



Acute toxicity studies of an novel natural polymer *vigna mungo* in swiss albino rats

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ABSTRACT

The aim of the present research is to investigate acute toxicity profiling of isolated Vigna mungo new natural polymer. Safety administration is the primitive criterion for any drug substance. To explore the safety and toxicity profiling of the novel polymer, this study was carried out. Vigna mungo novel polymer was isolated from the pulverised seeds of Vigna mungo which is part and parcel of our diet. This polymer is obtained using a non-solvent extraction method using acetone. Acute toxicity studies were performed according to the OECD guidelines 420. In this, the selected animal model is Swiss albino rats, grouped into control and test containing each three animals. 2000 mg/kg of Vigna mungo polymer was administered to a test group and did not produced any abnormalities and behavioural changes. Furthermore, histopathological studies, body weight, haematological parameters did not presented abnormal values. The observations found 2000mg/kg of a dose of the polymer did not cause lethality and death of any animal till 14 days of a period. It was concluded that Vigna mungo novel polymer is safe to administer up to 2000mg/kg dose. Hence, the novel *Vigna mungo* polymer is safer for therapeutic use in pharmaceutical formulations.



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INTRODUCTION

Polymers are the backbone of drug delivery systems. These are the carriers of inert nature. Polymers alter

the pharmacokinetics, pharmacodynamics properties of the dosage forms. Polymers are natural, synthetic and semisynthetic based on the source of origin. Many natural source origin polymers, especially proteins, polysaccharides used as carriers in the tissue engineering, targeting, and in bio response stimuli drug delivery systems (Gil and Hudson, 2004). These are used as binders, film formers, artificial organs linings, immunological testing, and as substrates for cell growth. Smart polymers are materials of choice in the dosage forms which undergo physical or chemical change in response to external stimuli. Hydrogels are the hydrophilic polymeric networks capable of taking a large amount of water or biological fluids (Qiu and Park, 2001).

Natural polymers are gaining popularity in the modern era due to biocompatibility, biodegradability,

no-toxicity, economic, safety, easy availability. Natural polymers are produced from plants, animals sources (Shanmugam *et al.*, 2005). Herbal polymers are largely contributing in many industries. These polymers are obtained from gummy exudates, and plant fibres are used in these areas (Sailaja *et al.*, 2011). Like Acacia, carrageenan, agar, Ispagula, tragacanth many polymers are used in various formulations (Gandhi *et al.*, 2019). Even though a lot of research work was done in the exploration of natural polymer associated dosage forms, yet to find and confirm the safety profile of the *Vigna mungo* polymer.

Novel *Vigna mungo* polymer is obtained from *Vigna mungo* (black gram) seeds pulverised portion using non-solvent acetone. Although research work regarding the *Vigna mungo* polymer was done still, there is a need to establish toxicity profiling to carry out *in vivo* administration of dosage form. To carry out this exploration, acute toxicity profiling was performed using OECD guideline 420 (Sravani *et al.*, 2011; Yadav *et al.*, 2009).

Toxicity studies are used in hazard identification and risk management in the context of production, handling, and use of chemicals (Aneela *et al.*, 2011). Acute oral toxicity study is imperatively required not exclusively to distinguish the scope of test substance that could be utilized, yet in addition to reveal the clinical signs evoked by the substances under scrutiny. It is likewise a valuable boundary to researching the helpful record of medications and xenobiotics (Sailaja *et al.*, 2011). For a traditional LD50 study, research facility mice and rodents are the species normally chose. Frequently both genders should be utilized for administrative purposes. In this study, female rats were used as per OECD 420 guidelines.

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance or multiple doses given within 24 hours. Toxicological studies are used to assess the acceptance for clinical use. Toxic doses and therapeutic indices of drugs can be determined from toxicity study. Chronic toxicity tests determines the affected organs after completion of toxicity study (Robinson *et al.*, 2009). Safety must be explored in animals to predict safe human doses (da Silva *et al.*, 2015).

MATERIALS AND METHODS

Plant Material

The *Vigna mungo* seeds were collected from the local market Nellore, Andhra Pradesh. It was authenti-

cated by Dr A.Sreenivasulu, Department of Botany, Visvodaya govt. Degree College, Venkatagiri, Nellore, Andhra Pradesh.

Preparation of *Vigna mungo* polymer

100g of powdered *Vigna mungo* seeds were soaked in 300ml of distilled water for 24 hours. Soaked material was filtered and to filtrate, add a double portion of acetone as non-solvent to separate the polymer. The polymer was dried at 40 °C. After drying, powder the material and sieve through sieve no.20.

Acute oral toxicity study

Experimental animals

Healthy adult Swiss Albino female rats weighing 130-150 g acclimatized for 14 days. The animals were housed under standard conditions, and room temperature (25±2°C) and the relative humidity was at 50-60%. Artificial lighting and 12 hours light and 12 hours dark was maintained (Walum, 1998). The animals were fed with a balanced pellet and water ad libitum (Sholikhah *et al.*, 2020). The experimental protocol of acute toxicity studies was approved by the Institutional Animal Ethical Committee of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study

The acute oral toxicity study was carried out according to OECD guideline 420, and a fixed-dose procedure was used (WHO, 1993). Considering the unavailability of *in vitro* and *in vivo* toxicological data of the *Vigna mungo* polymer extract, 300mg/kg of initial dose was chosen in the sighting study. At 300 mg/kg of dose did not produce any toxic symptoms on one rat.

In the preliminary tests, 2000mg/kg of dose did not produce toxic symptoms. In the main test, 2000mg/kg of the dose was continued on a test group containing 3 rats and a control group treated with distilled water. Animals were observed for a period of 24hrs and 14days to record the toxicity symptoms like body weight, treatment-related changes like respiration rate and heart rate and behavioural signs like apathy, reduced locomotor behaviour, licking activity (Pradeep *et al.*, 2020).

After the treatment period of 14 days, the vital organs (heart, liver, brain and kidneys) of animals under chloroform anaesthesia were preserved for histopathological examination (Saleem *et al.*, 2017). Then the blood samples were collected by cardiac puncture, subjected to haematological biochemical analysis (Chunlaratthanaphorn *et al.*, 2007).

Table 1: Body weight of a rat before and after treatment with *Vigna mungo* polymer at a dose of 300mg/kg (n=1), 2000mg/kg (n=3) and control(n=3) for a period of 14 days observations in an acute oral toxicity study

No. of rats	Group (dose mg/kg)	Bodyweight before and after treatment with <i>Vigna mungo</i> polymer			Weight gain(%)	
		Before	After 7 days	After 14 days	I week	II week
1	Control	132	135	139	2.27	5.03
2		135	138	140	2.22	3.70
3		139	140	142	0.71	2.15
1	300mg/kg	130	135	137	5.56	5.38
1	Test (2000mg/kg)	137	140	142	2.18	3.64
2		135	139	140	2.96	3.70
3		132	135	138	2.27	4.54

Table 2: The absolute body weight of rat before and after treatment with *Vigna mungo* polymer at a dose of 2000mg/kg in control (n=3), 2000mg/kg control (n=3) for a period of 14 days observations in an acute oral toxicity study

Group (dose mg/kg)	Organ	Absolute organ weight(g) in rats with numbers			Mean	Standard deviation
		1	2	3		
Control	Heart	0.52	0.56	0.54	0.54	0.011
	Liver	3.5	3.4	3.7	3.48	0.066
	Brain	1.46	1.47	1.49	1.46	0.008
	Kidney	0.56	0.58	0.59	0.56	0.012
Test (2000mg/kg)	Heart	0.54	0.52	0.56	0.54	0.011
	Liver	3.7	3.9	3.4	3.49	0.005
	Brain	1.48	1.46	1.45	1.47	0.008
	Kidney	0.59	0.56	0.55	0.56	0.0066

Table 3: Haematological parameters of control and test groups after treatment with *Vigna mungo* polymer for 14 days

Haematological parameter	Control group	Test group
Haemoglobin g/ml	14.26±0.02	14.53±0.001
Total RBC10 ⁶ /cmm	6.51±0.12	6.45±0.01
Haematocrit %	41.52±0.31	42.35±0.02
Mean corpuscular volume fL/red cell	57.54±0.14	57.68±0.02
Mean corpuscular haemoglobin (pg)	35.12±0.54	37.45±0.14
Total WBC(/cmm)	7,457±0.87	7,568±0.02
Eosinophil %	0±0.00	0±0.00
Basophil%	0±0.00	0±0.00
Neutrophil%	30.85±0.51	30.15±0.31
Lymphocytes%	67.21±0.24	64.11±0.21
Monocytes%	0±0.00	0±0.53
Platelets (/ cmm)	9,104,000±0.01	9,048,048±0.14

Values are expressed in mean ±SD

Statistical analysis

The obtained results were presented as mean \pm SEM, and the statistical significance between the groups was analysed by means of two way ANOVA. $P \leq 0.05$ was considered statistically significant.

RESULTS

Observations in the acute oral toxicity study in rats after 2000mg/kg of oral dose administration of *Vigna mungo* polymer

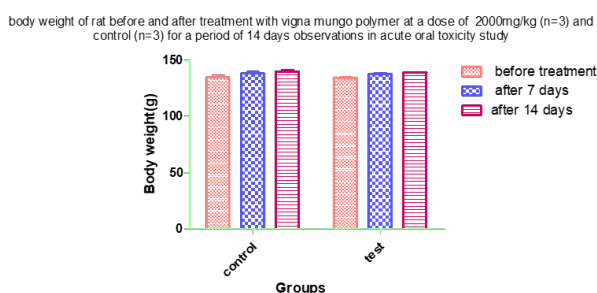


Figure 1: Bodyweight of rats before and after treatment with *Vigna mungo* polymer in acute toxicity studies

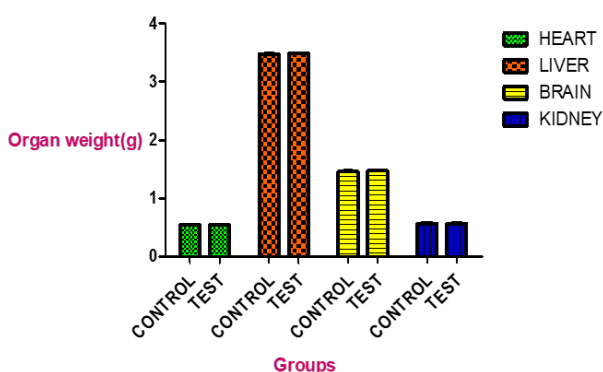


Figure 2: Mean absolute organ weight ($g \pm$ SEM) of Control vs test groups after treatment with *Vigna mungo* polymer after 14 days of period

DISCUSSION

Gums and mucilaginous substances are used in the medicinal system from the olden days (Anroop *et al.*, 2006). Polymers are macro molecular structures with a wide variety of physicochemical characteristics. Polymers are a significant part of drug delivery systems to alter the release profile characteristics. Polymers are used in the extension of the release pattern of both hydrophilic and hydrophobic drug molecules. With the exploration of polymers in the drug delivery systems well defined and predicted therapeutic benefit and release kinetics (Anepu

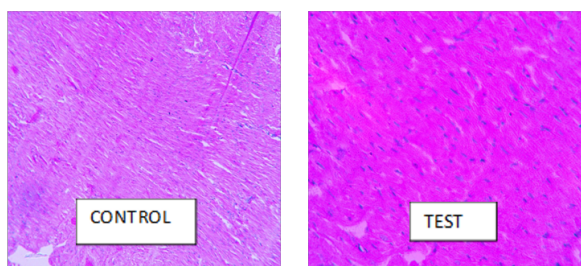
et al., 2016). Pharmaceutical active ingredients are associated with the carrier systems to allow transport and target the particular site. Polymer physicochemical characteristics allow understanding of the underlying mechanisms and behaviour in the biological environment (Liechty *et al.*, 2010). Sufficient concentration of the drug has to reach the target site to exert the desired therapeutic benefit. This is achieved by using a drug delivery system of suitable characteristics by employing polymeric structures (Santoshnaidu *et al.*, 2014; Durga *et al.*, 2020).

Table 1 clearly shows the bodyweight of each rat before and after treatment with *Vigna mungo* polymer. The observations exhibited normal after a week and 14 days of a period. The weight gain observations explored normal values during experimentation of 7 days and 14 days treatment with *Vigna mungo* polymer. The observations of body weight recorded at different time periods, i.e. before treatment, after 7 days of treatment and after 14 days of treatment and the same is illustrated in Figure 1. The body weight changes are the markers for identifying the toxicity in the animals. The experimental data is statistically analysed by 2-way ANOVA. It is clearly shown that there is no interaction overall, and the treatment periods effect was considered extremely significant ($p=0.001$). Comparing the bodyweight results in the control and test group were found to be not significant.

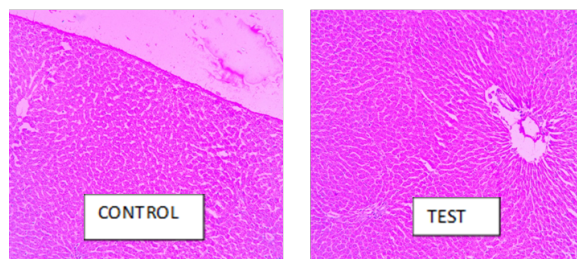
The vital organ weights (heart, liver, brain and kidney) of control and test groups after 14 days period presented in Table 2. Mean vital organ weight of control and test groups rats after treatment with *Vigna mungo* polymer after 14 days period illustrated in Figure 2. Many researchers studied liver and kidney in the acute toxicity study to evaluate the safety and toxicity of the Phyto chemicals. Pradeep D.P.*et al.* studied the effect of plant extract on vital organ weight. The study findings are in similarity with the present research results (Shende and Marathe, 2016). The results of test group animal and control group animals for gross pathological changes in view of size, shape and colour did not show noticeable variations. 2- ANOVA tests performed on experimental data. It was found that there is no significant difference observed in the test group compared to the control group.

Microscopic exploration of vital organs, heart, liver, brain and kidney were prepared for histo pathological studies. The prepared transverse sections of vital organs observed under a microscope. Histo pathological pictures represented in Figure 3 a, Figure 3 b, Figure 3 c and Figure 3 d were observed for toxic changes after treatment with a 2000mg/kg dose of

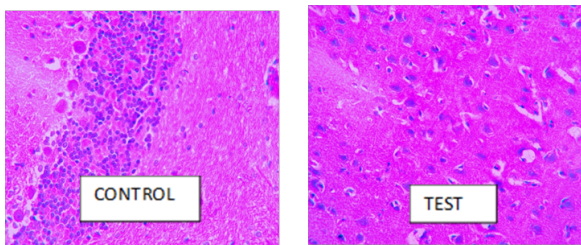
(a) Heart (Histopathology)



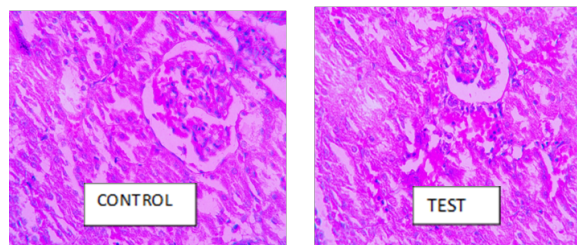
(b) Liver (Histopathology)



(c) Brain (Histopathology)



(d) Kidney (Histopathology)

**Figure 3: Histopathological studies of a. heart, b. liver, c. brain and d. kidney**

Vigna mungo polymer for the test group and compared with a control group. It was clearly found that there are no changes in the cell morphology, structure and no findings of altered conditions. These results concluded that administration of the *Vigna mungo* polymer up to 2000mg/kg did not produced toxic and necrotic signs in the cells and tissues of vital organs.

Blood parameters are sensitive indicators of any alterations in the physiological environment (Pradeep et al., 2020). Haematological examination results for haemoglobin, total RBC, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), total white blood cells (WBC), Eosinophils, Basophils, Neutrophils are presented in Table 3. By observing the haematological parameters of the control and test group were assessed (Jain et al., 2009). There is no significant difference, and the p-value is <0.0001. A significant difference compared to the control group using the two-way ANOVA was performed. There is no significant difference value of these groups to the control group was within the normal range (Robinson et al., 2009).

Information obtained from toxicity studies helps in the determination of organ toxicity, the relation between dose and response (Jothy et al., 2011). Toxicity levels of the medicinal substance makes it unsuitable for treatment (Ayodeji et al., 2019). Many plant-based products are safe, but it was proven false in many research findings (Pradeep et al., 2020). Mulchand A.Shende et al. documented acute and chronic oral toxicity findings of Hibiscus escu-

lentus mucilage on Swiss albino rats (Shende and Marathe, 2016). Divvela HN et al. explored acute oral toxicity studies of Araucaria heterophylla novel natural polysaccharide gum in albino mice (Divvela et al., 2016). Ez Zoubi Yassine et al. studied the acute toxicity using mucilage of aerial part (branches, flowers and leaves) of Lavandula stoechas L from morocco (Yassine et al., 2016). All the above Plant-based mucilage works justify the research findings of *Vigna mungo* polymer. Hence, it is found that *Vigna mungo* polymer obtained from the seeds of *Vigna mungo* is safe to use in animals up to the dose of 2000mg/kg and did not produce any sort of abnormal and toxic changes.

CONCLUSION

In the present research acute oral toxicity study, extracted *Vigna mungo* polymer was used up to 2000mg/kg. And this dose did not produce any toxicity and abnormal symptoms in the test group, i.e. in the preliminary study with one rat and in the main test with three rats. According to OECD guideline 420, 2000mg/kg dose produced no toxicity symptoms, and it is categorised as unclassified material. It is concluded that *Vigna mungo* polymer extracted using acetone is nontoxic material.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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