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EVALUATION OF ACUTE ORAL TOXICITY OF OMMIEZZ SYRUP (POLYHERBAL FORMULATION) WITH ITS ANTIOXIDANT AND ULCER PROTECTIVE ACTIVITIES IN PYLORUS LEGATED INDUCED PEPTIC ULCER

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ABSTRACT

Introduction: The traditional herbal formulations are becoming choice of drug for the treatment in present scenario because of their effectiveness without any side effect in compression to conventional drugs. The toxicity profile as well as pharmacological evaluation of newly developed drug is essential to provide scientific base and global acceptance. **Aim:** To evaluate acute oral toxicity with antioxidant and ulcer protective activities of Ommiezz Syrup (polyherbal formulation) against Peptic ulcer. **Method:** The study protocol was certified by IAEC (SKPCPER/IAEC/2016-02/01) as per the CPCSEA. The acute oral toxicity of test drug was evaluated by following OECD guideline AOT-425 to know single dose (2000mg/kg) toxicity. The efficacy of test drug on peptic ulcer was assessed in Pylorus ligation induced ulcer model in Albino Wister Rats. The effect of test drug on gastric parameters (gastric volume, pH, free and total acidity, ulcer index) and oxidative stress marker level (superoxide dismutase-SOD, catalase, malondialdehyde-MDA) were assessed comparatively with various controlled groups. **Results:** The acute oral toxicity study reveals neither any physical -behavioral changes nor mortality in any animal during study period. Significant increase in pH while decrease in gastric volume, total acidity, free acidity and ulcer index was observed in test drug treated group in comparison to various control and standard drug treated group. Significant effect of test drug was obtained on antioxidant marker level as compare to other groups. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Ommiezz syrup is 2000mg/kg as it did not produce any toxicity at that dose. The obtain results of test drug are favoring of its ulcer protective and antioxidant.

KEYWORDS

Poly herbal formulation, Ommiezz Syrup, OECD Guideline, NOAEL, Peptic ulcer and Antioxidant.

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INTRODUCTION

Traditional herbal remedies have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects[1]. In this age of globalization, the assessment of efficacy and safety of these

preparations should be based on the regular patterns of mainstream clinical medicine. These Herbal derived medicines need a scientific evaluation of their pharmacological properties and safety that actually can be assessed by new biologic technologies[2].

Open lesion or break in inner surface of stomach, duodenum or esophagus is called peptic ulcers[3]. It is characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid insufficient concentration and duration[4]. The causes of peptic ulcer are H.pylori infection, excessive use of NSAIDS, stress, alcohol, smoking and genetic factors etc. Among them H.pylori infection is major one (80%)[5,6]. Various synthetic anti-ulcer drugs are presently available in modern medicine. However each of these drugs have simpler to severe side effects such as diarrhea, itching, skin rash, dizziness and inactivation of some antifungal drugs (proton pump inhibitors), confusion in elderly patients, headache, antiandrogenic effect (H_2 receptor blockers), constipation, vomiting, indigestion, back pain, dizziness (Sucralfate), bleeding diathesis and abortion in pregnant women (Misoprostol)[7]. Thus, there is a growing interest in use of herbal drugs as they are comparatively safer and cure the condition without or with least side effect or adverse effect[8].

Considering all these issues, the present study has been conducted to develop NOAEL and evaluate ulcer protective and antioxidant effect of Ommiezz Syrup (a newly developed polyherbal formulation).

Aim and Objectives

1. To evaluate acute oral toxicity of Ommiezz Syrup on Swiss Albino Mice.
2. To evaluate antioxidant and ulcer protective effect of Ommiezz Syrup against peptic ulcer in pylorus ligation induced ulcer model.

MATERIAL AND METHODS

Test Material

The test drug (Ommiezz Syrup) was manufactured by maintained all the GMP standards during manufacturing. The detail of Ommiezz Syrup is mentioned below.

Method

The present study was performed after got approval from IAEC (SKPCPER/IAEC/2016-02/01) as per the CPCSEA, Ministry of Environment, Forest and Climate Change (MoFCC), Government of India.

Acute oral toxicity[9]

The OECD guideline AOT-425 was followed throughout the study to know single dose toxicity of Ommiezz syrup in swiss albino mice. All the Animals selected for this study were kept in standard condition stated in guideline. They were acclimatized and randomly divided in different groups with irrespective of their gender prior to dosing. Each mouse was treated with test drug at single oral dose (2000mg/kg) in sequence at 48 h intervals. All the animals were observed individually once during first 30 min after dosing, periodically during first 24 h and daily thereafter for a total of 14 days for any clinical sign of toxicity or mortality. The body weight of animals was also recorded once in a week. The dosing detail is mentioned below.

Effect on Peptic ulcer

The study was conducted in Pylorus ligation induced ulcer model in Albino Wister Rats. Animals allocated for study were maintained in standard condition. They were acclimatized for a minimum period of five days prior to dosing and subjected to randomization.

All the animals were divided in six different groups one day prior to surgery. They were kept fasted for 24 hand after that, the drug was given in standard dose according to weight of animal and interpretation of toxicological data. Group IV and V were administered orally with standard drug (Ranitidine - 27mg/kg) and Test drug (Ommiezz syrup - 25mg/kg) respectively before 1 h of surgery. First animal was anaesthetized then tied on surgical board. Hairs below xiphoid process were removed and midline incision was made. Then pylorus portion was ligated by lifting it out without damaging any blood supply of stomach. The incision was closed by interrupted suture and animals were kept for recovery in individual cage. After 24 h, they were sacrificed by cervical dislocation method. Stomach of animal was isolated and parameters were analyzed.

Evaluation of gastric parameters

Volume of Gastric juice

The gastric juice was centrifuged at 3000rpm for 15 min and then it was read from calibration on the centrifuge tubes.

pH

After centrifugation, pH of withdrawn liquid from centrifuge tubes was measured by pH strip.

Free acidity and Total acidity[10]

1ml of collected supernant liquid was taken and diluted up to 10ml by distilled water. Resulting mixture was titrated using 0.01N NaOH, phenolphthalein and methyl red (2-3 drops of both) as indicator. First end point was taken when yellow color solution turned in orange. The volume of titrant was noted, which gives amount of NaOH required to measure free acidity. Now same solution was kept titrated until pink color obtained and it persisted for more than 30 sec. Attend point amount of titrant was noted down which indicate amount of NaOH required to measure total acidity.

Ulcer Index[11]

Calculation and representation of ulcer index is highly complicated and controversial process. Bonny castle (1964) and Robert *et al* (1968) suggested a method in which the stomach was given grades (0 to 4) as follows:

Normal swelling and white spots

Red hemorrhagic spots ulcers,

Deeper hemorrhagic spots and white spot like ulcers,

Hemorrhagic ulcers and other type of ulcers,

Perforated stomach due to ulcers.

Ulcer index = % of animals having ulcers × average severity of ulcer (from scale 0 to 4) / Average number of ulcers per stomach.

Evaluation of oxidative stress markers:

Superoxide dismutase (SOD) activity[12]

Reagents

0.0001 M EDTA, 0.003 M Epinephrine, Carbonate buffer (pH 9.7)

The SOD calibration curve was prepared by taking 0.01, 0.1, 1 and 10 U/ml concentration of standard solution. Then solution of 1ml carbonate buffer, 0.2ml EDTA, 2ml epinephrine and 0.5ml supernant liquid were mixed. Absorbance of resulting solution was taken at 480 nm in spectrophotometer taking solution mixture without supernant as blank. Reading was taken at 30 sec interval for 3 min.

Catalase activity[13]

Reagents

50mM Potassium phosphate buffer (pH 7), 30mM H_2O_2

Solution of 1ml potassium phosphate buffer, 1ml hydrogen peroxide and 50µl sample (supernant) was prepared and absorbance of resulting mixture was taken at 240nm by UV Visible spectrophotometer taking solution mixture without supernant as blank solution. Reading was taken at 15 sec interval for 2.5 min.

Lipid peroxidation (LPO-MDA)[14]

Reagents

0.8% TBA, 20 % CH_3COOH in 0.27 M HCL (pH 3.5), 4 % W/V SLS, Distilled water. In 1ml of supernant liquid, 0.2ml of SLS, 1.5ml 20% CH3COOH in 0.27 M HCl and 0.8% 1.5ml of thiobarbituric acid (TBA) solution was added. Obtained mixture was heated at 85° C for15 min and centrifuged at 1000rpm for 15 min. After separation, upper organic layer was taken and its absorbance was taken in spectrophotometer at 532nm against blank prepared by omitting sample solution.

Estimation of total protein[15]

Reagents

NaOH 2gm, $NaHCO_3$ 10g, Sodium potassium tartrate 0.gm

All above reagent added and 500ml volume was made up with distilled water.

5% CuSO4 in dis. H2O.

10ml and 0.2ml of solution A and B taken respectively.

In 0.2ml of sample, 4ml of solution C and 0.6ml distilled water were added and kept aside for15 min at 37°C. 0.4ml of Folin-phenol reagent was added in that mixture after 15 min and resulting solution was again incubated for 30 min. After that, absorbance of prepared solutions were taken at 540nm in spectrophotometer by taking solution without sample as blank. Total protein was obtained in mg/ml of sample from standard albumin calibration curve.

STATISTICAL ANALYSIS

Graph Pad Prism computer software was used[16]. Result was expressed as Mean ± S.E.M, numbers of rats represented by n. Statistical significance between two means are determined by performing one way analysis of variance (ANOVA) followed by Dunnett's post hoc-test. P value <0.05 was considered significant.

OBSERVATIONS AND RESULTS

Acute oral toxicity

The animals were observed continuously for behavioural changes, autonomic profiles and other signs of toxicity or mortality up to period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14thday. There were no physical and behavioural changes observed in swiss albino mice during observation period. Body weight of all animals did not reveal any significant change as compared to vehicle control group and mortality was found Nil.

Effect on Peptic ulcer

The results of Ommiezz Syrup on Pylorus Ligation Induced Gastric Ulcer Model are as mentioned below [Table No.5, Graph No.1, 2, 3, 4, 5].

Effect on oxidative stress markers

The results of Ommiezz Syrup on various oxidative stress markers are as mentioned below [Table No.6, Graph No.6, 7, 8].

DISCUSSION

The toxicity screening of newly developed formulation is essential to assure its safety and effectiveness. This study can consider as a pioneer step for the establishment of safety profile and efficacy of Ommiezz syrup.

The acute oral toxicity study was performed on Swiss Albino Mice of both the gender for 14 days to rule out any toxic effect of Ommiezz Syrup at the single dose of 2000mg/kg. Individual animal weekly body weight was recorded and found to be increasing during the observation period [Table No.4]. Animal daily observation was recorded and found to be same and mortality was Nil [Table No.4]. There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Ommiezz Syrup which is indicated as antacid have no oral toxicity effect on Swiss albino mice. Hence, this can be used safely for therapeutic purposes.

The Ommiezz Syrup is combination of various proven ingredients for their ulcer protective effect. *Shatavari* (*Asparagus racemosus*)[17] and *Yastimadhu* (*Glycyrrhiza glabra*) have proven properties such as antisecretary and antiulcerative activity[18,19]. *Shati* (*Hedychium spicatum*) is also effective to protect histamine-induced gastric ulcer[20]. *Shankha Bhasma* is alkaline in nature and has excellent acid neutralizing capacity with speedy antacid as well as prolonged buffering action. It has been also proven for its anti-ulcer effect and indicated in various pathological conditions of GIT like hyper acidity (*Amlapitta*), loss of appetite (*Agnimandhya*), dysentery (*Grahani*) and duodenal ulcer (*Parinama Shula*)[21]. *Kamadudha Rasa*[22] and *Kapardika Bhasma*[23] are also proven formulations for their antacid action.

The ulcer protective effect of Ommiezz Syrup treated group in compression to various control and standard drug treated group showed statistically significant increase in pH and decrease in gastric volume, total acidity, free acidity and ulcer index was found in [Table No.5] which proves potential ulcer protective and antacid effect of this formulation.

The reactive oxygen species are generated and its accumulation is controlled by specific enzymes like superoxide dismutase, catalase and glutathione peroxidase during normal metabolic process. Any disturbance in such enzyme activity leads to accumulation of free radicals which can cause peptic ulcer[24]. The antiulcer and healing mechanism can be obtained by antioxidant activity of any medicinal plant or herbal formulation. The results of test drug on oxidative stress markers favors antioxidant properties of this formulation [Table No.6].

Table No.1: Ingredients of Ommiezz Syrup (Each 5ml contains)

S.No	Name of ingredient	Quantity
1	Ext. Asparagus racemosus	60mg
2	Ext. Hedychium spicatum	50mg
3	Ext. Glycyrrhiza glabra	20mg
4	Kamdudha Rasa	60mg
5	Shankha Bhasma	60mg
6	Kapardika Bhasma	50mg

Table No.2: Individual animal dosing record of test drug

S.No	Animal No	Gender	Experiment Day	Dose (ml)
1	H	M	1st day	1
2	B	M	3rd day	1
3	T	F	5th day	1
4	HT	F	7th day	1
5	UM	F	9th day	1

H: Head, B: Body, T: Tail, HT: Head and Tail, UM: Unmarked, M: Male, F: Female

Table No.3: Grouping of Animals

S.No	Group No	Group Name	Dose (Oral)	No. of animals
1	I	Normal control (NC)	Normal saline	6
2	II	Disease control (DC)	Normal saline	6
3	III	Sham operated control (Sham)	Normal saline	6
4	IV	Standard drug (Ranitidine) treated (Std.)	27mg/kg	6
5	V	Ommiezz Syrup (OS)	25mg/kg	6

Table No.4: Individual animal weekly body weight and Mortality record

S.No	Animal No	Gender	Experiment Day, Unit: gm			Mortality
			1st	7th	14th	
1	H	M	22	24	25	NIL
2	B	M	27	28	29	NIL
3	T	F	26	27	28	NIL
4	HT	F	25	26	26	NIL
5	UM	F	27	28	29	NIL

H: Head, B: Body, T: Tail, HT: Head and Tail, UM: Unmarked, M: Male, F: Female

Table No.5: Effect of test drug on various gastric parameters

Group	Dose (Oral)	Gastric volume	pH	Free acidity	Total acidity	Ulcer Index
I (NC)	Normal saline	0.3±0.0707	4.333±0.1667	--	--	0.0
II (DC)		8.625±0.4250###	2.167±0.1667###	31.33±3.75###	135.7±8.686###	213.8±23.75###
III (Sham)		0.3250±0.0853	4.167±0.0.1667	--	--	0.0
IV (Std.)	27mg/kg	2.875±0.3683***	4.833±0.1667***	7.00±1.00***	48.00±7.234**	16.25±3.75***
V (OS)	25mg/kg	5.475±0.6824***	3.333±0.3333***	12.33±4.910***	77.00±11.36**	60±15.00***

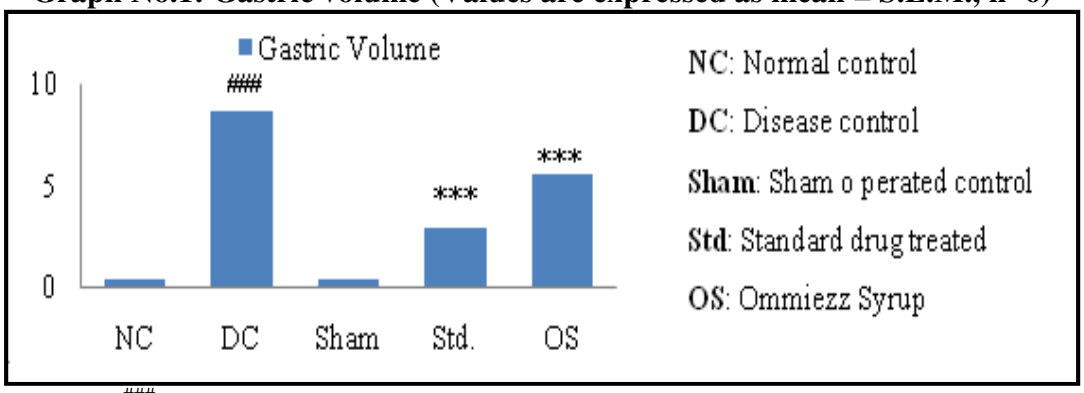
###p < 0.001 Vs Normal control, ***p <0.001, **p <0.01, *p <0.05 Vs Disease control

Table No.6: Effect of test drug on oxidative stress markers

Group	Dose (Oral)	SOD activity Units/mg protein	Catalase activity Unit/min/mg tissue protein	LPO MDA m moles/mg tissue peotein
I (NC)	Normal saline	16.28±1.071	0.04983±0.0008	17.55±1.360
II (DC)		3.724±0.3645###	0.0146±0.0011###	59.41±2.883###
IV (Std.)	27 mg/kg	14.37±0.2256***	0.04361±0.00063***	19.04±0.7666***
V (OS)	25 mg/kg	9.304±0.9348**	0.02510±0.0046*	32.60±2.415***

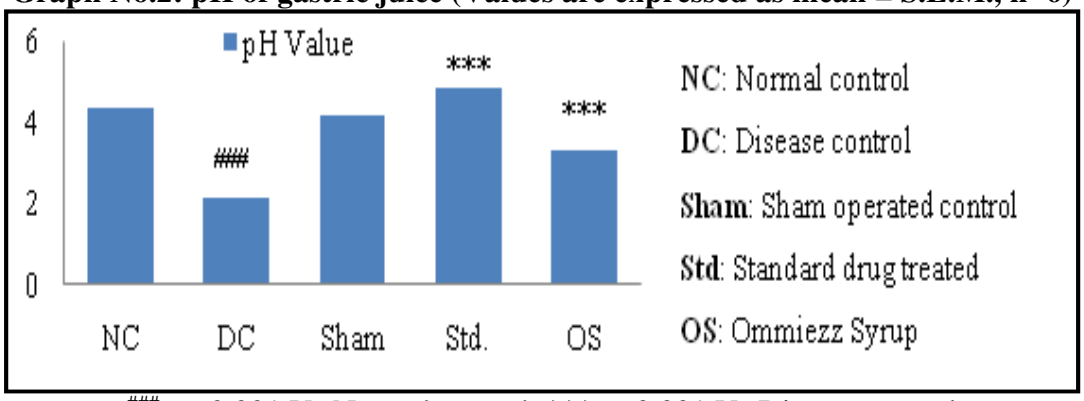
###p < 0.001 Vs Normal control, ***p < 0.001, **p < 0.01, *p < 0.05 Vs Disease control

Graph No.1: Gastric volume (Values are expressed as mean ± S.E.M., n=6)



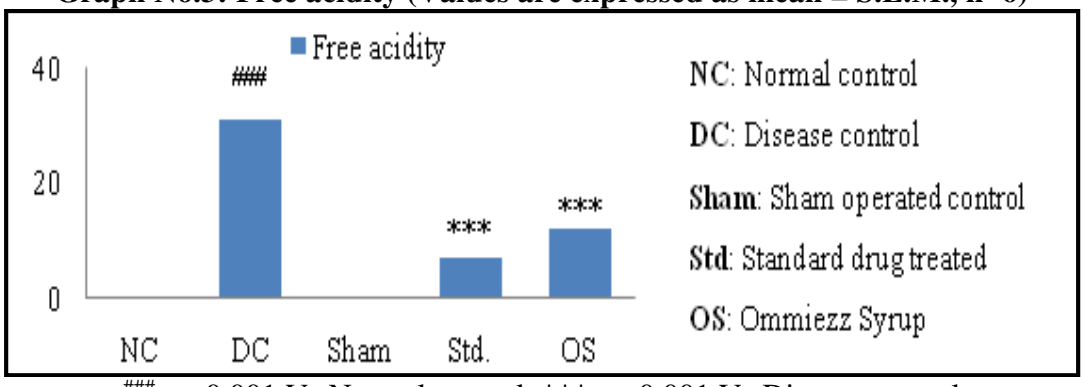
###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control

Graph No.2: pH of gastric juice (Values are expressed as mean ± S.E.M., n=6)



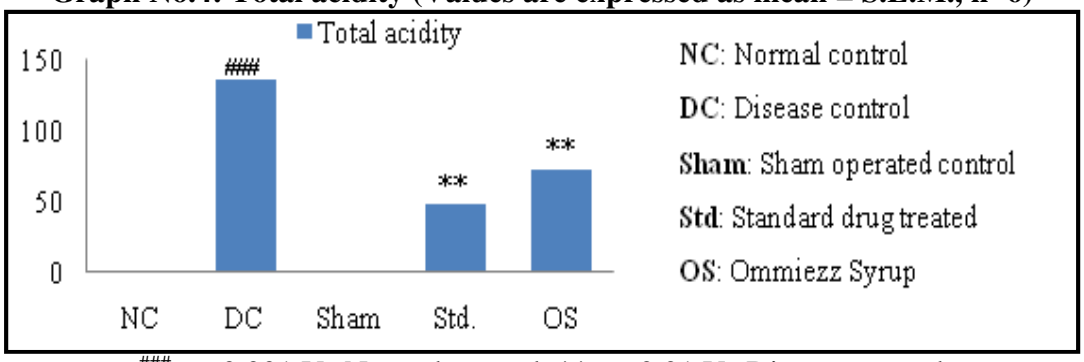
###p < 0.001 Vs Normal control, ***p <0.001 Vs Disease control

Graph No.3: Free acidity (Values are expressed as mean ± S.E.M., n=6)



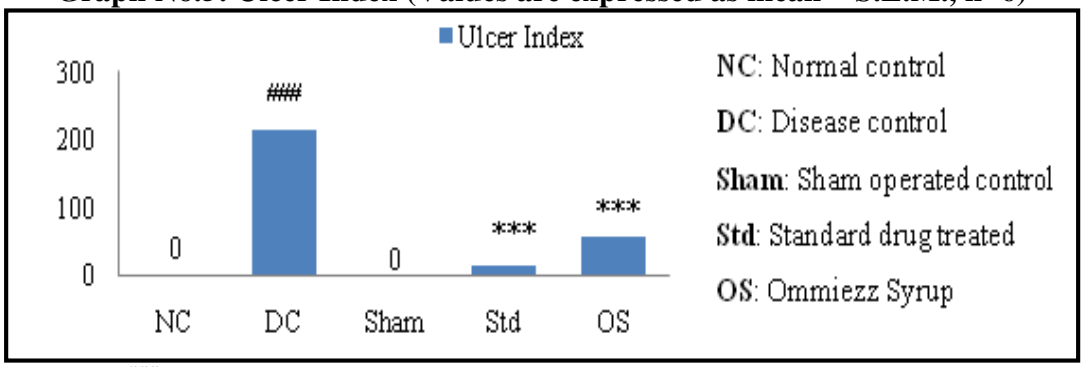
###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control

Graph No.4: Total acidity (Values are expressed as mean ± S.E.M., n=6)



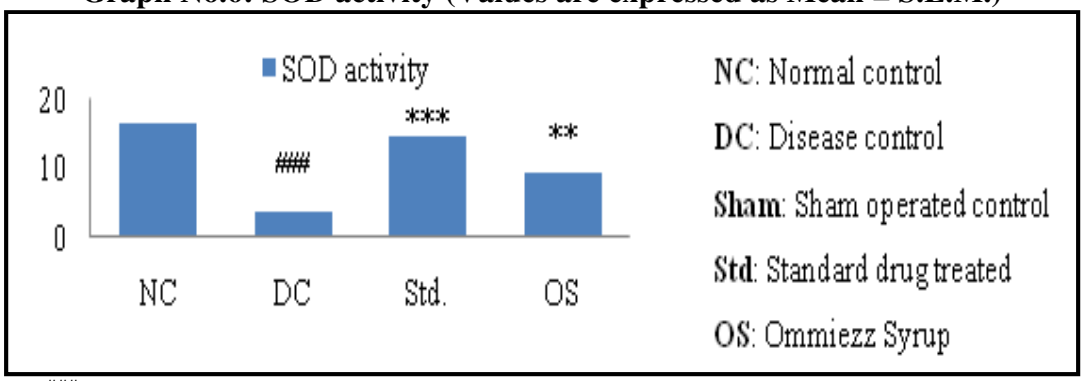
###p < 0.001 Vs Normal control, **p < 0.01 Vs Disease control

Graph No.5: Ulcer Index (Values are expressed as mean ± S.E.M., n=6)



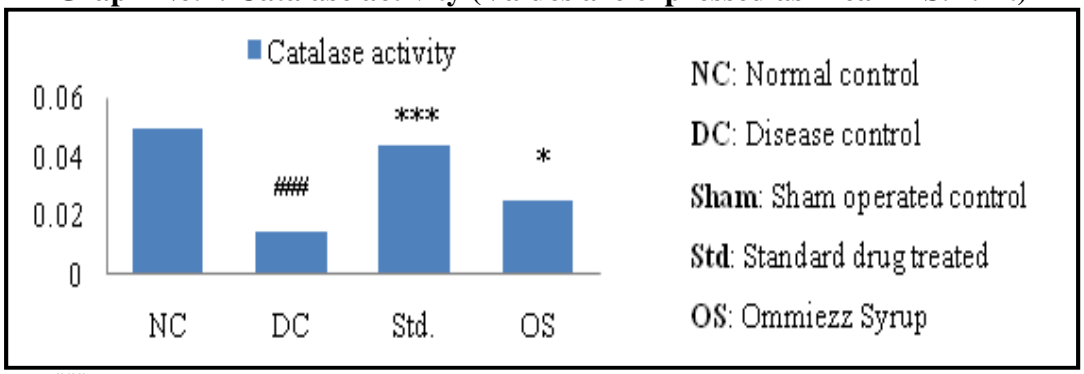
###p < 0.001 Vs Normal control, ***p <0.001 Vs Disease control

Graph No.6: SOD activity (Values are expressed as Mean ± S.E.M.)



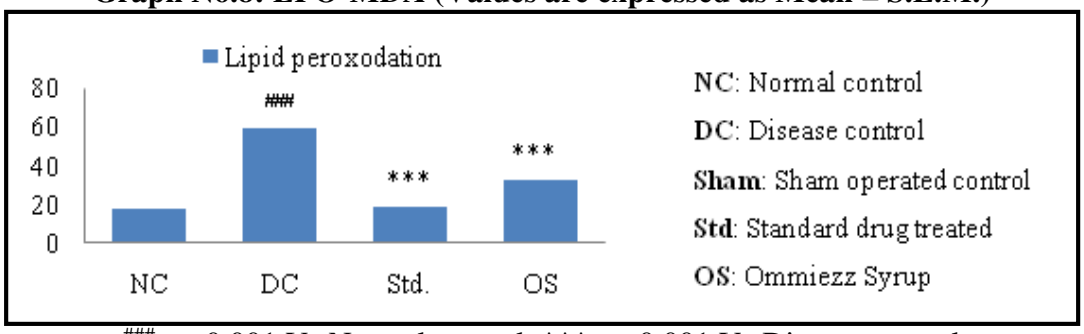
###p < 0.001 Vs Normal control, ***p < 0.001, **p < 0.01 Vs Disease control

Graph No.7: Catalase activity (Values are expressed as Mean ± S.E.M.)



###p < 0.001 Vs Normal control, ***p < 0.001, *p < 0.05 Vs Disease control

Graph No.8: LPO-MDA (Values are expressed as Mean ± S.E.M.)



$^{###}p < 0.001$ Vs Normal control, $^{***}p < 0.001$ Vs Disease control

CONCLUSION

This study reveals that Ommiezz Syrup (polyherbal formulation) does not have any toxic effect at dose of 2000mg/kg. So No-Observed-Adverse-Effect-Level (NOAEL) of Ommiezz Syrup is 2000mg/kg. The obtained results suggest that, tested polyherbal formulation (Ommiezz syrup) has antiulcer and antioxidant effect without any major side effects or mortality.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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