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## The toxicity of vanadium on gastrointestinal, urinary and reproductive system, and its influence on fertility and fetuses malformations

Szkodliwe działanie wanadu na układ pokarmowy i moczowo-płciowy oraz jego wpływ na płodność i wady rozwojowe płodu

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### Summary

Vanadium is a transition metal that has a unique and beneficial effect on both humans and animals. For many years, studies have suggested that vanadium is an essential trace element. Its biological properties are of interest due to its therapeutic potential, including in the treatment of diabetes mellitus. Vanadium deficiencies can lead to a range of pathologies. However, excessive concentration of this metal can cause irreversible damage to various tissues and organs. Vanadium toxicity mainly manifests in gastrointestinal symptoms, including diarrhea, vomiting, and weight reduction. Vanadium also exhibits hepatotoxic and nephrotoxic properties, including glomerulonephritis and pyelonephritis. Vanadium compounds may also lead to partial degeneration of the seminiferous epithelium of the seminiferous tubules in the testes and can affect male fertility.

This paper describes the harmful effects of vanadium on the morphology and physiology of both animal and human tissues, including the digestive system, the urinary tract, and the reproductive system. What is more, the following study includes data concerning the correlation between the above-mentioned metal and its influence on fertility and fetus malformations.

Additionally, this research identifies the doses of vanadium which lead to pathological alterations becoming visible within tissues. Moreover, this study includes information about the protective efficacy of some substances in view of the toxicity of vanadium.

**Keywords:** vanadium • toxicity • stomach • intestine • kidney • liver • reproductive system

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## INTRODUCTION

Vanadium is a transition metal that has a unique and beneficial effect on both humans and animals. Its primary uses are found in metallurgy and in the aerospace, chemical, glass, and photographic industries [38]. For many years, studies have suggested that vanadium is an essential trace element, of which only about 10 µg are required per day. Vanadium is consumed in unrefined oils, soy bean oil, olive oil, peanut oil, cottonseed oil, black pepper, mushrooms, parsley, spinach, and shellfish [30]. Vanadium deficiencies can lead to many different pathological changes, such as growth limitations and pathologies of teeth and bone calcification and of the reproductive system [2]. However, according to the International Agency for Research on Cancer (IARC),  $V_2O_5$  is possibly carcinogenic in humans and has been classified as a group 2B carcinogen (indicating limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals). In one study, inhalation exposure resulted in increased incidence of alveolar and bronchiolar neoplasms in mice and male rats; however, no data on human carcinogenicity were available to the IARC working group.

Furthermore, excessive concentrations of this metal can cause many alterations in tissues and organs, including the central and peripheral nervous systems. The kidneys and liver are particularly sensitive to vanadium. The mechanism of its toxicity has not been fully elucidated. However, it is believed that vanadium induces oxidative stress that causes, among other problems, lipid peroxidation, DNA degeneration, and denaturation of proteins, ultimately leading to the disintegration of cell membranes [35]. The harmful effects of vanadium are dependent on how it is administered and in what chemical form: its toxicity increases with valence, and the pentavalent forms (e.g.,  $V_2O_5$ ) seem to be the most harmful [17,32,38].

Given its broad use, it should be noted that high doses of vanadium may lead to pathological and often irreversible changes in tissues and organs. This paper describes the harmful effects of vanadium on the morphology and physiology of both animal and human tissues, including the digestive system, the urinary tract, and the reproductive system. Moreover, this study includes information about the protective efficacy of some substances in view of the toxicity of vanadium.

## DIGESTIVE SYSTEM

Vanadium is a transition element with a major impact on many organs, including the digestive system, though it is known that the metal is poorly absorbed there. It is rapidly excreted by kidneys, which to a large degree reduces its toxic effects. Vanadium compounds can cause gastrointestinal problems, diarrhea, vomiting, general dehydration with weight reduction, intestinal

inflammation, and a characteristic green tongue. The mechanisms by which vanadium mediates its metabolic effects *in vivo* are still not completely understood. However, oral and intraperitoneal vanadium administration in high doses can lead to death in animals.

There is very little data concerning the toxic effect of vanadium compounds on the digestive system, and especially on the oral cavity. It has been postulated that there are differences in the prevalence of dental caries between different regions of the USA and other countries, and this fact has been associated with exposure to trace elements, including vanadium [5]. Galli et al. [13] conducted an experiment concerning the osteoblastic differentiation of human mesenchymal stem cells and human dental pulp stem cells on poly-L-lysine-treated titanium-6-aluminium-4-vanadium (Ti6Al4V). Poly-L-lysine-treated titanium-6-aluminium-4-vanadium is a widely used biomaterial for orthopedic prostheses and dental implants, on account of its high mechanical strength and resistance to corrosion. Human mesenchymal stem cells and dental pulp stem cells play an important role during bone regeneration following the colonization of prostheses or dental implants. Dental pulp stem cells have a lower ability to colonize this biomaterial than do tissue culture-treated plastic and they further show a lack of focal adhesion kinase activation when grown on Ti6Al4V [13].

On the other hand, administration of vanadyl sulfate at a dose of 100 mg/kg body weight (b.w.) demonstrates an ameliorative effect against oxidative stress in the stomach tissues of streptozotocin-induced diabetic rats. In the diabetic group, the activities of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione S-transferase (GST) significantly increased, while that of catalase (CAT) insignificantly increased in stomach tissue, compared to the control group. It was observed that vanadyl sulfate had no significant toxic effect on stomach tissue treated with vanadyl sulfate alone, compared to the control group. The authors of that experiment suggest that this metal could be used as antioxidant in diabetic complications [42].

Because vanadium compounds have antidiabetic properties, it is extremely important to collect as much data as possible concerning its toxicity. A small number of publications have dealt with the effect of vanadium on the small and large intestines. In the small and large intestines of mice treated with 10 and 20 mg of ammonium metavanadate, focal shortening of the intestinal villi and mild dilatation of crypts were noted, respectively. Furthermore, at a dose of 20 mg V, moderate to marked dilatation of crypts and degeneration, as well as necrosis of the mucosal epithelial cells, were observed. The toxic influence of vanadium on these tissues was also confirmed by immunochemical methods. Positive reactions with antibodies against caspase-3, which is the protease involved in the end stage of apoptosis, were

observed. This end stage of apoptosis was visible within the small intestine and affected the tip of the villi [16].

Pathological changes also occurred in the spleen, where atrophy of lymphatic nodules was noticed. A dose of 20 mg V had a clearly worse effect on the spleen and the alterations in the organ were more visible than in the case of the 10 mg dose [16]. Concerning the immunological system, in which the spleen plays an important role, experimental data is available on the cecal tonsil in birds. The cecal tonsil in the cecum-rectum junction is known to be the largest lymphoid organ of the avian gut-associated lymphoid tissue (GALT). Dietary vanadium in excess of 30 mg/kg can reduce the T-cell population and cause pathological alterations of the cecal tonsil, impairing cecal tonsil function and affecting the local mucosal immune function of the intestines in broilers [11]. Moreover, it has been demonstrated that excess dietary vanadium reduces the numbers of IgA<sup>+</sup> cells and alters the levels of interleukin-6, interleukin-10, interferon gamma, and tumor necrosis factor alpha in the cecal tonsil of broilers [11].

Moreover, the toxicity of ammonium metavanadate depends on the diet. More severe pathological changes within the intestine after the amplification of vanadium in a high-fat diet have been observed in mice. Severe degeneration, necrosis, and loss of mucosal epithelial cells in the small intestine were observed. A TUNEL assay showed a decreased number of positive cells, and cells positive for acrolein immunohistochemistry, in the mucosal epithelial cells of the small intestine, indicating degeneration and necrosis in the vanadium-treated group of mice. This can be associated with the oxidative stress induced by vanadium [16].

There is also a report on the systematic effects of trace elements, including vanadium, on broilers' intestinal microbiota, which plays an important role in controlling enteric bacterial pathogens. Ammonium metavanadate at doses of 45 and 60 mg/kg decreased counts of *Bifidobacterium* spp. in the intestinal tract at 21 and 42 days of age, compared with the control group. With increasing levels of dietary vanadium, *Escherichia coli* counts significantly increased in the ileum, cecum, and rectum and decreased in the duodenum at 21 and 42 days of age. However, *Lactobacilli* decreased in the cecum and rectum and increased in the ileum of the groups receiving the doses of ammonium metavanadate at 45 and 60 mg/kg [41].

The main organ involved in the accumulation of vanadium is the liver. Hepatotoxicity induced by vanadium has been well examined both *in vivo* and *in vitro*, mostly in animal and bird studies. This metal disrupts the cell cycle and initiates apoptosis of hepatocytes in broilers [21]. Hepatocytes in the G0/G1 phase significantly increased in number in the 45 and 60 mg/kg groups, whereas liver cells in the S, G2, and M phases decreased in the 45 and 60 mg/kg groups; the proliferation index (PI) of hepatocytes in the 30, 45, and 60 mg/kg groups decreased compared

to the control group. This data further shows that dietary vanadium supplied as ammonium metavanadate in the 45–60 mg/kg range increases the population of cells staining positive with annexin V, which is considered to be an early marker of apoptosis, and negative PI, which represents the increased population of hepatocytes in both the early and end stages of apoptosis [21].

Experiments on broilers have clearly shown the degradation of hepatocytes at doses of 45 mg/kg of ammonium metavanadate after 21 days of treatment. Vacuolar and fatty degeneration were more pronounced in 60 mg/kg – group compared with 45 mg/kg group [20]. Comparing this data to the experimental results on laboratory animals, 10 mg V injected into mice caused vacuolization of hepatocyte cytoplasm [16]. Roy et al. [30] showed that a dose of 45 mg/kg damages the structure of the mouse liver, while 90 mg/kg resulted in fatty degeneration, degeneration of hepatocytes, structural damage, and focal inflammation. These histopathological changes coexisted with biochemical changes. The levels of liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were significantly higher in the experimental group than in the control [30].

Furthermore, *in vivo* and *in vitro* studies strongly suggest that vanadium induces liver toxicity, which is associated with the metal's effect on mitochondrial respiratory complexes I, II, and III. These induce reactive oxygen species (ROS) formation and ATP depletion in hepatocytes, ultimately leading to cell death signaling by mitochondrial pore opening and cytochrome c release, as has been demonstrated in experiments on rats [15]. In addition, metavanadate salt (V<sup>5+</sup>) induces cytotoxicity markers prevented by ROS scavengers, antioxidants, and mitochondrial permeability transition pore sealing agents. Vanadium-induced cytotoxicity can be attributed to oxidative stress that begins with glutathione-mediated metal reductive activation and continues with the mitochondrial-lysosomal toxic reaction [15].

According to Shrivastava et al. [34], the treatment of rats with vanadyl sulfate at a dose of 18.7 mg/kg per 7 days resulted in degenerative changes in the mitochondria, endoplasmic reticulum, and hepatocyte nuclei. It was also demonstrated that vanadium contributes to an increase in glycogen content in the liver, which may be associated with the activation of glycogenesis and the inhibition in the activity of glucogen phosphorylase enzyme. The vanadium compounds stimulate glucose uptake and lipid synthesis in muscle, adipose, and hepatic tissues and inhibit the activities of gluconeogenic enzymes [34].

## NEPHROTOXICITY OF VANADIUM

The kidneys are critical to the action of vanadium and studies on its nephrotoxic activities have been carried out for years, mainly on animal tissues.

The experiment of Sarsebekov et al. [31] clearly confirmed the pathological effects of vanadium on various structures of the kidneys. Samples of a vanadium-rich oil were applied subcutaneously to hybrid mice. In more than half of the experimental animals, renal failure was observed, which revealed itself mainly in the form of glomerulonephritis. Furthermore, the same experiment showed that intraperitoneal administration of vanadium to the tested animals provoked acute glomerulonephritis with partial tubular and glomerular necrosis associated with acute renal failure. It was also shown that the vanadium induced the hypertension, which indeed is connected with renal dysfunction. In addition, this metal disrupts the biochemical and electrolyte balance [3].

All the studies conducted on animals treated with various doses of vanadium have shown that the level of kidney damage depends on the so-called dose–time effect [20]. In the kidneys of animals treated with doses of 30, 45, and 60 mg/kg of ammonium metavanadate for 42 days, significantly larger lesions were observed than in the kidneys of the animals after 21 days of the experiment. The alterations of the organ mainly concerned the renal tubular epithelial cells. Beyond that, swelling of the cells, vacuolization, and granular cell degeneration were observed [20].

The studies of Roy et al. [30] emphasize the toxic effect of the vanadium–rutin complex. This experiment applied various doses of vanadium compounds to the Balb/c mice. A dose of 20 mg/kg showed no pathological changes within the renal structure. In the kidneys of the animals treated with a 90 mg/kg dose of vanadium, pyelonephritis (an inflammation of the pelvis and calyces), thickening of basement membranes, pyknotic nuclei, vacuolization, and cytoplasmic debris were seen. Significantly, the animals' creatinine and urea (essential biochemical parameters showing renal status) were significantly higher than in the control group. At a dose of 45 mg/kg, an improvement in the basement membrane of glomerulus, less cytoplasmic debris, fewer pyknotic nuclei, and less vacuolization were visible, similarly to the 90 mg/kg group.

Furthermore, the results of studies conducted by Wang et al. [40] also revealed a significant relation between the dose and pathological changes in renal tissue. Lesions in the kidneys of rats treated with vanadium at different doses (3, 15, and 30 mg/kg respectively) were observed. The most significant alterations were found in the kidneys from the last group. Granular degeneration and vacuolar degeneration in the cells of the renal tubules and the endothelial cells of glomeruli were observed [40].

As mentioned, the mechanism of the vanadium toxicity is not yet fully understood and requires further experimental work. However, it seems that the harmful effects of this metal may be associated with oxidative stress, in

which cells produce excessive amounts of ROS. This can result in many negative consequences, including peroxidation of lipids, denaturation of the proteins, DNA degradation, and disintegration of cell membranes. The study of Marouane et al. [22] showed that even 30 days' exposure of rats to 60 mg/kg of a vanadium compound (ammonium metavanadate) resulted in a significant increase (of over 100%) in lipid peroxidation within the tissue homogenates of the kidneys. In addition, an increase in the activity of antioxidant enzymes, such as SOD, GPX, and CAT, was observed [22]. These enzymes are the first line of defense against the negative effects of ROS on tissues. The increase in SOD activity was probably associated with the toxic effects of vanadium. Superoxide dismutase is responsible for superoxide anion neutralization. As a consequence of this process, hydrogen peroxide is released. Both the GPX and CAT enzymes neutralize hydrogen peroxide. Apart from the increase in the parameters of oxidative stress, Marouane et al. [22] observed some histopathological changes within the renal corpuscle, including shrinking of Bowman's space and reduced lumen of the proximal tubules through epithelial cell hyperplasia.

Studies on the concentration of vanadium in human organs have mostly described incidents of metal poisoning. Such a case was noted by Boulassel et al. [4], who described a fatal case of vanadium poisoning in a 24-year-old woman. The main symptoms of poisoning were extensive abdominal pain, nausea, vomiting, and diarrhea. The concentration of vanadium in the blood of the patient was 6.22 mg/L exceeding the norm of 0.07–1.1 mg/L by a factor of approximately 6000 [18,24]. Such a high concentration of vanadium doubtless resulted in impaired renal function, as confirmed by the fact that the creatinine level was 265 mmol/L (normal range 49–90 mmol/L) while the glomerular filtration rate (GFR) did not exceed 21 mL/min. Chronic kidney disease is diagnosed when the GFR is lower than 60 mL/min/1.73 m<sup>2</sup> for several measurements over the course of at least three months.

The effects of vanadium-contaminated commercial albumin solutions on renal tubular function in patients following coronary revascularization were also described. The urinary excretion of alpha-glutathione S-transferase (GST), a marker of proximal tubular damage, was examined. The mean excretion rate was significantly different from that of the control group. These data suggest that the use of commercial albumin solutions with high levels of vanadium can lead to renal failure [14].

## VANADIUM AND THE REPRODUCTIVE SYSTEM

The performance of the male and female reproductive systems can be disturbed by many chemical environmental factors, including metals. Vanadium has already been associated with reprotoxic effects [29]. Decreased weight of the body, testes, epididymides, ventral prostate, seminal vesicles, and coagulating gland were only

some of the consequences of chronic (70 and 90 days) exposure to ammonium metavanadate at a dose of 200 mg/kg b.w. or sodium metavanadate at a dose of 1 mg/kg b.w. in adult rats [25,39]. Furthermore, exposure to vanadium may result in prominent spermatogenic arrest, affecting the conversion of round spermatids to mature spermatids, reducing the epididymal sperm number, and increasing the percentage of morphologically abnormal sperm. The most commonly observed abnormal forms of spermatozoa included a banana-shaped head, an abnormal hook, and a coiled flagellar end [1,6,7,39]. There have also been other studies on rats treated with sodium metavanadate ( $\text{NaVO}_3$ ) at doses of 0.2, 0.4, and 0.6 mg/kg intraperitoneally. The medium dose led to a deterioration in the number of spermatogonia, preleptotene spermatocytes, midpachytene spermatocytes, and step 7 spermatids. The high dose resulted in testicular lesions characterized by the presence of degenerating cells in the seminiferous tubules, germinal epithelium disruption with moderate tubular necrosis, the presence of few spermatogenic cells, a reduction in round spermatids, and their failure to mature.

Vanadium can form organic complexes called vanadocenes, in which vanadium is in the +4 oxidation stage. The best known so far are vanadocene dichloride (VDC), bis (methylcyclopentadienyl) vanadium dichloride (VMDC), vanadocene dibromide (VBD), vanadocene diiodide (VDI), vanadocene diazide (VDA), vanadocene dicyanide (VDCN), vanadocene dioxycyanate (VDOCN), vanadocene dithiocyanate (VDSCN), vanadocene diselenocyanate (VDSeCN), vanadocene ditriflate (VDT), vanadocene monochloro oxycyanate (VDCO), and vanadocene monochloro acetoneitrilo tetrachloro ferrate (VDFE). Each of these has been found to exhibit spermicidal activity, even at nanomolar concentrations. The order of efficacy is  $\text{VDSeCN} > \text{VDSCN} > \text{VBD} > \text{VMDC} > \text{VDA} > \text{VDC} > \text{VDI} > \text{VDT} > \text{VDFE} > \text{VDCO}$ , according to D'Cruz et al. [9]. These researchers also reported that the exposure of highly motile human sperm to VDC resulted in a dose-dependent irreversible inhibition of sperm motility. At concentrations  $> 25 \mu\text{M}$ , VDC damaged the motility of 96% of the sperm treated. The observed decrease in sperm motility was associated with significant changes in the movement characteristics of the surviving sperm, especially with respect to the track speed (VCL), path velocity (VAP), and straight-line velocity (VSL). Furthermore, electron microscopy revealed ultrastructural alteration within the sperm subsequent to the VDC treatment.

Cells undergoing apoptosis exhibit some specific characteristics, and alteration of the plasma membrane appears very early. In apoptotic cells, some components of the plasma membrane (e.g., phosphatidyl serine) are translocated from the inner to the outer surface of the plasmalemma, which results in the exposure of these components to external factors [23]. The protein annexin V binds to the phosphatidyl serine residues exposed on the surface of apoptotic cells. Almost 60% of

VDC-treated spermatozoa exhibited an increase in the binding of annexin V. Further analysis showed that this reaction was time-dependent, with the maximum evident by 12 h after incubation. Sperm apoptosis was also confirmed by the TUNEL method [9].

The harmful effects of vanadium also concern the epididymis. Sperm cells leaving the testis are not yet fully mature. During transit through the epididymis, spermatozoa undergo a series of morphological, biochemical, and physiological changes in the process of epididymal sperm maturation. Phosphorylation, glycosylation and processing are among the posttranslational modifications of existing sperm proteins, resulting in changes in protein function and localization in the mature spermatozoa [8,36]. During epididymal maturation, the lipid, phospholipid, and cholesterol contents are modified, as is the composition of the spermatozoa [8,10]. The removal of cholesterol, the relative enrichment of particular phospholipids, and the selective gain of specific polyunsaturated fatty acids (PUFA) are physiologically significant for sperm functional maturity [8,28]. According to Chandra et al. [7], 13 days of treatment with  $\text{NaVO}_3$  resulted in a decreased number of mature spermatozoa in the tubular lumen, regressive and degenerative alterations in the epithelium of the cauda epididymis, as well as severe degenerative changes and almost complete absence of mature spermatozoa in the epididymal lumen (26 days' treatment) [7].

Furthermore, the toxicity of vanadium depends on the duration of exposure. Bharathi et al. [39] have shown that long-term (90-days) exposure of rats to sodium metavanadate at a dose of 1 mg/kg b.w. resulted in a noticeably increased sperm DNA fragmentation index (sDFI) and numerous pathological changes within the seminiferous tubules of the testes. The alterations ranged from vascular congestion to focally diffuse interstitial edema and focal areas of tubule degeneration. In addition, the microscopic analysis revealed some alterations within the seminiferous tubules, a moderate thickening of the basement membrane, a decreased number of spermatogonia and Leydig cells, an enlargement of the lumen of the seminiferous tubule, and coagulative necrosis of spermatozoa [39]. The studies on male mice also confirmed the toxic influence of vanadium. An electron microscopy analysis of spermatocytes and Sertoli cells from the seminiferous epithelium followed vanadium pentoxide inhalation ( $\text{V}_2\text{O}_5$ ) at dose of 3,64g/h, twice a week for 12 weeks showed necrotic cell death, evident from kariolysis and swelling [12]. The spermatocytes appeared to be undergoing phagocytosis by the Sertoli cells and, a fact that is also important, they exhibited cytoplasmic vacuolation. As far as the spermatogonia are concerned, some pseudoinclusions were seen, probably as a consequence of the convolution of the nuclear membrane [12]. The nuclear distortion and intracellular edema in the seminiferous epithelium were evident. Nuclear modifications in spermatids with ectoplasmic specialization (ES) edema were also observed in

the seminiferous epithelium. Furthermore, they were surrounded by edematous Sertoli cells. Ectoplasmic specializations are the most studied actin-based testis-specific type of adherens junctions and are believed to have an important function in spermatogenesis, since the basal ES support the tight junctions of the blood-testis barrier between adjacent Sertoli cells. The apical ES between Sertoli cells and spermatids participate in intracellular adhesion and the movement of germ cells through the seminiferous epithelium [26].

It is believed that the unfavorable alterations described above are due to the downregulation of the steroidogenic enzymes 17 $\beta$ -HSD, 3 $\beta$ -HSD and the consequent decline of testosterone and FSH levels. This leads to hormonal and redox imbalances within the gonads, reflected by an increase in testicular malondialdehyde level (MDA) and testicular lipid peroxidation and significant inhibition of SOD and CAT activity in a dose-dependent and time-dependent manner [7,39].

There is little data concerning the influence of vanadium on the female reproductive system, although the metal is known to be a reprotoxic factor since, among other effects, it disrupts estrous cycle regularity [19]. In the experiment of Shirastava et al. [34], female lactating rats were orally exposed to vanadium (VOSO<sub>4</sub>) at a dose of 7.5 mg/kg for 20 days. Compared to the control group, the glycogen content of the fresh uterus and ovary tissue was significantly decreased, as were the protein content and acid phosphatase activity. The uterine activity of adenosine triphosphatase and alkaline phosphatase also decreased following vanadium treatment. Additionally, a microscopic analysis of an ovary revealed a decreased diameter of the mature follicles, disintegration of the ovum, disorganized and hypertrophied developing follicles, as well as fibrotic stroma [34].

Besides its toxic effects on male and female sex organs, it seems that vanadium also particularly affects fertility, which presently poses a serious problem, with almost 20% of couples worldwide infertile. It is believed that one of the causes of infertility is the increasing pollution of the environment, including the air. This hypothesis is supported by a study of 3–12 weeks' inhalation of vanadium pentoxide at a dose of 3.46 mg/kg, which showed a decrease in actin in testicular cells and cytoskeleton damage which may be associated with impaired fertility [29].

Because vanadium crosses the blood–placenta barrier and tends to accumulate in the fetus, especially in the skeleton, pregnant females and developing fetuses are especially at risk from vanadium [37]. The study of Morgan and El-Tawil [25] confirmed the detrimental effects of a metavanadium aqueous solution at a concentration of 200 mg/kg on the fertility of both male and female

rats. The study confirmed a reduced number of pregnancies and of viable fetuses and an increased number of resorption and dead fetuses. Furthermore, the administration of metavanadium resulted in higher preimplantation and postimplantation losses. Litter weight was significantly lower at termination and on days 4, 7, 14, and 21, than in the control group. Similarly, the uterine and placental weights were reduced. Exposure to vanadium from the very first day of gestation and during lactation caused deterioration in the litter: stunted growth, micrognathia, and subcutaneous hemorrhages. Also, numerous visceral abnormalities were seen in the fetuses, including dilated brain ventricles, dilated nares, hypoplasia of olfactory pulp, cerebral hemisphere hypoplasia, microphthalmia, and anophthalmia. Anomalies in the thorax also occurred: heart hypertrophy, lung hypoplasia, and intrathoracic hemorrhages. The urinary tract was also affected by vanadium, with renal hypoplasia, hydroureter, and renal pelvis dilation found in the litter. As with the urinary tract, the detrimental effects were related to the skeleton and included wide separation of the parietal bones; incomplete ossification of the parietal or interparietal bones; incomplete ossification of sternebrae; reduced number of sternebrae; wavy and extra ribs; absence of carpals, metacarpals, tarsals, and metatarsals; and absence of caudal bones and phalanges [25].

Additionally, pathological alterations of the above mentioned organs have been shown in Table 1. Moreover, toxic influence of vanadium on fertility and fetuses after metavanadium treatment during pregnancy and lactation in rats has been presented in Table 2.

## CONCLUSIONS AND FUTURE DIRECTIONS

Due to the increasing use of vanadium in medicine, including its use as a potential drug to treat diabetes mellitus, the threats posed by the adverse effects of this metal on many organs including the stomach, intestines, liver, kidneys, and male and female reproductive systems need to be considered. Knowledge of this should be further expanded, not only by studies based on blood, biochemical, and histological analysis, but also by those dealing with the subcellular level.

Finally, it is worth adding that studies on the protective effects of many dietary supplements against the effects of vanadium have recently been carried out; these supplements include alpha glucosyl hesperidin [15], *Sesamum indicum* [27], vitamin E [25], tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) [34], zinc sulfate [27], testosterone propionate [6], selenium [34], and *Malva sylvestris* [22]. Positive results in such experiments would allow the full use of the medical aspects of vanadium even given its adverse effects.

**Table 1.** Toxic influence of vanadium in mg/kg on different tissues/organs

| Compound and dose of V                            | Pathological changes                                                                                                                                                                                                                                                                                                                                                                   | Species  | Source |
|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|--------|
| intestine                                         |                                                                                                                                                                                                                                                                                                                                                                                        |          |        |
| ammonium metavanadate, 10 mg/kg                   | focal shortening of intestinal villi, mild dilatation of crypts                                                                                                                                                                                                                                                                                                                        | mice     | [16]   |
| ammonium metavanadate, 20 mg/kg                   | marked dilatation of crypts, degeneration and necrosis of mucosal epithelial cells                                                                                                                                                                                                                                                                                                     |          |        |
| spleen                                            |                                                                                                                                                                                                                                                                                                                                                                                        |          |        |
| ammonium metavanadate, 10 mg/kg                   | atrophy of lymph follicles and loss of lymphocytes                                                                                                                                                                                                                                                                                                                                     | mice     | [16]   |
| Liver                                             |                                                                                                                                                                                                                                                                                                                                                                                        |          |        |
| ammonium metavanadate, 45 mg/kg                   | degradation of hepatocytes, the vacuolar degeneration and fatty degeneration                                                                                                                                                                                                                                                                                                           | broilers | [20]   |
| vanadium (IV) oxide sulfate monohydrate, 90 mg/kg | fatty degeneration, degeneration of hepatocytes, structural damage and focal inflammation, increasing of AST and ASP                                                                                                                                                                                                                                                                   | mice     | [30]   |
| vanadyl sulfate, 18.7 mg/kg                       | degenerative changes in the mitochondria, endoplasmic reticulum and nucleus of hepatocytes, increasing of glycogen content                                                                                                                                                                                                                                                             | rats     | [33]   |
| Kidney                                            |                                                                                                                                                                                                                                                                                                                                                                                        |          |        |
| ammonium metavanadate, 30, 45, 60 mg/kg           | pathologically altered renal tubular epithelial, swelling of the cells, vacuolization and granular cell degeneration                                                                                                                                                                                                                                                                   | broilers | [20]   |
| vanadium (IV) oxide sulfate monohydrate, 90 mg/kg | pyelonephritis, thickening of basement membranes, pyknotic nuclei, vacuolization and cytoplasmic debris                                                                                                                                                                                                                                                                                | mice     | [30]   |
| Testis                                            |                                                                                                                                                                                                                                                                                                                                                                                        |          |        |
| sodium metavanadate, 0.2; 0.4; 0.6 mg/kg          | decreased number of: spermatogonia, preleptotene spermatocytes, mid pachytene spermatocytes and step 7 spermatid, testicular lesions: degenerating cells, germinal epithelium disruption in the seminiferous tubules, moderate tubular necrosis, presence of few spermatogenic cells, reduction in the number of round spermatides, failure spermatids maturation to mature spermatids | rats     | [7]    |
| vanadium pentoxide 3.64 g/h                       | actin decrease in testicular cell, cytoskeleton damage                                                                                                                                                                                                                                                                                                                                 | mice     | [29]   |
| sodium metavanadate, 1 mg/kg                      | interstitial oedema, focal areas of seminiferous tubules degeneration, vascular congestion, mild thickening of basement membrane, decreases number ob spermatogonia and Leydig cells, enlargement of central lumen                                                                                                                                                                     | rats     | [39]   |
| vanadium pentoxide 3.64 g/h                       | pseudoinclusion of spematogonia, convolution of the nuclear membrane, kariolysis, cytoplasmic vacuolation, swelling, nuclear distortion, intracellular edeme of spermatocytes and Sertoli cells, nuclear modifications of spermatids                                                                                                                                                   | mice     | [12]   |
| ammonium metavanadate, 200 mg/kg                  | decreased weight                                                                                                                                                                                                                                                                                                                                                                       | rats     | [25]   |

| Compound and dose of V                   | Pathological changes                                                                                                                                                                                      | Species | Source |
|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------|
| Epididymis                               |                                                                                                                                                                                                           |         |        |
| sodium metavanadate, 0.2; 0.4; 0.6 mg/kg | decreased number of mature spermatozoa in the tubular lumen, regressive and degenerative changes in cauda epididymis, degenerative changes with almost absence of mature spermatozoa in lumen             | rats    | [7]    |
| ammonium metavanadate, 200 mg/kg         | decreased weight                                                                                                                                                                                          | rats    | [25]   |
| Sperm                                    |                                                                                                                                                                                                           |         |        |
| sodium metavanadate, 1 mg/kg             | increased sperm DNA fragmentation index (sDFI), coagulative necrosis of spermatozoa                                                                                                                       | rats    | [39]   |
| prostate gland                           |                                                                                                                                                                                                           |         |        |
| ammonium metavanadate, 200 mg/kg         | decreased weight                                                                                                                                                                                          | rats    | [25]   |
| semianl vesicles                         |                                                                                                                                                                                                           |         |        |
| ammonium metavanadate, 200 mg/kg         | decreased weight                                                                                                                                                                                          | rats    | [25]   |
| Uterus                                   |                                                                                                                                                                                                           |         |        |
| vanadyl sulfate, 7.5 mg/kg               | decreased glycogen and protein content, decreased activity of acid phosphatase, inhibition of adenosine triphosphatase and alkaline phosphatase activities                                                | rats    | [34]   |
| ammonium metavanadate, 200 mg/kg         | decreased uterine mass                                                                                                                                                                                    | rats    | [25]   |
| ovary                                    |                                                                                                                                                                                                           |         |        |
| vanadyl sulfate, 7.5 mg/kg               | decreased matured follicles, disintegration in ovum, disorganized and hypertrophied developing follicles, fibrotic stroma, decreased glycogen and protein content, decreased activity of acid phosphatase | rats    | [34]   |

**Table 2.** Toxic influence of vanadium on fertility and fetuses after metavanadium treatment at dose 200 mg/kg during pregnancy and lactation in rats [25]

| Test factor              | Result |
|--------------------------|--------|
| Number of implants       | ↓      |
| Number of viable fetuses | ↓      |
| Number of resorption     | ↑      |
| Number of dead fetuses   | ↑      |
| Litter weight            | ↓      |
| Placenta mass            | ↓      |
| Mean fetal body weight   | ↓      |

| Test factor                                     | Result                                                         |
|-------------------------------------------------|----------------------------------------------------------------|
| Incidences of gross anomalies in the fetuses    | stunted growth                                                 |
|                                                 | subcutaneous hemorrhages                                       |
|                                                 | micrognathia                                                   |
| Incidences of head anomalies in the fetuses     | dilated brain (hydrocephaly)                                   |
|                                                 | dilated nares                                                  |
|                                                 | olofactory pulp hypoplasia                                     |
|                                                 | cerebral hemisphere hypoplasia                                 |
|                                                 | microphthalmia and anophthalmia                                |
| Incidences of thorax anomalies in the fetuses   | heart hypertrophy                                              |
|                                                 | lung hypoplasia                                                |
|                                                 | intrathoracic hemorrhages                                      |
| Incidences of pelvic anomalies in the fetuses   | dilated renal pelvis (hydronephrosis)                          |
|                                                 | renal hypoplasia                                               |
|                                                 | hydrourether                                                   |
| Incidences of skeletal anomalies in the fetuses | wide separation of parietal bone                               |
|                                                 | incomplete ossification of parietal and/or interparietal bones |
|                                                 | incomplete ossification of sternbrae                           |
|                                                 | reduced sternbrae number                                       |
|                                                 | wavy and extra ribs                                            |
|                                                 | absence of carpal, metacarpal, tarsal and metatarsal bones     |
|                                                 | absence of caudal bones and phalanges                          |

↓: decreased

↑: increased

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