Mini Review

Dimethyl Sulfoxide and Their Toxicity

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1- Abstract:

The aim of the present mini-review was to report a toxic effect of dimethyl sulfoxide (DMSO) that has indicated to change the histology of the liver and kidney of rats. DMSO is a powerful co-solvent and often is used in early metabolic studies when compounds are poorly characterized and difficult to become soluble in water. To become soluble, they have to be dissolved in organic lipophilic solvents (vehicles). Following its conventional use, plant extract has been exposed to various intense and sub-acute toxicity studies to legitimize its toxicological security. Normally, DMSO has low fundamental toxicity yet plant extract when dissolved in 10% DSMO can cause noteworthy confined toxic impacts in the liver and kidney of rats. Be that as it may, vehicles currently in use have pleiotropic impacts, which are regularly obscure. Therefore, researchers ought to be cautious in the preparation and storage of substances before they are dispensed to animals. If this is not done, it may lead to accidental adverse effects on the animals and frequently result in

inaccurate outcomes. In this mini-review, we summed up data on biological impacts of the DMSO most generally utilized lipophilic medication vehicles. Other than in experimental models, the information, where accessible, are presented on the impacts of solvents in therapeutic use in rodents. All in all, a few suggestions are given on the utilization of medication solvents in tests.

Key Terms: DMSO, *Clinacanthus nutans*, mice, rat, toxicity.

2- Introduction: DMSO is an organosulfur compound with the equation $(CH_3)_2SO$, and a widely recognized solvents for hydrophobic substances for *in vivo* and *in vitro* reasons. Consequently, it is generally utilized in experimental biology and pharmacology [1]. Due to the weak solubility of some biologic agents in water, their extract ought to be dissolved in an organic vehicle which ought to be biocompatible, 100% dependable, with no biological impacts. Numerous components add to vehicle tolerance involving those pertaining to animals, the characters of the vehicle, the manners in which that are being performed, and the nature and length of the examination [2]. The toxicity of DMSO is low: lethal dose (LD50) in mice is 6.2 mL/kg when applied intraperitoneally (i.p) or 3.7 mL/kg applied intravenously (i.v.), LD50 in rats is 9.9 mL/kg (i.p) or 7.4 mL/kg (i.v.). It is noteworthy that some of the investigations cited in this review found a substantial effect even with doses as low as 0.1 mL/kg [3]. The Food and drug administration (FDA) to conclude that DMSO was highly toxic, and almost all United States-based research was stopped. Since 1965, the FDA has permitted very little work with DMSO [4], and currently approved, veterinary applications of DMSO are limited [5]. Selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation, including considerations for sterility if the substance is being administered perinentally; and dosing apparatus and animal restraint necessary for specific routes of delivery, should be scrutinized when deciding on delivering substances to animals. Also, research teams should take note of probable adverse effects related to substance administration to avoid confounding effects with other areas of the study design and to ensure accurate interpretation of research results [2].

3- Some Side-Effects of Dimethyl Sulfoxide

The liver is the fundamental organ in charge of the digestion of medications and lethal synthetic compounds, and numerous solvents (cationic and amphiphilic) can move in mitochondria because of the mitochondrial layer potential [6]. Collection of these

solvents inside the hepatocytic mitochondria represses unsaturated fatty acid β-oxidation (causing steatosis) and electron exchange along the respiratory chain [6]. Oxidative stress refers to raised intracellular levels of receptive oxygen species (ROS) that damages lipids, proteins, and DNA. Oxidative stress has been found to be connected to a series of pathologies [6]. Even though the pathophysiologic mechanisms of hepatotoxicity are as yet being investigated, studies have shown that they cause organic and functional harm to the liver in the following ways; (1) Alteration of the hepatocyte; with significant reduction in adenosine triphosphate (ATP) levels and a marked disassembly of actin fibrils at the surface of the hepatocyte with blistering and rupture of the cell membrane; (2) Alteration of the transport proteins; transport proteins may be affected by toxins at the canalicular layer which can inhibit bile flow; (3) Cytolytic T-cell activation: the covalent binding of a toxin to the P-450 enzyme acts as an immunogen resulting in the activation of T cells and cytokines and stimulating a multifaceted immune response; (4) Apoptosis of hepatocytes; this entails the activation of the apoptotic pathways by the tumor necrosis factor-alpha receptor of FAS which may trigger the course of intercellular capiases resulting in programmed cell death; and (5) Bile duct injury; toxic metabolites excreted in bile may make damage the epithelium of the bile duct [7]. Drugs that cause tubular cell toxicity do so by disrupting mitochondrial function, interfering with tubular transport, increasing oxidative stress, or by forming free radicals [8]. DMSO has low acute and chronic toxicity for animal, plant, and aquatic life [5]. Hepatic toxicity of DMSO is common among animals, and there have been reports of increased liver weight, steatosis, centrilobular single cell necrosis, transaminasemia, biliary hyperplasia and hepatic focal cystic degeneration [7, 9]. However, DMSO at low concentrations showed toxic properties in the blood cells, inducing RBCs hemolysis, reducing cryopreserved platelets activities, decreasing platelets aggregation levels, inhibiting the growth of EAhy926 cells, preventing cell progression from G1 phase to S phase, and elevating apoptosis rates [5]. Jourdon *et al*. [4] demonstrated that neuroblastoma × glioma hybrid NG 108-15 cells propelled by DMSO 0.5-1% DMSO (v/v) reversibly blocks sodium, potassium, and calcium currents and moves the sodium inactivation curve towards increasingly negative voltages, to expand membrane fluidity and to adjust its structure [10]. All these mechanisms (or probably a combination of them), which have been described in isolated systems and which induce a functional impairment in nerve conduction. DMSO is a highly polar compound with an exceptional ability to penetrate biological membranes and facilitates oxygen diffusion across these membranes. DMSO probably blocks arachidonic

acid conversion to prostaglandins (PG) and specifically blocks the receptors of PGFa, a most powerful vasoconstrictor. On the other hand, it increases the synthesis of PGE. This prostaglandin causes vasodilatation probably through its inhibition of calcium release, thus preventing the release of noradrenalin and its effect on the microcirculation [11]. The latter injected DMSO intraperitoneally in various concentrations (1 to 100%, 5 mL/kg) in rats, and showed that the incidence of aberrant femoral bone marrow cells increased from 10% at the 1% DMSO level to about 70% at the 100% DMSO level, and the incidence of aberrant cells in all treated groups was significantly elevated when compared to controls [12]. Three groups of 8 male Sprague-Dawley rats were subjected to an aerosol of 1600 mg DMSO per cubic meter of air for 4 hr. Control rats were subjected to a normal chamber condition. Groups were relinquished following exposure, then after another 24 hr and the third group was watched for about fourteen days after being subjected. There was no death and none of the rats showed outward indications of toxicity during and after they were exposed to DMSO. After been subjected to DMSO, their hair was clammy and slightly yellow, and the rats had a garlic-like scent. At necropsy, their organs seemed okay. Control rats and those treated with DMSO showed areas of hemorrhage during histopathologic test. Focal and diffuse accumulations of clear desquamated pneumocytes were noted inside lung alveoli in DMSO treated rats; identical edematous changes were not found in the controls [13]. Thioacetamide (400 mg/kg body weight, i.p.) was given to rats, and after 24 hr plasma glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities were greatly increased. These outcomes demonstrated that the necrotic procedure was started at around 12 hr and grew from that point. By co-administration of dimethyl sulphoxide (DMSO, 1 hr before, and 8 hr after administration of thioacetamide: each time, 2.5 mL/kg body weight) plasma GOT and GPT markedly decreased even similar to the control group, demonstrating that DMSO completely inhibited the necrotic activity of thioacetamide [14]. Since mammalian advancement and cellular differentiation are controlled epigenetically by DNA methylation and histone changes, DMSO likely influences the epigenetic system. The impacts of DMSO on transcription of three noteworthy DNA methyltransferases (Dnmts) and five very much considered histone modification enzymes were studied in mouse embryonic stem cells and embryoid bodies (EBs) by reverse transcription-polymerase chain reaction. Addition of DMSO (0.02%-1.0%) to EBs in culture prompted an increment in Dnmt3a mRNA levels with increasing dosage [15]. They recommend that the integrity of rat's chromosome structure is significantly distorted by DMSO [12].

Clinacanthus nutans (Burm. f.) Lindau is popularly referred to as Sabah snake grass in Malaysia. It belongs to the family Acanthaceae, but is known by several other vernacular names: Belalai Gajah (Malay), Dandang gendis (Javanese), Tajam (Sunda) in Indonesia; Phaya Yo, Phaya Plong Thong in Thailand; Twist of Flowers, Alligator Flower, Zuihua in Chinese. *Clinacanthus nutans* has been utilized in folk medicine for a long time in various parts of Asia because of its range of pharmacological effects. This plant is typically utilized as a natural medicine in Malaysia, Indonesia and Thailand treat various ailments as reported by Zulkipli *et al* [16]. Following its traditional use, has been subjected to several acute and subacute toxicity studies to justify its toxicological safety [17]. A recent study showed no histopathological changes in the liver and renal cortex of the kidney after oral administration of *Clinacanthus nutans* water extract (5000 mg/kg of body weight) for 14 days in Sprague Dawley rats [17]. However, Asyra *et al.* [18] reported that oral administration of ethanol extract of *Clinacanthus nutans* dissolved in 10% DMSO at doses of 75, 125 and 250 mg/kg of body weight daily for 90 days caused hepatotoxicity and renal toxicity in male Sprague Dawley rats. When the groups subjected to medium and high dose were observed, noteworthy unusual histopathological changes, were seen in their liver tissues. In their Kidney tissue, there were clear histopathology changes, for example, granular cast and cell cast were seen in these groups. The toxicity level observed here is in tandem with the findings of Chen *et al*. [19]. This study concluded that DMSO caused the most severe local toxicity due to the highest score on gallbladder presence of mild hepatocyte damage and bile duct injury in farm piglets. Moreover, when DMSO is not present, killer T (NKT) cells and natural killer (NK) cells do not play a pathologic role in acetaminophen-induced liver injury in C57Bl/6 mice. On the other hand, when DMSO is present, acetaminophen-induced liver injury was significantly attenuated in mice with reduced NKT and NK cells prior to acetaminophen treatment [20]. This level of toxicity agrees with what was reported in a study by Cavaletti *et al*. [1], where they reported neurophysiological and pathological changes after intraperitoneal administration of different DMSO solutions (1.8-7.2%) treated for 10 consecutive days and monitored for an additional 45 days in Wister rats. No structural changes were found in the sciatic nerve at 1.8% and 3.6% DMSO concentrations, suggesting that the mechanism of action of DMSO involves a functional impairment (i.e. conduction block) similar to that described earlier for this substance in isolated systems. Marked structural changes were however observed in the sciatic nerve, with obstruction of myelin and uncompacted myelin lamellae, when DMSO was

administered at 7.2% concentration, DMSO increased enzymatic activity of glucagon and effect on adenylate cyclase activity is reversible in rats [21]. Addition of DMSO (0.02% - 1.0%) to embryonic bodies in culture induced an increase in methyl transferases mRNA levels with increasing dosage [15]. In a study conducted by Zakaria *et al*. [22] they utilized methanolic leaf extract of *Clinacnthus nutans* with dosages of 50, 500 and 2500 mg/kg given over a time of 28 days and found no toxicity signs in the liver and kidney in both sexes of mice. This outcome is in accordance with a previous study by Kurdi *et al*. [23] where they demonstrated that when 1000 and 2000 mg/kg of methanolic extract was administered orally to mice for 28 days, there were no toxicity signs in the liver and kidney. Nevertheless, strain differences in response to xenobiotic are almost universal. They include differences in acute toxicity, neurotoxicity, carcinogenesis, teratogens and immune-toxicological reactions [24]. Research has also found that a high dose of streptozotocin (STZ) 100 to 200 mg/kg can cause diabetes in mice, whereas a dose of 35-65 mg/kg can cause the same effect in rats [25]. In other words, the mice are more resistant to the compound than the rats [26]. Factors ranging from distribution, absorption, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration; duration of treatment; pH, stability, homogeneity, and osmolality of the substance to be administered; selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation, including considerations for sterility if the substance is being administered perinentally; and dosing apparatus and animal restraint necessary for specific routes of delivery, should be scrutinized when deciding on delivering substances to animals. Also, research teams should take note of probable adverse effects related to substance administration to avoid confounding effects with other areas of the study design and to ensure accurate interpretation of research results [2].

4- Summary

We conclude that 10% DMSO enhances the toxicity of liver and kidney of rats based on the result from the study of Asyura *et al*. [17]. Researchers should be careful to reduce the amount of organic solvent (or reduce its concentration in vitro experiments) to the minimal dose possible. Besides that, we recommend that the plant extract is properly closed after the freeze dryer and kept in -20˚C because it is easily dissolved in aqueous solvents with changed pH or ionic strength and by thorough mixing of the solution. The

findings may serve as a useful guide for future researchers. It is also desired that the findings would reveal the attributes of this plant.

Abbreviations:

Dimethyl sulfoxide (DMSO); lethal dose (LD50); intraperitoneally (i.p); intravenously (i.v.); Food and drug administration (FDA); adenosine triphosphate (ATP); prostaglandins (PG); glutamate-oxaloacetate transaminase (GOT); glutamate-pyruvate transaminase (GPT).

Conflict of Interest

The authors have declared that no competing interests exist.

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