# Effect of Knotweed Extract on Alkeran-Induced Changes in Rat Ejaculate

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We studied the effects of aqueous knotweed extracts in alkeran-induced experimental pathozoospermia. Therapeutic effect of knotweed extract in experimental cytostatic hypogonadism was demonstrated (the preparation improved spermatozoon motility).

**Key words:** *pathozoospermia; sterility; knotweed*

Male sterility became now a very actual problem because of high number of infertile marriages and more pronounced role of the male factor in this pathology (from 24 to 60%) [4,6,9]. Male sterility is not a nosologic unit. It results from various pathological processes causing pathozoospermia. Sterile patients show decreased ejaculate volume, multiple decrease in sperm concentration and relative content of active forms associated with increased number of immotile ones [2]. In modern andrology, male sterility associated with pathozoospermia is usually treated by hormonal drugs [7,12,13]. Some of them, in particular, steroids, often cause complications, for instance inhibition of the anterior pituitary, total hormonal imbalance, disturbances of lipid metabolism, and exacerbation of undetected prostatic cancer. This makes important the search for the new drugs producing no unfavorable effects.

The aim of the present study was to examine the effects of aqueous extract of knotweed (*Polygonum ex gr. aviculare L.*) in pharmacological pathozoospermia modeled by antineoplastic cytostatic drug alkeran. Knotweed is among few plants used in traditional medicine of East Asia for the treatment of male sexual disorders.

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## MATERIALS AND METHODS

The experiments were carried out on adult random-bread albino male rats weighing 120-200 g. Our previous studies demonstrated that aqueous extracts of knotweed were superior to both alcoholic extracts and lyophilized preparations [5]. Therefore, aqueous extracts were used in the present study. The extracts were prepared according to the XI State Pharmacopeia and administered via a stainless steel probe.

The animals were divided into 5 groups ($n$=12 for each). Groups 1 and 2 animals received knotweed extract (1 g/kg calculated per dry weight of raw material) once a day. Groups 3, 4, and 5 received 1 ml distilled water. After 15 days the groups 1 and 3 animals were intraperitoneally injected with 10 μg/kg alkeran, groups 2 and 4 received 100 μg/kg alkeran. Controls (group 5) were injected with 1 ml distilled water. After 3 days the groups 1 and 2 rats started receiving the same daily doses of knotweed extract. The experiments were stopped on day 15 after single alkeran injection. Ejaculate was examined before and on days 3, 5, 10, and 15 after alkeran injection. To obtain ejaculate, a male rat was placed into a cage with a female in estrous phase induced by 4 injections of 0.05% folliculin (0.02 mg/kg/day). Ejaculate was collected from the vagina immediatelly after copulation. Spermatozoa were counted in a Goryaev chamber. Sperm motility was evaluated under a microscope at 125-fold magnification by counting motile and immotile forms. Morphological features of spermatozoa and immature

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cells were examined at 800-fold magnification. Ejaculate smears were fixed with 40% paraformaldehyde and stained according to Romanowsky. The number of normal, pathologically changed spermatozoa (with small, large or doubled head, with doubled or no tail), and immature cells (spermatocytes, spermatids and spermatozoa with cytoplasmic fragments) were counted in several visual fields and their total content was calculated.

The results were processed statistically using Student's *t* test.

## RESULTS

Administration of knotweed extract for 15 day had no effect on sperm concentration, ratio between motile and immotile forms, and the relative content of pathological and immature cells (data not shown). On day 3 after injection of 10 μg/kg alkeran, the number of immotile and pathological forms in groups 1 and 3 increased 1.7- and 2.3-fold, respectively. Alkeran in a dose of 100 μg/kg decreased sperm concentration by 28% and relative content of immature cells 2.4-fold (Table 1). On days 5 and 10 after alkeran injection all parameters of rat ejaculate significantly differed from the control. This effect was more pronounced on day 10: alkeran in doses of 10 and 100 μg/kg decreased sperm concentration 7.6- and 25-fold, respectively, all spermatozoa were immotile or carry pathological alterations, immature cells were absent. On day 15 sperm concentration increased, motile and immature cells appeared indicating recovery of normal spermatogenesis.

The protective effect of knotweed extract was observed on day 3 after injection of 10 μg/kg alkeran: the number of immotile and pathologically changed cells was lower than in rats receiving alkeran alone (Table 1). Knotweed extract markedly changed sperm motility on days 5, 10, and 15, and had no effect on sperm concentration and the number of immature cells (Table 1).

These results show that cytostatic alkeran impairs sperm motility and inhibits production of mature gametes probably by disturbing spermatogonium mitosis and meiotic processes. These features are typical of secretory-toxic forms of male sterility [1] associa-

**TABLE 1.** Effect of Knotweed Extract (1 g/kg) on Spermogram Parameters in Rats after Single Injection of Cytostatic Alkeran (*M*±*m*)

| Parameter | Days | Control | Alkeran, μg/kg | | | |
|---|---|---|---|---|---|---|
| | | | 10 | | 100 | |
| | | | -knotweed | +knotweed | -knotweed | +knotweed |
| Number of spermatozoa, $10^6$/ml | 3 | 7.5±0.3 | 7.0±0.3 | 6.8±0.6 | 5.4±0.3* | 5.7±0.5 |
| | 5 | 7.8±0.5 | 5.1±0.2* | 5.2±0.2 | 1.20±0.03* | 1.50±0.11 |
| | 10 | 7.6±0.5 | 1.0±0.1* | 1.2±0.1 | 0.30±0.03* | 0.40±0.03 |
| | 15 | | 2.8±0.2 | 3.0±0.3 | 0.9±0.1 | 1.0±0.1 |
| Motile forms, % | 3 | 93.7±4.9 | 89.2±5.1 | 92.7±2.6 | 81.6±6.5* | 80.8±7.2 |
| | 5 | 94.1±4.6 | 76.5±2.9* | 90.3±4.3+ | 56.1±2.3* | 85.0±4.7+ |
| | 10 | 92.9±4.2 | 18.6±1.4* | 27.9±0.8+ | 0 | 5.8±0.2+ |
| | 15 | | 40.5±2.7 | 59.3±3.4+ | 14.2±1.0 | 26.9±1.7+ |
| Immotile forms, % | 3 | 6.3±0.4 | 10.8±0.3* | 7.3±0.5+ | 18.4±1.1* | 19.2±1.4 |
| | 5 | 5.9±0.5 | 23.5±1.2* | 9.7±0.2+ | 43.9±2.7* | 15.0±0.9+ |
| | 10 | 7.0±0.7 | 81.4±4.2* | 72.1±2.7+ | 100.0±0.0* | 94.2±4.4+ |
| | 15 | | 59.5±3.4 | 40.7±2.6+ | 85.8±5.2 | 73.1±7.9 |
| Pathological forms, % | 3 | 5.3±0.2 | 12.2±0.7* | 6.3±0.2+ | 19.3±1.7* | 20.1±1.9 |
| | 5 | 5.1±0.2 | 15.6±1.0* | 10.3±0.4+ | 47.5±3.6* | 45.9±4.1 |
| | 10 | 5.4±0.2 | 24.8±1.9* | 25.0±2.4 | 100.0±0.0* | 100.0±0.0 |
| | 15 | | 19.0±0.7 | 18.4±1.8 | 86.1±5.2 | 82.5±7.9 |
| Immature cells, % | 3 | 6.6±0.5 | 5.8±0.5 | 6.0±0.5 | 2.7±0.1* | 2.5±0.2 |
| | 5 | 6.7±0.6 | 3.5±0.1* | 3.7±0.2 | 0 | 0 |
| | 10 | 6.6±0.6 | 0.9±0.1* | 1.1±0.1 | 0 | 0 |
| | 15 | | 1.7±0.1 | 1.5±0.1 | 0.3±0.1 | 0.4±0.1 |

**Note.** $p<0.05$: *compared to the control or +the corresponding parameter without knotweed.

ted with hypogonadism, which require conservative treatment [8]. As was shown on the model of cytostatic hypogonadism, knotweed extract affected mainly sperm motility. In rats receiving knotweed extract, some spermatozoa retained molitity on day 10 after alkeran injection in a maximum dose, while controls showed absolute necrospermia.

Previous experiments with rats and mice showed that ethanol and aqueous knotweed extracts did not stimulate gonadotropic and androgen activity or androgen secretion [10]. Therefore, we assume that the effect of these extracts on sperm motility is associated with increased number or sensitivity of androgen receptors. Many androgen receptors are localized in seminal vesicles, prostate, urethral and bulbourethral glands producing semen components responsible for sperm motility. The effect of knotweed can be associated with stimulation of these receptors.

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