*Biomedical Research 2018; 29 (15): 3149-3154* *ISSN 0970-938X*
*www.biomedres.info*

# Sub-chronic oral toxicity study of matrix tablets of micronized domperidone formulation in rodents.

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## Abstract

**The present study deals with sub-chronic oral toxicity studies in rodents of micronized domperidone matrix drug formulation. The matrix tablet of domperidone was formulated by using different polymers. After the completion of the formulation the matrix tablets the tablets were crushed into powder and a homogeneous suspension was prepared. The suspension was administered to rats at different dosages according to the study plan. During the studies animals' weight were taken at periodically. After 28$^{th}$ day of study period the animal's blood was collected for pathological and biological analysis. At the termination of study, all the animals were sacrificed and vital organs were taken out for autopsy studies. After the thorough studies the results shows that that the micronized formulation did not produce any significant toxicity in rodents.**

**Keywords:** Micronized domperidone, Matrix drug formulation, Sub-chronic oral toxicity study, Wistar strain rats.

*Accepted on August 20, 2018*

## Introduction

Toxicity study for dosage form is one of the important criterions for evaluation of safety profile of the drugs as well as novel pharmaceutical dosage forms. Acute and sub chronic toxicity studies in various species including rodents are two important tests which is used by various researchers as routine analysis [1,2]. Sub-chronic oral toxicity study in rodents is one of the most established and simple method to evaluate the toxicity and safety profile of any drugs and dosage forms. Researchers and scientists specifically designed sub-chronic toxicity study to perform and evaluate any drug and excipient interaction that may produce any toxic impurities. In addition sometimes micronized or nanoform of the drug also may increase the toxicity profile of the drug enormously. Regulatory bodies of various countries have also made it mandatory to evaluate toxicity studies for new drugs or novel dosage forms of old drugs also [1,2].

Domperidone is one of the most effective selective dopamine D2 receptor antagonists is mainly used for antiemetic activity [3]. Literature survey reveals that domperidone may be responsible for sudden cardiac arrest [4]. Oral administration of domperidone is safe and well established but as the domperidone was micronized so there may be a possibility of enhancement of potency and toxicity [5]. In previous research work we designed a formulation which consists of micronized domperidone. The micronized domperidone was formulated in matrix dosage form. The safety profile of the dosage form must be evaluated for human use. For this reason the sub-chronic study of the dosage form in rodents was emerged as an important research component for our entire experiment to evaluate the evidence of toxicity [6-9].

## Materials

48 Wistar strain rats having both male and female were purchased from IICB, Kolkata. All the animals were kept for seven days as acclimatization period before staring the experiment. Specification of the test is mentioned in Table 1.

## Methods

### *Randomization, numbering and grouping of animals*

Forty eight rats i.e. 24 male and 24 female healthy rats were divided into four groups of 6 rats per sex i.e. four dose groups receiving the dose of 0, 15, 30 and 60 mg/kg. Animals were

kept for acclimation period of 7 d to laboratory conditions prior to the initiation of dosing. Rats were assigned to six per cage sex wise and the individual animal was fur marked with picric acid markers. The females were nulliparous and not pregnant.

*Table 1. Test system of sub-chronic toxicity study in rodents.*

| Species | Rat |
|---|---|
| Strain | Wistar strain rats |
| Source | Animal house, IICB, Kolkata |
| Sex | Male and female |
| Age | 6 to 8 w |
| No. of animals per dose level | 6 per sex per dose |
| Acclimation | Seven days prior to dosing |
| Identification of animals | By cage number and individual marking on fur |
| Diet | Pelleted feed supplied by M/s Ghosh Enterprise, Kolkata |
| Water | Aquaguard pure water in glass bottles ad libitum |
| Housing | The rats were housed 6 each; of the same sex in polycarbonate cages provided with bedding of husk. The temperature was maintained in between 20°C to 24°C and relative humidity between 30% to 70%; 12 h each of dark and light cycle was maintained. |
| Dose | Male: 0, 15, 30 and 60 mg/kg body weight. |
| | Female: 0, 15, 30 and 60 mg/kg body weight. |
| Route of administration | Oral (gavages) |

## Dose preparation

The matrix tablets of domperidone were crushed into powder by mortar and pestle and then the powder was suspended in corn oil. The drug was then administered to rats at the dose levels of 15, 30 and 60 mg/kg in the dose volume of 1 ml/100 g body weight. The domperidone tablet formulation suspensions were freshly prepared every day for 28 d. The control animals were administered vehicle only.

## Observations

**Symptoms:** All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

**Mortality:** All animals were observed twice daily for mortality during the period of the study.

**Body weight:** The weight of each rat was recorded on d 0 and at weekly intervals throughout the course of the study. The group means body weights were calculated.

**Food consumption:** The quantity of food consumed by groups consisting of six rats each was recorded weekly and the food consumption per rat was calculated for control and dose groups.

## Terminal studies

**Laboratory investigations:** Following laboratory investigations were carried out prior to sacrifice on completion of dosing period of 28 d in animal's fasted over-night. Blood samples were collected from orbital sinus following morning using heparin as anticoagulant.

**Haematological investigations:** Following haematological parameters were studied using Sysmax-K250 Cell Counter-Hb: Hemoglobin (g (%)), Rt: Reticulocyte (%), Platelets: ($\times 10^5$/cmm), WBC: White Blood Corpuscles ($\times 10^3$/cmm).

**Biochemical investigations:** Following biochemical parameters were studied using Robonik ASP-300-total serum protein (g (%)) [10], BUN: Blood Urea Nitrogen (mg (%)) [11], SGPT: Serum Glutamic Pyruvic Transaminase (IU/L) and SGOT: Serum Glutamic Oxaloacetic Transaminase (IU/L) according to the method described by Reitman and Frankel [12].

**Necropsy:** All animals were sacrificed on d 29, using $CO_2$ asphyxiation technique (body weights mentioned in the table section are fasting body weights). Necropsy of all animals was carried out and the weights of the following organs were recorded: Liver, kidneys and heart. The organ weights were recorded as absolute values and their relative values (i.e. percent of the body weight) were calculated.

## Histopathology

Following tissue samples of organs from control and animals treated at the highest dose level of 60 mg/kg were preserved in 10% formalin for histopathological examination. Adrenals; heart; kidneys; liver; lungs; stomach; adrenals, heart, kidneys, liver, lungs and stomach of low and intermediate dose group animals were preserved for possible histopathological examination, in case the histopathological examination of high dose group animals is indicative of abnormalities associated with the treatment [13-15].

## Statistical evaluation

All the animals were assigned into four different treatments i.e. control, low dose, middle dose and high dose. After 28 d vigorous studies all pathological and biochemistry parameters had been evaluated statistically. Analysis of Variation (ANOVA) is the suitable statistical tool to compare the means of three or more groups. The test compares the variation (variance) in the mean between treatments with those within treatments. The ratio of variations (variance) in the mean between treatments with those within treatments determines the F value. If the F value for different studies found to be less than recorded F value was tabulated against 2 d.f for between mean square and 45 d.f within mean square at 5% level of significance then the results were found to be non-significant [16-18].

## Results

### Sub-chronic oral toxicity study (28 d) on rats

All the animals taking various levels of doses were free of intoxicating signs throughout the dosing period of 28 d. The mortality and intoxication symptoms were shown in Table 2.

### Variation of body weight of experimental rats

Both male and female animals from control and the different dose groups exhibited normal body weight gain throughout the dosing period of 28 d as illustrated in Table 3.

### Food consumption of experimental animals

During the dosing period and at termination the quantity of food consumed by both male and female animals from different dose groups was found to be comparable with that of control animals which is tabulated in Table 4.

### Haematological investigations of blood parameters

The hematological parameters of male and female-were analysed at termination of dosing on d 29, no significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits which are shown in Table 5.

The ANOVA was applied to evaluate the statistical difference of the mean hematological parameters among four independent drug treatments referring to the Table 6, F against 3 degree of freedom for between mean square and 44 degree of freedom for within mean square; we find a value 2.8 at 5% level of significance. Since the value for F obtained in the present experiment is less than the recorded value, we conclude that the difference between the treatment means is not significant ($p<0.05$).

*Table 2. Effect of drug treatment on clinical signs of intoxication.*

| Group no. | Dose (mg/kg) | Observed Signs | Total no. of animals | Animal nos. | Period of signs in days | | Mortality |
|---|---|---|---|---|---|---|---|
| | | | | | From | To | |
| Sex: Male | | | | | | | |
| I | Control | NIL | 6 | 1-6 | 1 | 28 | 0/6 |
| II | 15 | | 6 | 13-18 | 1 | 28 | 0/6 |
| III | 30 | | 6 | 25-31 | 1 | 28 | 0/6 |
| IV | 60 | | 6 | 37-42 | 1 | 28 | 0/6 |
| Sex: Female | | | | | | | |
| I | Control | NIL | 6 | 7-12 | 1 | 28 | 0/6 |
| II | 15 | | 6 | 19-24 | 1 | 28 | 0/6 |
| III | 30 | | 6 | 31-36 | 1 | 28 | 0/6 |
| IV | 60 | | 6 | 43-48 | 1 | 28 | 0/6 |

*Table 3. Group means body weight (g).*

| Gr. no. | Dose (mg/kg) | | Day | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 7 | 14 | 21 | 28 |
| I | Control | Mean ± SD | 104.23 ± 6.62 | 104.58 ± 7.49 | 105.97 ± 8.00 | 106.13 ± 7.01 | 107.18 ± 7.37 |
| II | 15 | | 103.53 ± 5.38 | 104.93 ± 5.43 | 105.48 ± 4.68 | 106.43 ± 4.34 | 107.13 ± 5.41 |
| III | 30 | | 105.95 ± 5.78 | 104.15 ± 5.44 | 104.92 ± 4.93 | 105.95 ± 5.46 | 106.07 ± 5.18 |
| IV | 60 | | 103.95 ± 5.44 | 104.77 ± 5.38 | 105.35 ± 4.81 | 106.07 ± 4.57 | 106.28 ± 5.03 |

### Biochemical investigations

At termination on d 29, all biochemical parameters were studied i.e. total serum protein; SGPT, SGOT, BUN were found to be comparable with controls and were within the normal biological and laboratory limits (Table 7).

The ANOVA was applied to evaluate the statistical difference of the mean biochemical parameters among four independent drug treatments. Referring to the Table 8, F against 3 degree of freedom for between mean square and 44 degree of freedom for within mean square, we find a value 2.8 at 5% level of significance. Since the value for F obtained in the present

experiment for different biochemical parameters is less than the recorded value, we conclude that the difference between the treatment means is not significant ($p<0.05$).

*Table 4. Group mean food consumption (g/animal).*

| Gr. no. | Dose (mg/kg) | Day | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 | 28 |
| I | Control | Mean | 4.86 | 5.46 | 5.94 | 6.15 | 6.49 |
| II | 15 | | 5.31 | 5.98 | 5.52 | 5.86 | 6.21 |
| III | 30 | | 4.94 | 5.56 | 5.84 | 5.72 | 6.09 |
| IV | 60 | | 5.15 | 5.42 | 5.17 | 5.58 | 5.44 |

*Table 5. Group mean-haematology.*

| Gr. no. | Dose (mg/kg) | | Hb (g (%)) | Platelets (× $10^5$/c) | Rt (%) | Total WBC (× $10^3$/cmm) |
|---|---|---|---|---|---|---|
| I | Control | Mean ± SD | 14.14 ± 0.97 | 5.58 ± 0.84 | 1.07 ± 0.20 | 7.13 ± 0.87 |
| II | 15 | | 14.56 ± 1.34 | 6.02 ± 0.71 | 1.13 ± 0.22 | 7.08 ± 0.54 |
| III | 30 | | 14.38 ± 0.64 | 6.03 ± 0.84 | 1.17 ± 0.19 | 7.14 ± 0.40 |
| IV | 60 | | 14.51 ± 0.64 | 6.42 ± 0.81 | 1.23 ± 0.23 | 7.28 ± 0.46 |

*Table 6. ANOVA of Hematological parameters of 4 different groups.*

| Parameters | No. of animals | Mean square (Between treatment) | Mean square (Within treatment) | F value | Statistical significance |
|---|---|---|---|---|---|
| Hemoglobin (g %) | 48 | 0.4 | 0.67 | 0.61 | NS |
| Platelets (× $10^5$/cmm) | 48 | 1.38 | 0.64 | 2.17 | NS |
| Rt (%) | 48 | 0.126 | 0.12 | 1.05 | NS |
| Total WBC (× $10^3$/cmm) | 48 | 47 | 0.35 | 0.23 | NS |

*Table 7. Group meta-blood chemistry.*

| Gr. no. | Dose (mg/kg) | | Total serum protein (g (%)) | BUN (mg %) | SGPT (IU/L) | SGOT (IU/L) |
|---|---|---|---|---|---|---|
| I | Control | Mean ± SD | 6.92 ± 0.57 | 28.33 ± 4.47 | 55.25 ± 8.14 | 114.5 ± 17.15 |
| II | 15 | | 7.04 ± 0.37 | 28.33 ± 3.96 | 50.01 ± 4.39 | 119.67 ± 9.88 |
| III | 30 | | 6.78 ± 0.27 | 26.67 ± 4.09 | 52.5 ± 2.81 | 126.75 ± 18.00 |
| IV | 60 | | 7.06 ± 0.40 | 26.75 ± 2.83 | 51.58 ± 2.67 | 111.92 ± 19.44 |

*Table 8. ANOVA of biochemical parameters of 4 different groups.*

| Parameters | No. of animals | Mean square (Between treatment) | Mean square (Within treatment) | F value | Statistical significance |
|---|---|---|---|---|---|
| Total serum protein (g %) | 48 | 0.2 | 0.17 | 1.18 | NS |
| BUN (mg %) | 48 | 15.87 | 15.12 | 1.02 | NS |
| SGPT (IU/L) | 48 | 85.65 | 52.01 | 1.64 | NS |
| SGOT (IU/L) | 48 | 755.96 | 345.75 | 2.18 | NS |

## *Variation of organ weights*

The animals from control and the different dose groups exhibited normal organ weight after the sacrifice at 29$^{th}$ d. The ANOVA was applied to evaluate the statistical difference of the mean weight of vital organs among four independent drug treatments (Table 9). Referring to the Table 10, F against 3 degree of freedom for between mean square and 44 degree of freedom for within mean square, we find a value 2.8 at 5% level of significance. Since the value for F obtained in the present experiment for different organ weights is less than the recorded value, we conclude that the difference between the treatment means is not significant ($p<0.05$).

### Necropsy

Summary of necropsy findings in male and female animals are described in Table 11. The gross pathological examination revealed no abnormality attributable to the treatment.

*Table 9. Group mean-relative values (%) of different organ weights in different groups.*

| Gr. no. | Dose (mg/kg) | | Liver (g) | Kidneys (g) | Heart (g) |
|---|---|---|---|---|---|
| I | Control | Mean ± SD | 3.95 ± 0.31 | 0.63 ± 0.04 | 0.43 ± 0.06 |
| II | 15 | | 3.99 ± 0.16 | 0.65 ± 0.05 | 0.41 ± 0.02 |
| III | 30 | | 3.87 ± 0.33 | 0.62 ± 0.04 | 0.42 ± 0.02 |
| IV | 60 | | 4.09 ± 0.29 | 0.63 ± 0.04 | 0.43 ± 0.04 |

*Table 10. ANOVA of hematological parameters of 4 different groups.*

| Parameters | No. of animals | Mean square (Between treatment) | Mean square (Within treatment) | F value | Statistical significance |
|---|---|---|---|---|---|
| Liver | 48 | 0.1 | 0.08 | 1.32 | NS |
| kidney | 48 | 0.012 | 0.01 | 1.25 | NS |
| Heart | 48 | 0.086 | 0.01 | 0.86 | NS |

*Table 11. Summary of gross pathology findings.*

| Site and lesion observed | Group | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| | | Male | | |
| No abnormality detected (NAD) | 1-6 | 13-18 | 25-30 | 37-42 |
| | | Female | | |
| No abnormality detected (NAD) | 7-12 | 19-24 | 31-36 | 43-48 |

### Histopathology of different organs of high dose groups

Summary of histopathological observations of vital organs of different dose group is given in Table 12. Histopathological examination of animals from high dose group revealed no abnormality attributable to the treatment.

*Table 12. Summary of histopathology findings of different vital organs of low and high dose.*

| Group | | 0 mg/kg | 60 mg/kg |
|---|---|---|---|
| No. of animals | | 6 | 6 |
| | | # SEV | # SEV |
| Adrenals | # Ex | 6 | 6 |
| Heart | # Ex | 6 | 6 |
| Kidneys | # Ex | 6 | 6 |
| Liver | # Ex | 6 | 6 |
| Lungs | # Ex | 6 | 6 |
| 6=normal histopathology | | | |

## Discussion

The experimental study has been performed to evaluate the potential of micronized drug domperidone to induce a systemic response after exposure. Domperidone is a very popular anti-emetic drug, acts on D2 receptor with low toxicity [19]. Our experimental study is aimed to evaluate toxicity studies of micronized form of domperidone. Due to higher exposed surface area micronized form of domperidone is considered to have better oral absorption. Sub-chronic toxicity studies were executed to investigate any overall short-term toxicity produced by the micronized form of the drug due altered bioavailability.

The study animals of either sex were found alive, no significant alteration of body weight was noticed. Food consumption of the entire study group was found to be normal that indicates domperidone didn't change the appetite of the treated rodents.

Evaluation of hematological profile was performed, and hence any significant change with reference was not found. However, drug induced change in hematology indicates a number of possible serious or life threatening health hazards in individuals.

Domperidone pass through an extensive first-pass metabolism in the liver so different serum protein and enzyme was determined and treatment groups were compared with control group as a biochemical marker to investigate any hepatotoxicity. The obtained result was found to be satisfactory, and no significant alteration in serum protein and enzymes (i.e. SGOT, SCPT) were noticed. Urea is a metabolic waste product produced during metabolism, excreted after filtration in kidney and is used as a surrogate marker for identifying renal toxicity [20-22]. The serum concentration of BUN was found within normal range throughout the 28 d study period.

The organ weights of all the study animals of either sex was determined and compared after post study sacrifice of animal, no marked alteration was observed in statistical comparative values.

The entire study shows a promising result that micronized domperidone when formulated in matrix tablet did not produce any significant toxicity. Previous research work shows some

toxicity but the micronized form of domperidone matrix tablet formulation did not produce any major toxicity.

## Conclusion

Sub chronic oral toxicity is generally used to find any short term or long term toxicity induced by the use of the drug. Domperidone is a drug of highly water insoluble in nature. As a result the bioavailability of domperidone formulation is also compromised in conventional dosage form. To overcome this problem we have developed a novel sustained release dosage form using domperidone in micronised form.

After development of matrix tablet formulation, there was a need to evaluate their *in-vivo* performance in healthy human volunteers. So prior to this, a preclinical sub-chronic oral toxicity study of the final formulation was carried out in rats to see for any toxic effects due to pharmacological interaction between the drugs and the excipients as well as the effects of the micronized particles of domperidone in formulation. Sub-chronic toxicity showed no significant abnormality in haematological parameters, biochemical parameters, organ weights and histopathology findings.

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