal sperm morphology, such as large head, headless and pin head sperm were also observed in GnRH-BSA + Kamdhenu ark treated group when compared with GnRH-BSA, especially in the later part of the experiment ($p<0.01$).

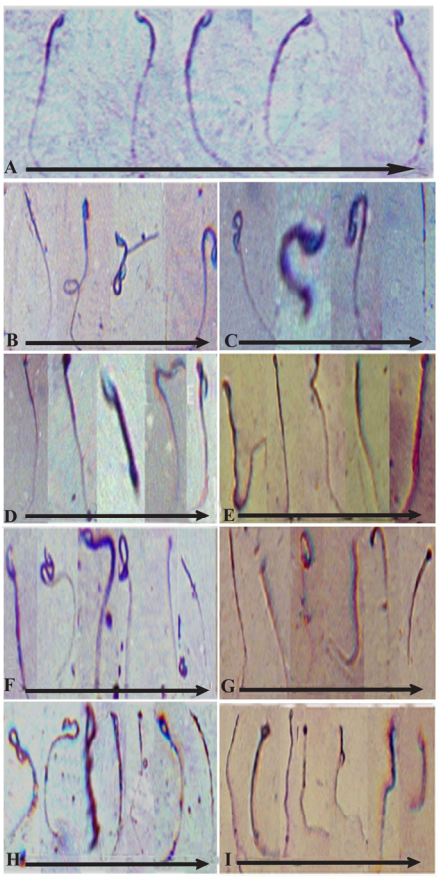

Figure 1. Normal morphological sperm forms in the controls (A) and morphologically abnormal sperms after GnRH-BSA and Kamdhenu ark along with GnRH-BSA administration (B-I) in male *Mus musculus*.

Discussion

The endocrine effects of active immunization against GnRH have been studied in a variety of young adult male and female animals (15-17). Experimental studies have demonstrated decreases in gonadotropins, sperm production, follicular development, ovulation and conception after immunization against GnRH, chemically conjugated to a carrier protein. GnRH immunization affected sperm motility and sperm counts in ram lambs, boars and colts (18, 19). Several other experimental studies have revealed the deleterious effects of immunization against GnRH on different sperm parameters in rats, bulls, stallions, cats and dogs (20-24).

Cow urine has been tested for its immunomodulatory properties that enhance both cellular and humoral immune responses (25, 26). Kamdhenu ark (distilled cow urine) has been reported to increase the humoral immunity in rats (27). Chauhan *et al.,* (2004) observed that Kamdhenu ark may modulate the immune responses because

Table 3. Percentage of normal and abnormal sperm morphology in the experimental and control groups of male mice, *Mus musculus*, after different intervals

Days	Groups	Normal (%)	Abnormal (%)									
			Pin head	Large head	Oval head	Double head	Head less	Bent neck	Looping mid piece	Coiled tail	Double tailed	Tail-less
30	Control	58.44±2.12	5.0±0.35	1.0±0.35	4.0±0.79	1.2±0.65	4.0±1.17	3.0±0.86	4.4±1.60	1.6±0.30	0.0±0.0	2.5±1.00
	GnRH-BSA	27.77±0.78 [a]	8.4±1.03	5.0±0.79	5.80±1.29	2.2±0.74	5.8±1.08	6.4±1.03	6.2±0.65	2.4±0.75	2.2±0.96	5.4±1.15
	GnRH-BSA+ KA	24.00±1.22 [ab*]	7.6±0.57	4.20±0.65	6.4±1.03	2.4±0.75	8.6±0.90	5.6±1.3	6.5±2.19	1.80±0.24	1.66±0.51	2.5±0.75
60	Control	64.00±1.41	5.4±0.57	4.2±0.65	3.4±0.57	0.60±0.00	5.0±1.36	3.4±0.57	4.2±0.65	2.5±0.55	1.33±0.51	0.80±0.14
	GnRH-BSA	23.20±1.16 a	11.2±0.65	10.20±1.74	7.20±0.50	2.60±0.90	7.80±0.65	9.4±1.15	10.80±1.38	4.0±0.70	2.00±0.00	3.5±0.87
	GnRH-BSA+ KA	20.65±0.94 a	10.80±0.45	6.4±0.59	7.4±0.90	0.00±0.00	11.80±1.43	10.00±1.76	9.60±0.51	2.50±0.48	3.0±1.11	5.80±2.38
90	Control	67.10±1.74	4.5±0.65	2.60±1.03	2.2±0.41	0.00±0.00	3.401.71	2.60±0.90	1.60±0.83	0.00±0.00	2.60±1.03	1.0±0.21
	GnRH-BSA	14.24±0.48 a	13.60±1.15	5.80±0.93	6.0±1.00	1.20±0.65	10.40±0.75	12.0±0.79	17.80±1.94	2.50±1.15	1.0±0.25	8.0±0.65
	GnRH-BSA+ KA	12.72±0.23 ab	13.20±1.19	8.80±0.96	5.80±1.55	2.2±0.96	13.40±1.03	9.5±2.07	10.80±1.29	3.0±0.79	2.5±0.83	11.0±1.76
120	Control	72.00±1.66	2.60±0.41	2.20±0.39	1.0±0.50	0.00±0.00	2.80±1.29	1.20±0.54	2.0±0.44	1.00±0.00	0.00±0.00	2.5±1.15
	GnRH-BSA	10.33±0.25 [a]	12.80±1.55	7.00±0.79	6.00±0.79	2.80±0.96	16.00±1.36	17.20±0.96	20.60±1.95	5.00±1.11	2.80±0.41	9.50±1.68
	GnRH-BSA+ KA	7.95±0.40 [ab]	14.60±1.03	11.00±0.79	7.20±0.82	3.80±0.96	17.60±1.07	15.00±1.83	14.25±2.21	4.60±1.15	4.0±1.17	12.50±0.65

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)
a = Significant difference with the controls in the same column ($p< 0.01$)
b = Significant difference with GnRH-BSA groups in the same column ($p< 0.01$)
* = Significant differences ($p< 0.05$)

it increases the secretion of interleukin-1 and 2 (28). Recently, Ganaie and Shrivastava reported the modulatory effects of Kamdhenu ark on GnRH-BSA immunization in female mice (29).

In corroboration to above studies, our study also revealed that GnRH-BSA immunization significantly decreased the values of GSI, sperm motility, count and morphology in male *Mus musculus*. The aforesaid parameters diminished more significantly in the group supplemented with Kamdhenu ark along with GnRH-BSA immunization. All these changes in GSI and sperm parameters suggested that GnRH-BSA immunization could have directly suppressed the activities of gonadotropins and testosterone through hypothalamo-hypophysial-gonadal axis or might have indirectly affected the testicular tissue. However, more significant decreases in the parameters after Kamdhenu ark supplementation may be because of its modulatory and bioenhancing properties.

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