Effect of *Portulaca oleracea* and its interaction with vecuronium on neuromuscular junction in experimental animals

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**ABSTRACT**

**Background:** The extracts from the plant *Portulaca oleracea* have been mentioned to have muscle relaxant properties in traditional medicine literatures and used as a food supplement. The site of therapeutic action is directly on muscle fibres rather than neuromuscular transmission. The muscle relaxant property is found to be similar with dantrolene in frog rectus abdominis. So objectives of our study to analyze the effect of *Portulaca oleracea* and its interaction with vecuronium on the neuromuscular junction in experimental animal.

**Methods:** The study was conducted in two parts i.e. study 1 and study 2. In study 1:2 ml and 4 ml *Portulaca oleracea* fresh aqueous leave juice was given orally to 12 number rabbits and vecuronium 5 microgram/ml was given intravenously to all 6 rats through marginal ear vein with the help of scalp vein attached with 10 ml syringe. The head drop time is recorded and reversal of neuromuscular blockade was done with neostigmine (0.25 mg/kg) along with atropine (2 mg). Study 2: 2 ml and 4 ml of *Portulaca oleracea* fresh aqueous leave juice was tested on frog’s isolated rectus abdominis muscle in Dales’s isolated organ bath and its interaction with vecuronium (1 mmole-32 mmole) was recorded.

**Results:** *Portulaca oleracea* has muscle relaxant properties, which was recorded which was indicated by decreasing the time of head drop and complete block in study 1. In study 2, it also decreased the Ach induced contraction in presence of vecuronium.

**Conclusions:** The results indicate the muscle relaxant properties fresh juice of *Portulaca Oleracea* probably due to high content of potassium.

**Keywords:** *Portulaca oleracea*, Vecuronium, Neuromuscular junction, Muscle relaxation

**INTRODUCTION**

Neuromuscular blocking drugs promote skeletal muscle relaxation and administered routinely for tracheal intubation during abdominal surgery, orthopaedic manipulation, laryngoscopy, bronchoscopy, oesophagoscopy etc.1 *Portulaca oleracea* (puruni sag in odia) widely grown all around mostly in dry climate and consumed as salad and vegetables.2 *Portulaca oleracea* medicinally used for headache, stomach-ache, painful urination, enteritis, mastitis, lack of milk flow in nursing mother and post-partum bleeding1,2. Externally used to treat burns, earache, insect stings, inflammations, skin sores, ulcers pruritus, eczema and abscesses.3,4 Present study was undertaken to know the effect of *portulaca oleracea* and its interaction with Vecuronium on neuromuscular transmission on animal models.

**METHODS**

This study was approved by institutional animal ethics committee, V.S.S. Medical College, Burla, Odisha, India. The *Portulaca oleracea* plant was freshly collected from local area and was identified and authenticated by the Botany department of Sambalpuri University.

**Drugs and fluids used**

The following drugs were used in the study - Vecuronium bromide (Neon Laboratories Limited, Mumbai, India), Neostigmine methyl sulphate (Neon Laboratories...
Limited, Mumbai, India). Perfusion fluid used was frog ringer solution for study 2.

Collection and identification of plant

Portulaca oleracea was freshly collected from local area from single source during the month of March (as it is grown in ample during January to April) around Hirakud dam on bay of the river Mahanadi were used for the study. Recognition and certification of the leaves of Portulaca oleracea was done by head of the department, department of botany, sambalpur university, Odisha, India. A specimen sample was stored in our department of pharmacology for future reference.

Plant material extraction

The leaves were cleaned, washed and grounded to paste in pestle and mortar. Then was filtered in whatman filter paper no 40 and fresh juice collected (method of rosenthaler-1930).The prepared juice was used as 2 ml and 4 ml respectively.

Experimental animals

Eighteen healthy male albino rabbits weighing 2.5 kg to 3.5 kg bred in the animal house of the department of pharmacology, VSS Medical College, Burla, Odisha, India were used. They were kept under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water and were acclimatized for three weeks to laboratory conditions before the commencement of the experiments.

Statistics

One way anova was used.

Two types of study were done as per the designed study protocol (Table 1).

Study 1

For this study eighteen healthy male albino rabbits weighing 2.5 kg to 3.5 kg were randomly selected from departmental animal house divided into three groups, having six in each. Group I was given, Vecuronium (5 μg/ml) diluted with 10 ml of normal saline in a 10 ml syringe, given slow intravenously through marginal ear vein of rabbit with the help of a scalp vein, which served as negative control. Group II and III were given Portulaca oleracea, 2 ml and 4 ml per orally and Vecuronium (5μg/ml) diluted with 10 ml of normal saline in a 10 ml syringe, given slow intravenously through marginal ear vein with the help of a scalp vein respectively after 30 minutes of Portulaca oleracea, which served as tests.

Table 1: Protocol design-different drugs administered with their route and dose.

| Group   | Drugs                      | Route                      | Type       |
|---------|----------------------------|----------------------------|------------|
| I       | Vecuronium (5 μg/ml)       | Intravenous                | Control    |
| II      | Portulaca 2 ml+Vec (5μg/ml)| Per Oral+Intravenous       | Test       |
| III     | Portulaca 4 ml+Vec (5μg/ml)| Per Oral+Intravenous       | Test       |

All the doses were selected as per literature. Vecuronium was freshly prepared before administration. Time of head drop and complete block was recorded. Data was analysed by one way ANOVA mentioned in (Table 2).

Table 2: Time of onset of head drop and complete block in minutes.

| Group | Drug                  | Time for onset of head drop (in Minutes) (Mean ± SD) | Time for complete block (in Minutes) (Mean ± SD) |
|-------|-----------------------|------------------------------------------------------|--------------------------------------------------|
| 1     | Vecuronium 5 mcg/ml i.v. | 9.36±1.2                                              | 15.8±0.2                                         |
| 2     | Per oral 2 ml+Vec 5 mcg/ml i.v. | 6.25±2.3***                                           | 10.5±0.5***                                      |
| 3     | Per oral 4 ml+Vec 5 mcg/ml i.v. | 5.36±1.6***                                           | 10.2±0.3***                                      |

One way ANOVA F (df); 11.13 (2, 15); 10.27 (2, 15) p< 0.01 <0.01

Rabbit head drop method

The procedure of rabbit head drop was followed as described by Verny.7

Study 2

Isolated frog rectus abdominis muscle was mounted in Dale’s isolated organ bath. Graded dose response of acetylcholine was recorded and submaximal dose (SMD) of acetylcholine was determined. Then fresh juice of Portulaca oleracea was added into the organ bath in graded doses. Then the tissue was left for thirty minutes was given for the action of portulaca oleracea,

Figure 1: Effect of portulaca oleracea on acetylcholine induced muscle contraction.
submaximal dose (SMD) of acetylcholine was added into the bath and response was recorded.

RESULTS

Time for onset of head drop for different groups and time for complete block was recorded (in minutes) and was expressed in (Table 2).

Study 2

In isolated frog rectus abdominis muscle preparation, 2 ml and 4 ml of portulaca oleracea leaf juice was tested. It showed, reduced skeletal muscle contraction in dose dependant manner in presence of vecuronium and the effect was found reversed after 1 hour (Figure 1).

DISCUSSION

In study 1 it was found that Portulaca oleracea showed muscle relaxant properties by increasing the time of head drop and complete block. After putting one way ANOVA, the result was found to be very highly significant. In study 2, it also decreased the contraction induced by acetylcholine in presence of vecuronium. The probable mechanism may be due to high concentration of potassium ion in the juice. It also rich in omega 3 fatty acid, noradrenaline, vitamin A, B, C, niacin, nicotinamide, a tocopherol, bcarotin, minerals, fattyacid, glutathione, glutamic acid and aspartic acid. Our observations on potentiation of neuromuscular relaxation with Portulaca oleracea is in accordance with previous studies.

Though muscle relaxant property of Portulaca oleracea probably largely depends on the level of potassium which could not be found out due to the unavailability of high performance thin liquid chromatography (HPTLC) which is the limitation of the study, but the study regarding muscle relaxant activity of Portulaca oleracea is very sparse. Further studies should be in line to find out whether the increased extracts potassium could have either augmented the phytoconstituents muscle relaxant activity and whether direct juice or aqueous extract or ethanolic extract is far more effective.

CONCLUSION

Portulaca Oleracea has muscle relaxant properties that of the vecuronium. To know the actual mechanism of action further study may be under taken. Its possible use in different conditions can be for reducing the adverse effects of vecuronium.

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