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# PRECLINICAL SAFETY AND TOXCITY STUDY OF WAMUPGEL IN WISTAR RATS

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## ABSTRACT
WAMUP is an aqueous based gel formulation containing 5% L-Arginine for topical application for both males & females. It is indicated for Female Sexual Arousal Disorder (FSAD), Dyspareunia and Lubrication of Dry Vagina and other menopausal disorders in females and mild to moderate erectile dysfunctions of males. The present study was performed to asses the systemic toxic effects of WAMUP GEL, when administered sub cutaneously to male and female Wistar Rats. All the animals were conditioned, randomized and divided in three groups (3 Females and 3 Males). Two groups of animals (TG-I and TG-II) were administered with 150mg/ml and 300mg/ml dose of L-Arginine respectively and another Group of 3 male and 3 female were kept as control (Group II-Control) and treated with vehicle solution. Animals were observed for a period 14 days after the administration. Parameters evaluated including clinical signs of toxicity, live phase of animals, cage side observations, and body weight and food consumption. Blood samples were collected on day 7 and 14 for hematology and clinical chemistry analysis. Animals were necropsied and vital organs were weighed and subjected to histopathological examination.[2,8] No mortality was observed in any group. There were no differences in body weight gains, live phase, physical activity and abnormal neurological observation between the control and test groups Rats throughout the study period. No major abnormal clinical signs were observed at the site of application. Their biochemical and hematological profiles were in normal range in all groups.[15] No significant differences were observed in the histopathology of various organs between control and test groups at different time points.[16]

**KEYWORDS:** Clinical laboratory investigations, hematology, histopathology.

## 1. INTRODUCTION
L-Arginine is the precursor of NO. In animal models of cardiovascular disease, supplementation with L-arginine improves vasodilation and increases vascular NO synthesis. In preclinical studies, long-term administration of l-arginine inhibits atherosclerosis and myointimal hyperplasia and enhances angiogenesis. More importantly, short-term studies in patients with cardiovascular risk factors or disease indicated that l-arginine supplementation may increase NO synthesis and improve vasoreactivity. In patients with severe peripheral arterial disease (PAD), intravenous infusion of l-arginine (30 g over 30 minutes) increases limb blood flow 2-fold and to the same extent as prostaglandin E1 The increase in limb blood flow is associated with parallel increases in urinary nitrogen oxides, as well as plasma and urinary cGMP, consistent with increased NO synthesis.[14]

WAMUP is an aqueous based gel formulation containing 5% L-Arginine for topical application for both males & females. It is indicated for Female Sexual Arousal Disorder (FSAD), Dyspareunia and Lubrication of Dry Vagina as in cases of Menopause and other disorders. L-arginine in WANUP GEL releases nitric oxide (NO) through nitric oxide synthase enzyme (NOS).[8] NO activates the enzyme guanyl cyclase which in turn converts GTP to cyclic GMP and cGMP dilates blood vessels to ensure tissue engorgement and heightened sensitivity. The tissue changes result in less vaginal spasm (vaginismus) and greater suppleness of the genitals with facilitation of penetration. The act of applying Wamup mutually helps relax not only the vaginal wall and muscles, but also the muscles and soft tissues of pelvic floor and even the skeletal muscles of the inner thighs. The heightened sense of pleasure automatically reduces the sense of discomfort and pain.[56]

## 2. OBJECTIVE
The objective of the study is to investigate the systemic toxic effects of WAMUP GEL in male and female Rats (Wistar).

**3. Experimental details.**

| S.No | No. of Animals & sex | Group details | Duration of Exposure | Study duration # |
|---|---|---|---|---|
| 1 | 10(5M+5F) | **Group I (Control)** Phosphate buffer saline | Single exposure daily | 14 days |
| | | | | |
| 2 | 10(5M+5F) | Group II Group II (Test I) 150 mg/mL DEBRIDACE | Single exposure daily | 14 days |
| 3 | 10(5M+5F) | Group II Group III (Test II) (Test))II) | Single exposure daily | 14 days |

**3.1 Experimental methodology**

Systemic toxicity study was conducted in thirty Rats (15 males + 15 females), which were divided into three groups *viz.*

(i) Group-1 Vehicle Control (VC).
(ii) Group-2 Test Group (TG I) and,
(iii) Group-3 Test Group (TG II).
(iv) Animals were conditioned for a period of 7 days after randomization.

**3.2 Experimental procedure**

As mentioned in the above table all the animals were randomized in to 3 groups and test compound administered to each group of 5 males and 5 females sub-cutaneously. Before the test compound administration hair at the site of the injection was removed by clipping.[9,10] At most care was taken to avoid abrading the skin while clipping of the hair. Test compound was administerd sub-cutaneously between the shoulder blades under the perfect restraint position by using 23 G, 1 inch needle attached with guarded syringe.[4,6]

The animals were monitored for behavior, cage side activity, bio chemical and hematology during the experimental period. On 14$^{th}$ all animals subjected to hematology, biochemistry and necropsy. Collected the vital organs and examined the same for histopathology including site of injection.[12]

**4. OBSERVATIONS**

**4.1. PHYSICAL EXAMINATION**

Physical examinations were made periodically and the following observations were recorded: The physical appearance of hair coat whether it is clean/groomed/soiled, Lacrimation the presence of eye secretions if any (normal, excessive), salivation (normal, excessive etc.), animals were observed for eyelids closure (wide open, drooping and fully closed)Respiration character and Respiratory rate were observed (viz. normal, decreased, increased, shallow, deep, and gasping), Biting character was also recorded, the presence of specific tremors (body, head, impaired locomotion) if any was recorded, convulsions if any were recorded immediately after the exposure to the test compound and daily during the post exposure phase. All the above-mentioned parameters were recorded at least twice a weekly.[2]

**4.2. Clinical Laboratory Investigations**

**4.2.1 Clinical Chemistry**

Plasma glucose, creatinine, total protein, ALP, SGOT, SGPT, Bilirubin, Potassium, Magnesium and Calcium were estimated using Alfa Biotech PLD 951 autoanalizer, supplied by Wipro Biomed. All analytical kits were purchased from Wipro Biomed.[11,13]

Animals were starved for 22hrs blood samples were drawn from orbital venous plexus, collected in commercially available heparinized vacuette tubes, and centrifuged at 3000 rpm for 10 minutes to separate the plasma for clinical chemistry analyses.

Quality control samples supplied by TRANSASIA BIO-MEDICALS LTD (ERBA controls for biochemistry) were used to establish the precision and accuracy of the analyses. Quality control samples at two levels (level 1 and level 2) supplied by Wipro Biomed were used to establish the precision and accuracy of the analyses. Following are the principles underlying various analyses carried out1.[7]

**i) Glucose (mg/dl)**

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red – violet quinoneamine dye as indicator. The absorbance of the reaction was monochromatically measured at 500 nm.

**ii) Creatinine (mg/dl)**

Creatinine reacts with Picric acid reagent under alkaline medium to form an orange colored complex**.** The rate of formation of this complex is measured by reading the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinine.

**iii) Proteins**

Copper ions complex with the peptide bonds of protein under alkaline conditions will form a violet-coloured compound. The amount of the violet complex is proportional to the increase in absorbance was measured monochromatically at 546 nm.

**iv) Blood urea**

In the presence of urease, urea was hydrolyzed to yield ammonia and carbon dioxide. The ammonia formed then

reacts with 2-oxoglutarate in the presence of glutamic dehydrogenase (GD) and NADH, to produce L-glutamate. The resulting decrease in absorbance of NADH at 340 nm is proportional to the level of urea nitrogen in the sample.

**v) ALP**

The enzymatic sequence employed in the essay of alkaline phospatase is as follows:

P-Npp + H2O p-Nitriphenol+H3PO4

p-Npp is colorless but p-Nitriphenol has strong absorbance at 405 nm. The rate of increased absorbance at 405 nm is proportional to the enzymatic activity.

**vi) SGOT (IU/L)**

Aspartate aminotransferase was assayed based upon the transamination of aspartic acid to 2-Oxoglutaric acid. The resulting Oxaloacetate was enzymatically transformed to malate by the NADH-dependent malate dehydrogenase (MDH)**.** A redox system involving NADH - NAD was adopted for this purpose.

**vii) SGPT (IU/L)**

Alanine aminotransferase was assayed based upon the transamination reaction mediated between L-Alanine and 2-Oxoglutarate resulting in the formation of Pyruvate and L- Glutamate. The resulting pyruvate was enzymatically transformed to lactate by the NADH-dependent Lactate dehydrogenase (LDH)**.** A redox system involving NADH - NAD was adopted for this purpose.

**viii) Magnesium (mEq/L)**

Magnesium combines with calmigate in an alkaline medium to form a red coloured complex. Interference of calcium and proteins is eliminated by the addition of specific chelating agents and detergents. Intensity of the color formed is directly proportional to the amount of magnesium present in the sample.

**ix) Sodium (mEq/L)**

Sodium and proteins are precipitated with magnesium and Uranyl acetates to form Uranyl magnesium sodium acetate. The precipitates are separated by centrifugation. The excess of Uranyl ions in supernatant will react with Potassium Ferrocyanide to produce a brownish color in acidic medium. The absorbance of color is measured at 550nm and inversely proportional to the concentration of the Sodium in the sample.

**x) Calcium (mg/dl)**

In alkaline solution, Calcium binds with metal complex one dye O-Cresolphthalein Complex one (OCPC) to form a Bluish-Purple complex, which is measured at 578 nm. The intensity of color formed is proportional to Calcium concentration in the sample.

**xi) Potassium (mEq/L**)

Potassium is measured turbid metrically. Potassium reacts with Sodium Tetra phenyl Boron, resulting in an insoluble turbid suspension. The extension of turbidity is measured at 630 nm and is proportional to the concentration of the Potassium in the sample.

### 4.2. HEMATOLOGY

Hematological investigations included - Total white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and mean platelet volume (MPV) and differential leucocyte count. These investigations were carried out using the blood samples drawn after 7 and 14 days of post exposure. Samples were drawn into tubes containing EDTA K2 from the orbital plexus using heparinized microhaematocrit tubes and analyzed on an automated blood cell counter (Medonic-CA 620).[7]

### 4.3. HISTOPATHOLOGY

The following organs and tissue samples were collected from all the animals and preserved in 10% buffered neutral formalin: Brain, thymus, spleen, kidney, heart, lung, trachea, sternum, liver, gastro intestinal tract, testes and ovaries.[17]

### 4.4. Physical examination

The clean groomed hair coat, no piloerection, excessive lacrimation, salivation was observed in the experimental animals during study period. There were no significant abnormalities in respiration character & rate in all groups of animals during the experiment. The eye prominence was found to be normal in all the groups of animals during the experimental period. There were no convulsions, aggressive biting character and tremors recorded immediately after the administration of test compound and during the post exposure in any group of animals (Table-1).

## 5.0 RESULTS

**Table 1: Ophthalmological Examination**

**Male**

| **Group (N)** | | **1(5)** | **2(5)** | **3(5)** |
|---|---|---|---|---|
| **Dose (mg/kg BW)** | | **Control** | **150 mg/ml** | **300 mg/ml** |
| Eye | Observation | No. of Animals Showing Observation | | |
| Right | Normal | 5 | 5 | 5 |
| Left | Normal | 5 | 5 | 5 |

Key: N = Number of animals in group, BW: Body weight.

**Female**

| **Group (N)** | | **1(5)** | **2(5)** | **3(5)** |
|---|---|---|---|---|
| **Dose (mg/kg BW)** | | **Control** | **150 mg/ml** | **300 mg/ml** |
| Eye | Observation | No. of Animals Showing Observation | | |
| Right | Normal | 5 | 5 | 5 |
| Left | Normal | 5 | 5 | 5 |

Key: N = Number of animals in group, BW: Body

weight.

**4.2 Clinical Laboratory Investigations**

**4.2.1 Clinical chemistry**

The statistical analysis indicating that there were no significant abnormalities between the test and control groups in clinical chemistry parameters viz. blood glucose, total protein, SGOT, SGPT, ALP, creatinine and electrolytes (Magnesium, Potassium and Calcium) in mice exposed to test compound at various time points. All the parameters were within the physiological range (Table – VIII). Means for all clinical chemistry parameters were found to be similar between test groups and vehicle control.(Table-2).

**Table 2: Clinical Chemistry.**

**Male**

| Group (N) | 1(5) | | 2(5) | | 3(5) | |
|---|---|---|---|---|---|---|
| Dose (mg/kg BW) | Control | | 150 mg/ml | | 300 mg/ml | |
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| Glucose mg/dl | 66.71 | 5.89 | 72.81 | 13.34 | 87.878 | 7.72 |
| Cholesterol mg/dl | 107.87 | 15.73 | 111.63 | 12.31 | 135.12 | 22.78 |
| Triglyceride mg/dl | 117.69 | 35.86 | 130.24 | 67.28 | 115.126 | 26.97 |
| ALT IU/L | 65.75 | 24.62 | 64.28 | 12.76 | 59.318 | 8.72 |
| AST IU/L | 185.35 | 30.03 | 168.13 | 12.47 | 154.30 | 20.03 |
| GGT IU/L | 3.93 | 2.64 | -1.80 | 4.95 | 1.475 | 1.28 |
| ALP IU/L | 208.57 | 98.70 | 164.09 | 43.20 | 212.47 | 79.13 |
| Calcium mg/dl | 12.02 | 1.18 | 16.93 | 5.49 | 15.304 | 3.09 |
| Urea mg/dl | 49.59 | 9.19 | 43.86 | 7.47 | 50.92 | 5.83 |
| Phosphorus mg/dl | 6.42 | 0.49 | 7.75 | 2.12 | 8.8 | 1.02 |
| Albumin g/dl | 4.58 | 0.15 | 4.70 | 0.21 | 4.608 | 0.21 |
| T. Protein g/dl | 7.32 | 0.19 | 7.55 | 0.44 | 7.352 | 0.30 |
| T.Bilirubin mg/dl | 0.11 | 0.04 | 0.17 | 0.11 | 0.214 | 0.17 |
| Creatinine mg/dl | 0.29 | 0.22 | 0.3 | 0.11 | 0.262 | 0.19 |
| Sodium mmol/L | 148.11 | 2.33 | 148.89 | 1.01 | 149.768 | 0.97 |
| Potassium mmol/L | 2.94 | 0.30 | 2.52 | 0.09 | 2.558↓ | 0.21 |
| Chloride mmol/L | 99.28 | 0.85 | 99.90 | 0.90 | 101.86↑ | 1.20 |
| BUN mg/dl | 23.17 | 4.29 | 20.50 | 3.49 | 23.792 | 2.73 |

Key: SD = Standard deviation, N = Number of animals in group, BW = Body weight.

**Females**

| Group (N) | 1(5) | | 2(5) | | 3(5) | |
|---|---|---|---|---|---|---|
| Dose (mg/kg BW) | Control | | 150 mg/ml | | 300 mg/ml | |
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| Glucose mg/dl | 89.99 | 13.80 | 73.99 | 10.67 | 87.49 | 11.54 |
| Cholesterol mg/dl | 122.40 | 15.96 | 120.89 | 22.12 | 115.15 | 17.13 |
| Triglyceride mg/dl | 193.91 | 70.28 | 177.77 | 62.99 | 239.70 | 89.85 |
| ALT IU/L | 72.74 | 7.29 | 72.79 | 13.73 | 69.61 | 14.69 |
| AST IU/L | 181.67 | 32.07 | 175.92 | 31.89 | 177.74 | 41.55 |
| GGT IU/L | 1.74 | 0.58 | 2.22 | 1.31 | 2.24 | 1.42 |
| ALP IU/L | 337.31 | 45.46 | 365.30 | 56.14 | 398.62 | 65.08 |
| Calcium mg/dl | 13.78 | 2.04 | 14.95 | 1.56 | 19.37 | 4.83 |
| Urea mg/dl | 57.95 | 7.01 | 52.23 | 7.18 | 59.40 | 7.85 |
| Phosphorus mg/dl | 8.79 | 0.69 | 9.11 | 0.41 | 9.22 | 0.83 |
| Albumin mg/dl | 4.24 | 0.04 | 4.45 | 0.17 | 4.57↑ | 0.14 |
| T. Protein g/dl | 7.16 | 0.22 | 7.71 | 0.39 | 7.78↑ | 0.25 |
| T.Bilirubin mg/dl | 0.23 | 0.10 | 0.29 | 0.07 | 0.48 | 0.24 |
| Creatinine mg/dl | 0.23 | 0.18 | 0.19 | 0.16 | 0.30 | 0.10 |
| Sodium mmol/L | 141.34 | 1.63 | 148.25 | 3.69 | 150.26 | 0.95 |
| Potassium mmol/L | 6.36 | 0.20 | 6.09 | 0.39 | 5.85 | 0.37 |
| Chloride mmol/L | 96.50 | 1.66 | 99.27 | 1.75 | 97.84 | 4.42 |
| BUN mg/dl | 26.97 | 3.07 | 24.41 | 3.35 | 27.76 | 3.67 |

Key: SD = Standard deviation, N = Number of animals in group, BW = Body weight.

**4.2.2 Haematology**

There were no significant differences in the hematological parameters in animals exposed to test compound as compared to those in the vehicle control group (Table -3).

**Hematology**

Details of different haematological parameters are summarised in Summary Table.

**Table 3: Hematology.**

**Males**

| Group (N) | 1(5) | | 2(5) | | 3(5) | |
|---|---|---|---|---|---|---|
| Dose (mg/kg BW) | Control | | 150 mg/ml | | 300 mg/ml | |
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| RBC x $10^6$ µl | 8.48 | 0.59 | 9.09 | 0.49 | 8.99 | 0.50 |
| WBC x $10^3$ µl | 8.62 | 0.87 | 7.74 | 0.96 | 7.54 | 1.23 |
| Hgb g/dl | 15.58 | 0.58 | 16.48 | 0.80 | 15.38 | 1.16 |
| HCT % | 44.26 | 2.16 | 46.64 | 2.13 | 43.94 | 3.35 |
| MCV $\mu m^3$ | 52.20 | 1.64 | 51.06 | 0.66 | 48.78 | 2.08 |
| MCH pg | 18.40 | 0.73 | 18.06 | 0.19 | 17.10 | 0.78 |
| MCHC g/dl | 35.24 | 0.46 | 35.40 | 0.16 | 35.06 | 0.25 |
| Platelet x $10^3$ µl | 543.60 | 88.98 | 566.20 | 38.85 | 619.20 | 48.31 |
| Neutrophil % | 15.20 | 3.70 | 13.80 | 5.76 | 14.40 | 4.93 |
| Lymphocyte % | 80.80 | 3.19 | 83.20 | 4.82 | 81.75 | 6.60 |
| Monocyte % | 2.60 | 0.55 | 1.40 | 0.55 | 2.20 | 0.84 |
| Eosinophil % | 1.40 | 0.89 | 1.60 | 0.55 | 1.60 | 0.55 |
| Basophil % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Reticulocyte % | 0.86 | 0.15 | 0.88 | 0.18 | 1.06 | 0.09 |
| Clotting Time(Sec) | 150.00 | 21.21 | 126.00 | 39.12 | 108.00 | 16.43 |

Key: SD = Standard deviation, N = Number of animals in group, BW = Body weight.

**Females**

| Group (N) | 1(5) | | 2(5) | | 3(5) | |
|---|---|---|---|---|---|---|
| Dose (mg/kg BW) | Control | | 150 mg/ml | | 300 mg/ml | |
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| RBC x $10^6$ µl | 8.45 | 0.47 | 8.52 | 0.37 | 8.19 | 0.61 |
| WBC x $10^3$ µl | 6.10 | 1.83 | 4.54 | 0.67 | 6.00 | 1.21 |
| Hgb g/dl | 14.98 | 0.75 | 15.20 | 0.43 | 14.74 | 1.10 |
| HCT % | 41.94 | 2.02 | 42.36 | 1.00 | 41.12 | 3.40 |
| MCV $\mu m^3$ | 49.66 | 0.52 | 49.76 | 1.03 | 50.14 | 0.70 |
| MCH pg | 17.78 | 0.36 | 17.86 | 0.38 | 18.00 | 0.14 |
| MCHC g/dl | 35.78 | 0.43 | 35.90 | 0.45 | 35.94 | 0.37 |
| Platelet x $10^3$ µl | 457.20 | 200.21 | 537.60 | 12.16 | 546.40 | 92.27 |
| Neutrophil % | 12.40 | 4.51 | 14.00 | 4.74 | 11.60 | 2.30 |
| Lymphocyte % | 83.80 | 4.87 | 82.60 | 5.13 | 85.00 | 3.54 |
| Monocyte % | 2.60 | 0.89 | 2.00 | 0.71 | 1.80 | 0.84 |
| Eosinophil % | 1.20 | 0.84 | 1.40 | 1.14 | 1.60 | 0.55 |
| Basophil % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Reticulocyte % | 1.02 | 0.08 | 1.04 | 0.09 | 1.08 | 0.08 |
| Clotting Time(Sec) | 120.00 | 21.21 | 120.00 | 30.00 | 108.00 | 16.43 |

Key: SD = Standard deviation, N= Number of animals in group, BW = Body weight.

**4.2.4 Histopathology**

The histopathology observations (deviations from normal histology) indicate the following.

- All the observed changes were seen randomly.
- There were no statistically significant differences between the vehicle control and test group.
- It may be concluded that under the given experimental conditions and based on the results obtained, there were no histopathological changes in test compound.

**4.3 CONCLUSION**

1) The Rats administered to WAMUP GEL on single exposure did not show any abnormal findings in body weight and food consumption profiles. Live phase, physical activity of animals in all groups is

observed to normal,

2) Physiological, biochemical and hematological parameter of treated animals, when compared with control animals is appeared to be normal and all values are with in the physiological range.[1]
3) No abnormal changes in gross necropsy and histopathology of major organs were observed in all groups.

No specific statistically significant abnormalities in physical, physiological, biochemical and clinical chemistry parameters were observed in test group (TG-1 and TG-II) when compared with the control group. Hematological and pathological profiles were also similar in all groups. With this study it can be concluded single exposure of different concentration of WAMUP GEL is not exerted any systemic toxicological effect in Rats.

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