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REPRODUCTIVE TOXICITY INDUCED BY THE HERBICIDE QUIZALOFOP-P-ETHYL IN SWISS ALBINO MALE MICE

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ABSTRACT

Reproductive effects from herbicide exposure in mammals and the ecological assessment of chronic exposure is currently seen to be matter of concern. Quizalofop-p-ethyl is a herbicide used for controlling grass weeds in various crops such as soybeans, peanuts, cotton, potatoes, sugar beets and flax. The general toxicity of herbicide is well studied but reproductive toxicity studies are lacking. So an attempt has been done to assess the male reproductive toxicity. The LD50 of Quizalofop-p-ethyl in mice is 2350 mg/kg. Male mice were given oral doses of 1/25th of LD50 of the Quizalofop-p-ethyl (587.5 mg/kg) and 1/50th LD50 (1175 mg/kg) dissolved in water for 30 and 60 days respectively. Symptomatic behavioural changes were noticed, body weight and food consumption was decreased gradually. Treated mice group at 60 days showed significant decrease in testis, epididymis and seminal vesicle weight as compared to group which was given 30 days treatment. The biochemical analysis shows significant decrease in protein, fructose and sialic acid values at both doses levels 587.5mg/kg and 1175mg/kg for 30 and 60 days, increase in cholesterol and glycogen level has been seen at both days of treatment. The male reproductive toxicity of Quizalofop-p-ethyl suggests the restricted use of herbicide in public places.

Keywords: Quizalofop-p-ethyl, Biochemical analysis, reproductive toxicity, male swiss albino mice

INTRODUCTION:-

Environment is sum total of biological, economic, social, physical, chemical and cultural elements which are interlinked. Toxicity refers to chemical substance or mixture of substances which can damage an organism, such as an animal, bacterium or plant **[1]**. Toxic agents can cause potential harm to an organism such as bioaccumulation, issues of carcinogenicity, teratogenicity, mutagenic effects or the impact on reproduction and severely upsets biological balance. These agents can be herbicides, insecticides, rodenticides etc. One of the most common deleterious human activity that is threatening our own existence is the extensive use of these chemicals. Trade name for Quizalofop-p-ethyl is Targa Super, Assure 2, Matador, Leopard etc **[2]**. Quizalofop-p-ethyl is a selective phenoxy herbicide and is used to eliminate or control annual or perennial grass weeds in potatoes, sugar beets, vegetables, cotton and flax **[3]**. The continuous indiscriminate use of Quizalofop-p-ethyl may result in its direct release to the environment by various mode/sources or by waste streams. Quizalofop-p-ethyl remain in particulate phase in the atmosphere if released to air, because of vapour pressure of $4.49*10^{-9}$ mm Hg at 20ºC. By wet and dry deposition the particulate phase of Quizalofop-p-ethyl is removed from the atmosphere. Repeated use of herbicide is very common to control or eliminate the annual or perennial grass weeds. That's why an assumption has been made that lowest dose of Quizalofop-p-ethyl i.e of $1/25^{th}$ of LD50 (587.5 mg/kg) which is used in experiment could be found in food of plant origin which are treated with different concentration as recommended. The aim of this experiment was to investigate the effect of Quizalofop-p-ethyl herbicide on the reproductive parameters of Swiss Albino male mice

MATERIAL AND METHOD:-

HERBICIDE: Quizalofop-p-ethyl was purchased from local market of Jaipur by trade name Targa super.

ANIMAL: Healthy Swiss Albino male mice (25-30 gm) were procured from the animal house of IIS(deemed to be University), Jaipur. The usage of mice was approved by Institutional animal ethical committee (Approval No. IAEC/2019/II/3). A standard pellet diet was given to mice and water was provided *ad libitum*. A 12 hour light/ 12 hour dark schedule, controlled temperature and humidity conditions were maintained in animal house.

EXPERIMENTAL DESIGN AND GROUPING:-

The male mice were divided into 6 groups and in each group 6 animals were taken Group 'A' served as control for 30 days

treatment, Group 'B' 1/25th LD50 of Quizalofop-p-ethyl i.e. dose of 587 mg/kg was given and Group 'C' 1175 mg/kg i.e. 1/50th LD50 of Quizalofop-p-ethyl was given for 30 days. For 60 days Group 'D' served as control, Group 'E' 1/25th LD50 of Quizalofop-p-ethyl i.e. 587 mg/kg and Group 'F' is of 1175 mg/kg were given treatment for 60 days. The treatment was given orally through intubation tube and mice were dissected on 31st and 61st day respectively.

METHODS OF BIOCHEMICAL ANALYSIS:-

Biochemical tests were performed by respective methods i.e. Protein estimation **[4]**, Cholesterol **[5]**, Fructose, Sialic acid **[6]**, Glycogen **[7]**. Markers of Steroidogenesis: 3β Hydroxysteroid dehydrogenase and 17β Hydroxysteroid dehydrogenase **[8]** and sperm parameters were: Sperm Motility and Sperm count, Sperm viability and sperm deformities **[9]**.

STATISTICAL ANALYSIS:-

Data was analyzed and compared by using SPSS software. And the difference was calculated by their P values, significant if the ($^{*}P<0.05$) and highly significant ($^{**}P<0.01$) when experimental groups are compared to relative control and significant if the ($^{@}P<0.05$) and highly significant ($^{@@}P<0.01$) when compared between the groups.

RESULTS:-

The male mice were administered with two doses of Quizalofop-p-Ethyl. Experimental animal showed lethargy, red nose, sneezing, rubbing of nose and decrease in food intake **(Table 1)**.

Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) resulted in highly significant decrease ($^{**}P<0.01$) in body weight of mice after 30 and 60 days of treatment as compared to respective controls. Highly Significant decrease ($^{**}P<0.01$) in the weight of testis was seen after the mice was treated with Quizalofop-p-ethyl at 30 and 60 days of treatment at both the doses. Significant decrease ($@P<0.05$) in testis weight has been seen in Group III as compared to Group II.

Highly significant decrease ($^{**}P<0.01$) in epididymis weight was observed as compared to their relative control at 60 days of treatment. Significant decline ($@P<0.05$) in epididymis weight has seen in Group III as compared to Group II.

After 30 days of treatment significant decrease ($^{*}P<0.05$) in the seminal vesicle weight as compared to control groups, similarly at 60 days of treatment highly significant decrease ($^{**}P<0.01$) in the seminal vesicle weight was seen when compared to relative control **(Table 2)**.

The values of protein level showed significant decline ($^{*}P<0.05$) in Quizalofop-p-ethyl group at dose (1175 mg/kg) after 30

days of treatment. Highly significant decrease (**P<0.01) was observed in protein activity at 60 days of treatment with Quizalofop-p-ethyl at both the doses. No significant change was observed between the groups at 30 days of treatment. But significant decline (@P<0.05) in protein level has seen in Group III at 60 days of treatment.

Quizalofop-p-ethyl treated mice resulted in highly significant decrease (**P<0.01) in sialic acid level in the testis after 30 and 60 days of treatment as compared to respective controls. No significant change was observed between the group III and group II at 30 and 60 days of treatment.

Higher dose of Quizalofop-p-ethyl (1175 mg/kg) resulted in significant increase (*P<0.05) in cholesterol values and as treatment was continued for 60 days, highly significant increase (**P<0.01) was seen in the cholesterol level as compared to control values. Slightly increase (@P<0.05) in cholesterol level has been seen at 60 days of treatment when compared between Group III and Group II.

When Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) was administered orally for 30 days, significant increase (*P<0.05) in glycogen level was observed as compared to control values. But when the treatment was continued for 60 days, highly significant increase (**P<0.01) in glycogen level was observed. Highly significant increase (@@P<0.05) in glycogen level has observed in Group III when compared with Group II at 60 days of treatment.

The changes in fructose level of seminal vesicle of male mice is depicted in table 2. Significant decrease was observed in fructose level after 30 days of Quizalofop-p-ethyl (587.5 mg/kg) (*P<0.05) and at dose 2 when the treatment was further continued for 60 days, the fructose level showed highly significant decrease (**P<0.01) in Group III when compared to respective control. Significant decrease (@P<0.05) in fructose level has been observed in Group III when compared to Group II **(Table 3)**.

3β -HSD and 17β -HSD levels has significantly decreased when compared to respective control after 60 days of treatment. Highly significant decrease has been seen at dose 1 and dose 2 at 60 days when compared to respective control but significant decrease has been seen in group III when compared to group II **(Table 4)**.

Table 1: Effects of administration of Quizalofop-p-ethyl (5487.5 mg/kg) and (1175 mg/kg) on relative body weight, testis weight, epididymis weight and seminal vesicle weight of *Swiss Albino* male mice after 30 and 60 days of treatment

PARAMETER	TREATMENT	30 DAYS	60 DAYS
BODY WEIGHT (gm)	CONTROL	32.25±1.02	35.41±2.03
	QUIZALOFOP-P-ETHYL TREATMENT (587.5 MG/KG)	28.36±2.16	22.48±0.81 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	27.38±1.31 **	20.12±0.98 **
TESTIS WEIGHT (mg)	CONTROL	124.33±0.58	124.29 ± 0.49
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	120.47 ± 1.38	111.95 ± 1.23 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	115.05 ± 1.15@	98.18 ± 0.56 **
EPIDIDYMIS WEIGHT (mg)	CONTROL	12.39 ± 0.05	15.5 ± 0.42
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	12.14 ± 0.26	11.95 ± 0.15 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	14.05 ± 0.50@	9.18 ± 0.11 **
SEMINAL VESICLE WEIGHT (mg)	CONTROL	149.11 ± 0.70	147.62 ± 1.04
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	147.93 ± 0.43	138.35 ± 2.48 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	143.1 ± 0.76 *	135.4 ± 0.10 **

Table 2: Effect of administration of dose of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on various biochemical parameters in testis of *Swiss Albino* male mice (n=6)

PARAMETER	TREATMENT	30 DAYS	60 DAYS
TOTAL PROTEIN (mg/g)	CONTROL	24.26 ± 1.41	23.94 ± 0.96
	QUIZALOFOP-P-ETHYL(587.5 MG/KG)	10.5 ± 0.97	11.85 ± 1.22 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	8.69 ± 2.49 *	5.76 ± 0.82@
SIALIC ACID LEVEL (µg/mg)	CONTROL	22.92 ± 1.19	20.77 ± 0.70
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	10.64 ± 0.67 **	6.83 ± 0.70 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	9.77 ± 1.59 **	4.80 ± 0.69 **
TOTAL CHOLESTEROL (mg/100mg tissue weight)	CONTROL	62.12 ± 5.01	56.12 ± 6.89
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	83.48 ± 4.43	123.68 ± 18.2 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	89.56 ± 2.38 *	115.32 ± 7.22@
GLYCOGEN (µg/100mg)	CONTROL	355.21 ± 2.50	372.84 ± 2.16
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	372.17 ± 10.35 *	416.22 ± 7.22 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	396.72 ± 12.73 *	445.78 ± 3.58@@
FRUCTOSE LEVEL in seminal vesicle (µg/mg)	CONTROL	34.95 ± 2.11	29.35 ± 0.52
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	25.3 ± 0.94 *	19.76 ± 0.78 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	16.89 ± 1.78 **	10.97 ± 0.70@

Table 3: Effect of administration of dose of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on markers of steroidogenesis after 30 and 60 days of treatment in *Swiss Albino* male mice (n=6)

PARAMETER	TREATMENT	30 DAYS	60 DAYS
3β -HSD (nano mole of and-rostenedione formed/mg protein/min)	CONTROL	1.86±0.004	1.94±0.006
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	1.75±0.007	1.35±0.02 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	1.65±0.03 *	1.16±0.02 **
17β -HSD (nano mole of and-rostenedione formed/mg protein/min)	CONTROL	1.87±0.004	2.01±0.05
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	1.75±0.003	1.48±0.02 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	1.59±0.07 *	1.33±0.001 **

Table 4: Effect of administration of the Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on sperm parameters of *Swiss Albino* male mice (n=6)

PARAMETER	TREATMENT	30 DAYS	60 DAYS
SPERM MOTILITY (%)	CONTROL	82.66±0.90	81±3.64
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	45.87±1.43 **	29.06±3.50 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	35.86±0.80 **	27.86±3.31 **
SPERM VIABILITY (%)	CONTROL	69.06±3.96	71.46±4.44
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	30±3.79 **	23.4±3.19 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	25.33±4.25 **	18.4±1.35@
SPERM COUNT (million/ml)	CONTROL	43.45±1.31	42.16±2.75
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	28.85±0.98 **	20.9±0.23 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	21.48±0.79@	18.33±2.03 **
SPERM ABNORMALITY (%)	CONTROL	70±1.31	76.83±0.36
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	54.16±1.54 *	38.5±1.03 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	36±0.38@	22.16±1.62 **

Highly significant reduction (**P<0.01) in the cauda epididymal sperm motility was seen after the mice was treated with the both doses of Quizalofop-p-ethyl after 30 and 60 days of treatment as compared to control values. No significant change was observed between the group II and group III.

When mice was treated with Quizalofop-p-ethyl at dose 587.5 mg/kg and 1175 mg/kg for 30 and 60 days. Highly significant decrease (**P<0.01) in the cauda epididymal sperm viability was seen as compared to the control values. Significant decrease (@P<0.05) is seen in Group III at 60 days when compared to Group II.

In the cauda epididymal sperm count highly significant decline (**P<0.01) was observed after 30 and 60 days at dose 1 and dose 2 of Quizalofop-p-ethyl treated mice as compared to control value. Significant decrease (@P<0.05) has been observed in Group III at 30 days when compared to Group II.

Quizalofop-p-ethyl at dose 587.5 mg/kg and 1175 mg/kg led to sperm abnormality in mice. Statiscally highly significant decrease (**P<0.01) was observed in the number of normal sperms when mice treated with Quizalofop-p-ethyl for 30 and 60 days. Significant decrease (@P<0.05) was observed in Group III when compared to Group II at 30 days. Few abnormalities of sperms like two headed sperms, banana shaped head sperms, long tailed sperms and double headed sperms were observed in the Quizalofop-p-Ethyl treated mice.

DISCUSSION:-

Although pesticides are double edge sword it has its own benefits as well as some drawbacks and having potential toxicity to all life forms. To protect crops and vegetables from any harm and hazard, herbicides are used. Herbicides affects field, soil and even consumers when exceeded from recommended value. The continuous use may lead to several deadly, hazardous diseases (Deshmukh *et al;*

2015). In this present study Quizalofop-p-ethyl treated mice show decrease in body weight and organ weight, eye discharge and decrease in food intake. Similar results such as decrease in food consumption, body weight, testis weight were observed in rats at dose of 433mg/kg and 1300mg/kg of Quizalofop-p-ethyl **[10]**.

The study indicates that body weight and organ weight (testis, epididymis and seminal vesicle) of Swiss Albino male mice has significantly decrease in experimental group treated with both doses of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) at time interval of 30 and 60 days of treatment as compared to relative control group and within the groups, similar results were seen by Jian-Xi *et al.,* 2005 in which he reported that their was decrease in organ coefficient of testis, seminal vesicle and epididymis when rats were treated with Quizalofop-p-Ethyl for 3 months of time interval at dose of 433mg/kg and 1175mg/kg. Significant reduction in the weight of testis has been seen when treated with cyhalothrin **[11]**. The decrease weight of testis, epididymis and seminal vesicles was reported by Mathur *et al;* 2019 in methoxychlor treated animal. Al-Thani *et al*; 2003 has observed decline in body weight, food intake and relative weight of reproductive organs when male mice were treated with Amitraz for 12 weeks **[12]**. Fertility was significantly reduced in male mice ingesting 10.56 ± 0.97 or 20.39 ± 2.17 mg/kg/day. Testicular sperm count and sperm production were significantly decreased in males that ingested amitraz (pesticide). Significant variations has been seen in biochemical parameters also.

The spermatogenic process in the testis is coordinated by sertoli cells during different stages of maturation of germ cells synthesize variety of proteins. In testis, the protein is considered as a marker of injury in the tissue, damage of cells and healing **[13]**.

[14] The protein level of testis in our study was decreased as compared to control group at 60 days of treatment suggesting defective spermatogenesis, similar findings were seen by Deshmukh *et al;* 2015. Similarly decline in protein level and increase in cholesterol level has been seen in rats which received the dose of 62.5 or 125mg/kg of Pirimiphos-methyl for 90 days **[15]**. Cholesterol is mandatory for the regular testicular activity. It is a necessary basic component of all cell membranes and is an initiator of steroid hormones and for biosynthesis of bile acid (Chen *et al.,* 2011). Significant increase in cholesterol level of testis has been observed when treated with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) for 30 and 60 days indicating metabolism has been

affected. In 2010 it has been reported that the cells in the testis need cholesterol for biogenesis of membrane, cell signalling and it act as a precursor for synthesis of androgen **[16]**. In the present study, cholesterol levels in testis was increased after treatment with Quizalop-p-ethyl in mice for 30 and 60 days. Increased cholesterol level in testes of male mice when treated with cypermethrin **[17]**.

Sialic acid is a sialo mucopolysaccharide which is required for maintaining the structural integrity of membranes of spermatozoa and also aid in maturation of their membrane **[18]**. It is a derivative of N-acetyl mannose and pyruvic acid and also called as N-acetylneuraminic acid. The amount of sialic acid in testis and epididymis is associated with androgen level **[19]**. Sialic acid also forms a part of glycoprotein and glycolipids **[20]**. Significant decline in levels of sialic acid in testis is observed in the present study when compared to their respective controls which suggested that structural integrity of acrosomal membrane of spermatozoa is affected. Sialic acid and protein content of testis were decreased and cholesterol level was increased in dose dependent manner in mancozeb treated animals **[21]**. Similarly biochemical profile of the testis revealed a significant decline in the contents of sialic acid, whereas significant increase in cholesterol level was observed when treated with malathion **[22]**. Fructose is one of the most important markers of seminal vesicular function. Fructose helps in the spermatozoa metabolism and provides energy to the sperms and increase in sperm motility **[23]**. The formation and secretion of fructose by seminal vesicle depends upon the exudation of testosterone from the Leydig cells of the testis. The values of fructose level in seminal vesicle has significantly decreased after 30 and 60 days of treatment which depicts towards impairment in the secretory function ascribed to androgen depletion. Glycogen is the storage form of glucose in animals and is considered as the energy source in the testis and epididymis. For maturation and functioning of gonads testicular glycogen plays a crucial role **[24]**. It is also a source of glucose 6- phosphate required for pentose phosphate pathway **[25]**. Significant increase in glycogen level was observed in control values and between the groups. Biochemical parameters are good diagnostic tool for reproductive toxicity. Increase in cholesterol level is interrelated with decline in the activities of steroidic enzymes (3β hydroxysteroid dehydrogenase and 17β hydroxysteroid dehydrogenase) which disturb the testicular steroidogenesis. Testicular androgenesis is controlled by two rate limiting enzymes 3β -HSD and 17β -HSD (Ghosh *et al.,* 1989; Ishii-Ohba, 1986). The enzyme 3β-

hydroxysteroid dehydrogenase (3β-HSDs) and 17β-hydroxysteroid dehydrogenase (17β-HSDs) are the key enzymes in the biosynthesis of all the steroid hormones. Decrease in level of 3β -HSD and 17β - HSD level has been seen when treated with Quizalofop-p-ethyl at 30 and 60 days of treatment. Similar results were seen in Kostic *et al.,* 2011 suggested that androgen production can inhibit its production in testis and its repression is regulated by 3β-HSD level **[26]**.. The changes in the synthesis of steroid hormones may be due to the changes in activity of steroidogenic enzymes. 3β-HSD is one of the important enzymes in the biosynthesis of androgens and all other physically active steroid hormones **[27]**.

The cauda epididymis is the primary storage for the mature sperm **[28]**. In another attempt, Smith et al; 1972 studied DDT and found that it causes male reproductive toxicity he stated that single oral dose of DDT found to cause a high level of radioactivity in the testis and seminal vesicle. Dikshith and Datta stated that it also causes spermatogenic cell degeneration and decreased spermatogenic activity in the testis of rodents when treated for longer period of time **[29]**. Methoxychlor another widely distribut ed organo chlorinated hydrocarbon, studied by Bal that inhibits spermatogenesis **[30]**. Hamilton 1975, reported that the epididymis of mammals secrete glyceryl phosphoryl choline (GPC), sialic acid, lipids and proteins and these protein and sialic acid undergo control of androgens and plays an vital role in sperm motility **[31-32]**. Testosterone and epididymal protein are needed for the maturation and development of epididymal spermatozoa and their motility **[33]**. Sperm motility, sperm viability, sperm count was decreased when treated with Quizalofop-p-ethyl for 30 and 60 days. Few abnormalities of sperms like two headed sperms, banana shaped head sperms, long tailed sperms and double headed sperms was observed in the Quizalofop-p-ethyl treated mice. When albino male albino rat was exposed to Roundup herbicide for 12 weeks at the dose of 50.4mg/kg and 248.4 mg/kg b.w. leading to reduction in sperm count, percentage motility and increase abnormal sperm cell has been seen consisting of double head sperm, double tail sperm, sperm with coiled tail **[34]**.

CONCLUSION:-

The Quizalofop-p-ethyl (a herbicide) when given for 30 and 60 days at dose of (587.5 mg/kg) and (1175 mg/kg) has induced profound changes in reproductive parameters evidenced by changes in several biochemical parameters such as protein, cholesterol, sialic acid, glycogen and cholesterol and physical parameters i.e. body weight and organ weight (testis,

epididymis, seminal vesicle). Significant changes has been observed in markers of steroidogenesis and in sperm parameters. Sub-chronic exposure of Quizalofop-p-ethyl leads to significant toxicity in male reproductive system of Swiss Albino mice. It is found to be hazardous on living kind if swallowed or inhaled, so avoid recurrence in order to minimize the risk of these chemicals.

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CONFLICT OF INTEREST

No conflict of interest

ETHICAL APPROVAL

The study was approved and the work was performed in accordance with the guidelines of Institutional Animal Ethical Committee (Approval No. IAEC/2019/II/3).

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