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In vitro antioxidant and renoprotective potential of methanolic extract of *Verbascum thapsus* leaf in rats

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ABSTRACT

Kidney disease are the most leading causes of death in world. In this study the effect of oral administration of methanolic extract of Verbascum thapsus leaf at the dose of 250 & 500 mg/kg was studied on gentamicin induced nephrotoxicity in wistar rats using Silymarin as a standerd drug at a dose of 50 mg/kg, and it significantly reduced nephrotoxicity by reducing the elevated creatinine, urea and blood urea nitrogen level. In addition to that, significant decrease in lipid peroxidation (MDA level) and increase in catalase, superoxide dismutase, reduced glutathione were observed in kidney tissue homogenate. Thus show nephroprotective effect threw antioxidant activity and anti oxidant activity is conformed by in vitro evaluation threw DPPH radical scavenging, Nitric oxide scavenging, Reducing power assay.

Keywords: *Verbascum thapsus* nephrotoxicity; gentamicin

INTRODUCTION

Verbascum thapsus is a biennial plant of Scrophulariaceae family botanical source is *Verbascum thapsus linn.* about 228 species are found all over world.. It is found wild in North America, Europe, and Asia, In India it is found mainly in uttrakhand, himachal and some area on north east states. *V. thapsus* is known by a variety of names commonly known as commen Mullein, van tambakhu, gedar tambakhu, ekal veer The standard English name used by authorities in its native area is Great Mullein**,.** It generally grow straight ,tall, woolly, winged by the decurrent base of the leaf and form 3 to 5 feet high and flowers are golden yellow in color [**1**] . Major chemical constituent are Resin, Glycoside (aucubin), Saponins, Flavonoids (hesperidin, verbascoside), Tannins, Carotene, Mucilage, Choline, Magnesuium, Iron, Potassium, Calcium phosphate, Sulphur [**2][3]**the general use of mullein are antiviral [4], anti microbial, antimalarial, antioxidant**[5]** antiinflammatory, antinociceptive, antitumor, anticancer, cytotoxic immunomodulatory, antiulcerogenic, antihepatotoxic, anti hyperlipidemic, antitussive and antigermination [**6]** anti bacterial**[7]** anti fungal **[8].**

Oxygen is essential for the survival of all of us in the earth . About 5% of oxygen utilize in metabolic process reduced in free radicals such as superoxide radicals, hydrogen peroxide, hydroxyl radical and nitric oxide radicals all of these are known as reactive oxygen species these are highly toxic, mutagenic and reactive [**9]** . These highly unstable molecules react rapidly by donating, abstracting, or even sharing outer orbital electron and generating a second free radical. There high reactivity is due to the generation of such molecular chain reaction and effectively multiply their deleterious effect in many folds and later the free radicals produce number of complication like cancer, inflammation, ageing, diabetes, liver damage, kidney damage, cardiovascular disorder & neurodegenerative disease. [**10]**

Our body is having a defense mechanism to scavenge these reactive oxygen species these are called endogenous antioxidants they neutralize the free radical before they attack to the cell. These endogenous antioxidants are superoxide dismutase, catalase, glutathione peroxidase**.[11]** . There are other type of antioxidants also available which are obtained from natural plants these are also called as exogenous antioxidants these are tocopherol, carotenoids, ascorbic acid, flavonoid, tannins etc**[12].**

Acute renal failure is a major complication of aminoglycoside antibiotics, which are widely used in the treatment of gram-negative infections. Sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water. A large amount of in vitro and in vivo evidence indicates that these partially reduced oxygen metabolites are important mediators of gentamicin nephrotoxicity. Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical. Gentamicin has been shown to lead to release of iron from renal cortical mitochondria and to enhance generation of hydroxyl radical. These in vitro observations have been supported by in vivo studies in which scavengers of reactive oxygen metabolites and iron chelators have shown to be protective in gentamicin induced acute renal failure.[**13][14]**

The present study aimed to evaluate invitro antioxidant and nephroprotective effect of *Verbascum thapsus* against Gentamicin induced nephrotoxicity in rats with special reference to biochemical and antioxidants parameters .

MATERIALS AND METHODS

2.1 Plant Collection:

The plants **Verbascum *thapsus*** were collected in the month of august 2010 from the nainital region, India. The plant material was taxonomically identified by the N.B.P.G.R. Niglat Bhawali, Nainital Uttrakhand A voucher specimen of plant was deposited in the RSB herbarium under the number RSB/Tech.Corrsp./2011-12/525.

2.2 Preparation of Extract:

The leaves of **Verbascum *thapsus*** were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further use and extracted with petroleum ether and methanol respectively using soxhlet apparatus. After complete extraction the solvent was recovered with the help of recovery unit. The extract was stored at room temperature till further use in the experiment. The Percentage Yield (methanolic Extract) was found to be 17.6%.

2.3 Phytochemical screening of extract:

Phytochemical screening of plant extract shows the presence of carbohydrate, tannins , flavanoids, Phenolic compounds Phlobatannin, Glycosides and saponin.

2.4. In vitro evaluation of antioxidant:

2.4.1. DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging activity was measured by spectrophotometric method. 2.95 ml of methanolic solution of DPPH (100 µM) was added to 0.05 ml of different concentrations (10-640 µg/ml) of methanolic extract of *Verbascum thapsus* dissolved in methanol. Equal amount of methanol was added to the control. Absorbance was recorded at 517 nm after 20 min **[15]** . Ascorbic acid was used as a standard and all the assays were carried out in duplicate. The purple colour of DPPH changes to yellow, based on the efficacy of antioxidants. Radical scavenging activity was expressed as percent inhibition and was calculated using the following formula.

Percentage inhibition (%) = [(A*control* – A*sample*) / A*control*)] x 100.

where, A*control* is the absorbance of control reaction (containing all reagents except test compound), and Asample is the absorbance of test compound.

2.4.2Nitric Oxide scavenging assay

Sodium nitroprusside (5 mM) in phosphate buffer saline was mixed with different concentrations of methanolic extract (10-640 µg /ml) dissolved in methanol and incubated at 25^{o}C for 30 min. After 30min, 1.5 ml of incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% napthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotisation of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine was measured at 546 nm along with a control **[15]** The percentage inhibition is calculated as above.

2.4.3 Reducing power
Reductive ability of the extract was measured according to the method of Oyaizu . Different concentrations (10-640 µg /ml) of extract were mixed with 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50oC for 20 min. Trichloroacetic acid (2.5 ml of 10%) was added to it, the mixture was mixed and centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of ferric chloride (1%) and absorbance was measured at 700 nm. Control reaction contains all the reagents except test compound. Higher absorbance indicated higher reducing power.

CHEMICALS USED:
D.P.P.H., nicotinamide adenine dinucleotide(from hi media lab.), nitroblue tetrazolium (from altop science) sodium nitroprusside, sulphanilic acid, napthylethylene diamine dihydrochloride, orthophosphoric acid, potassium ferricyanide, Trichloroacetic acid, ferric chloride etc. (loba chemicals)

2.5. Pharmacological evaluation for nephroprotective studies
2.5.1 Animals ;- Male Wistar rats (150-200 g) were procured from I.V.R.I. Berally, CPCSEA Reg. No. 490/01/a/CPCSEA, vide letter No. 25/1/99, Dated: 31st October 2001. Protocol No.: 07/2011. They were housed in microlon boxes with standard laboratory diet and water. The study was conducted after obtaining Institutional animal ethical committee clearance

2.5.2 Experimental protocol
Group 1 ;- only gentamicin 80 mg/kg
Group 2 ;- Silymarin(std)50 mg/kg with gentamicin
Group 3 ;- Methanolic extract 250mg/kg with gentamicin
Group 4 ;- Methanolic extract 500mg/kg with gentamicin
Group 5;- vehicle treated

At the end of experimental period,all the animals were sacrificed under diethyl ether anesthesia. But the Blood samples were collected on 0,3,5,9 day allowed to clot. Serum was separated by centrifuging at 4000 rpm for 10 min and analyzed for various biochemical parameters.

2.5.3. Drugs and Chemicals:
Gentamicin was obtained from micro labs Company and was used to induce nephrotoxicity in rats. Sylimarin was obtained from the Himalaya Drug Company Pvt. Ltd, and was used as a standard drug. All other chemicals and solvents used in this study were of analytical grade and purchased from commercial sources.

2.5.4. Serum Analysis:
During the experiment blood samples were collected on 0,3,5,9 day from retro-orbital plexus under anaesthetic conditions and allowed to clot. Serum was separated by centrifugation at 4000 rpm for 10 minutes and analyzed for Blood Urea , blood urea nitrogen (GLDH- urease method) & creatinine (Mod. Jaffe's kinetic method) by auto analyzer.

2.5.5.Kidney homogenate analysis:
On last day animals were sacrificed by cervical dacepitation and the abdomen was cut open to remove both kidneys from each animal. tissue is rinsed with 0.9% ice cold normal saline then 10% kidney homogenate with 1.17% ice cooled KCl and then centrifuged tissue at 800 g for 5 min at 4° c then seprate the supernatant and again centrifuge at 10,500 g for 20 min at 4° c and collect the supernatant. And performed lipid peroxidation assay by Lefevre et al. 1998**[17].** enzymes superoxide dismutase (SOD) and catalase (CAT) were estimated by using the methods described by Kakkar et al. 1984 **[18]** and Luck 1963 **[19]** respectively & reduced glutathione by ellman., 1959 **[20]**

2.6 Statistical analysis:
The results were expressed as the mean ± SEM and analyzed using one-way ANOVA followed by t comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to be statistically significant.

2.7 Biochemical parameters:
The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyser and U.V.Spectophotometer .

RESULTS

3.1. Invitro antioxidant results

3.1.1 NO scavenging assay of mevt = The MEVT effectively reduced the generation of nitric oxide radicals from sodium nitroprusside solution in a concentration dependent manner (Figure 1). This showed significant nitric oxide scavenging activity of the extract. Percentage inhibition at 640 µg/ml was found to be 41.12% and 94.59% by MEVT and ascorbic acid, respectively**.**

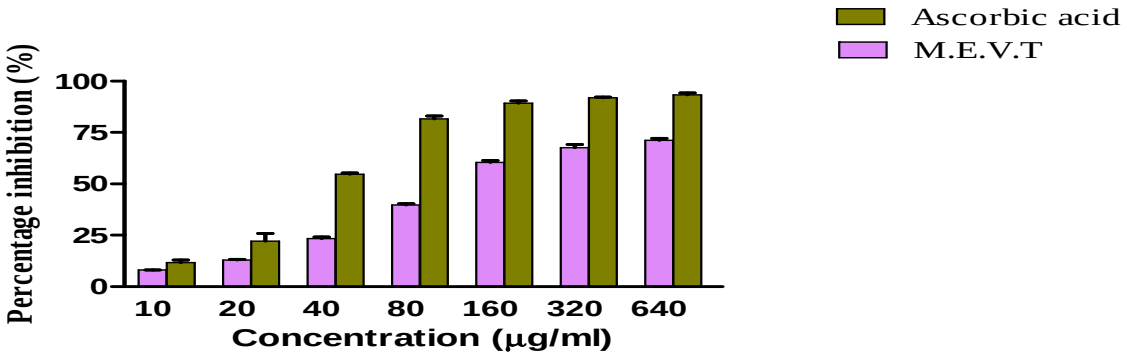

Figure 1; Effect of different concentration of M.E.V.T *on* NO scavenging assay.
Values are average of duplicate experiment and represented as mean ± standard error mean

3.1.2 D.P.P.H Assay = The results showed that the M.E.V.T has reduced the free radical (1,1-diphenyl-2-picrylhydrazyl) to corresponding hydrazine in a concentration dependent manner. Percentage inhibition at 640 µg/ml was found to be 51.83% and 98.27% by M.E.V.T and ascorbic acid, respectively. The results thus demonstrated good free radical scavenging activity of the extract.

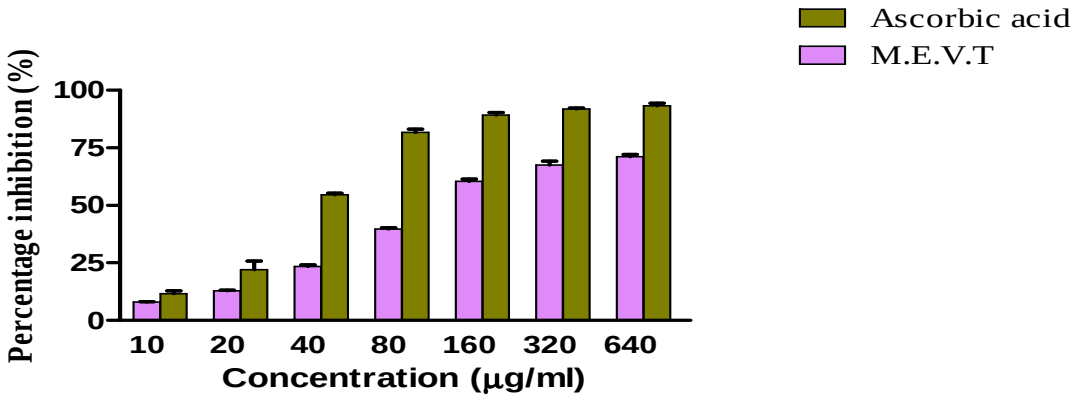

Figure 2; Effect of different concentration of MEVT *on* DPPH scavenging assay.
Values are average of duplicate experiment and represented as mean ± standard error mean

3.1.3 Reducing power assay

The extract exhibited concentration dependent increase in absorbance. Absorbance indicated by all the concentrations of extract was significantly higher than the absorbance of control reaction (0.031). Higher absorbance indicates high reducing power due to formation of reduced intermediate.

Table 1: Reducing ability of different concentration of MEVT
Values are average of duplicate experiment and represented as mean ± standard error mean. Ascorbic acid was taken as standard

Concentration (µg/ml)	Absorbance at 700 nm	
	Ascorbic acid	MEVT
10	0.2237±0.013	0.1905±0.011
20	0.4036±0.006	0.3480±0.018
40	0.7619±0.037	0.4723±0.024
80	1.317±0.026	0.7730±0.032
160	2.280±0.175	1.4439±0.039
320	3.675±0.238	1.8893±0.045
640	5.108±0.108	2.5011±0.047

3.2. Gentamicin induced nephrotoxicity results.

3.2.1 Biochemical parameters:

In gentamicin treated group of animals the concentration of serum urea, creatinine and blood urea nitrogen were considerably increased than the normal animals which indicates severe nephrotoxicity. Treating with methanol extract of *Verbascum thapsus* showed significant decrease ($p<0.001$) in concentration of serum urea, creatinine and blood urea nitrogen compared to gentamicin treated group. N=5 animals in a group; Values are expressed as Mean ± SEM;

*: $p<0.05$, **$p<0.01$, *** $p<0.001$. ns indicate no significant.

These colour bars indicates corresponding group

= Gentamicin treated

= Sylimarin treated

= MEVT 250

= MEVT 500

= Vehicle

Fig. 3 Creatinine ;- Group I show significant increase in the creatinie level as compare of vehical treated group as per the no.of dosing increase indicate nephrotoxic rats. And In the methanolic extract of *Verbascum thapsus* and standerd treated groups, creatinie level was significantly lower than that of group 1 . Results are shown in Table 2 and figure 3.

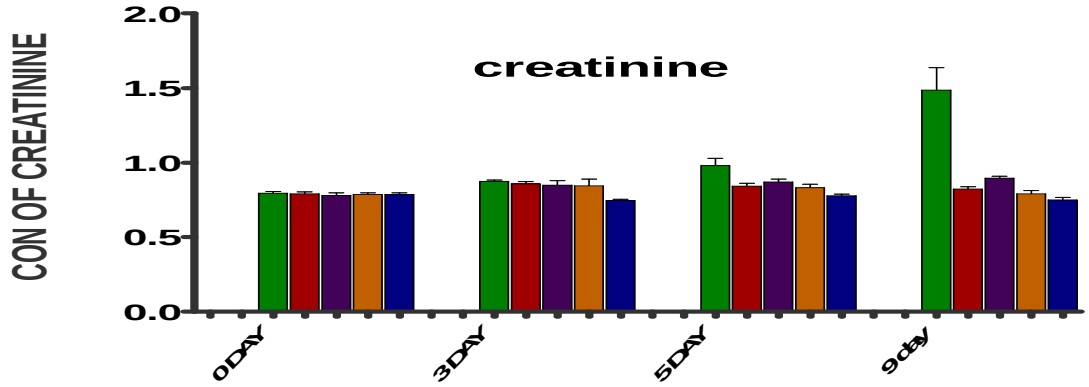

Fig. 4. Urea:- ;- Group I show significant increase in the urea level as compare of vehical treated group with no.of dosing increase indicate nephrotoxic rats. And In the methanolic extract of *Verbascum thapsus* and standard treated groups, urea level was significantly lower than that of group.1. Results are shown in Table 3 and figure no.4

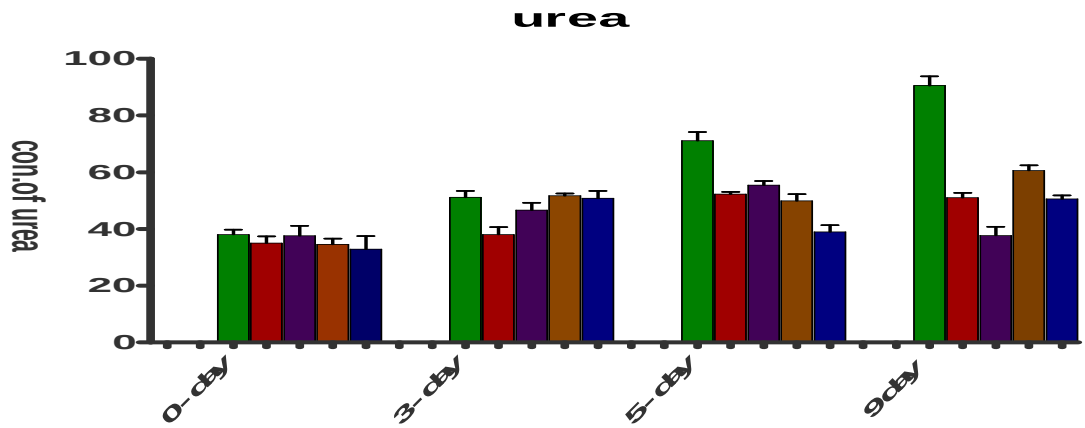

Table 2 ;- Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *MEVT* on serum creatinine; in treated rats for 9 days

GROUP	0 DAY	3rd DAY	5th DAY	9th DAY
CONTROL	0.79 ± 0.009	0.87 ± 0.009**	0.98 ± 0.049**	1.48 ± 0.149**
STANDARD	0.79 ± 0.014	0.85 ± 0.013^{ns}	0.84 ± 0.019*	0.82 ± 0.014**
MEVT 250	0.78 ± 0.016	0.85 ± 0.028^{ns}	0.87 ± 0.020^{ns}	0.89 ± 0.012**
MEVT 500	0.78 ± 0.012	0.84 ± 0.045^{ns}	0.83 ± 0.023*	0.79 ± 0.023**
VEHICAL	0.78 ± 0.015	0.74 ± 0.009	0.77 ± 0.010	0.75 ± 0.016

Table 3:- Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *MEVT* on serum urea in treated rats for 9 days

GROUP	0 DAY	3rd DAY	5th DAY	9th DAY
CONTROL	38.02 ± 1.74	51.14 ± 2.21**	71.13 ± 3.07***	90.53 ± 3.25***
STANDARD	35.05 ± 2.30	50.78 ± 2.67^{ns}	52.31 ± 0.82***	50.63 ± 1.19***
MEVT 250	37.64 ± 3.52	51.66 ± 0.89^{ns}	55.43 ± 1.48**	60.61 ± 1.76***
MEVT 500	34.64 ± 1.95	46.66 ± 2.59^{ns}	49.96 ± 2.27***	51.00 ± 1.68***
VEHICAL	32.82 ± 4.70	38.03 ± 2.68	39.02 ± 2.35	37.68 ± 3.13

Fig. 5. Blood urea nitrogen ;- Group I show significant increase in the Blood urea nitrogen level as compare of vehical treated group with no.of dosing increase indicate nephrotoxic rats. And In the methanolic extract of *Verbascum thapsus* and standard treated groups, Blood urea nitrogen level was significantly lower than that of group 1. Results are shown in Table 4 and figure no.5

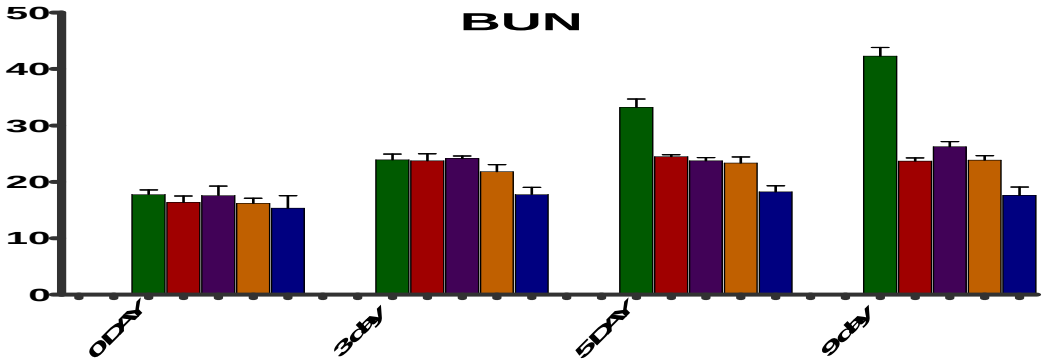

Table 4 - Effect of 80 mg/kg/day intraperitoneal gentamicin and graded oral *MEVT* on serum BUN in treated rats for 9 days

GROUP	0 DAY	3rd DAY	5th DAY	9th DAY
CONTROL	17.76 ± 0.85	23.90 ± 1.03**	33.23 ± 1.43***	42.30 ± 1.52***
STANDARD	16.38 ± 1.07	23.72 ± 1.24^{ns}	24.44 ± 0.38***	23.62 ± 0.56***
MEVT 250	17.58 ± 1.64	24.14 ± 0.41^{ns}	25.90 ± 0.89**	28.32 ± 0.85***
MEVT 500	16.19 ± 0.91	21.80 ± 1.21^{ns}	23.34 ± 1.06***	23.83 ± 0.78***
VEHICLE	15. 33 ± 2.19	17.77 ± 1.25	18.23 ± 1.10	17.61 ± 1.46

3.2.2 In vivo kidney tissue enzyme level: Kidney antioxidant status:
Considerably decrease in activity of Catalase, SOD and glutathione peroxidase in gentamicin treated animals when compared to normal animals. Treatment with methanol extract of V.T significantly prevented decrease in the level of catalase, SOD, GSH activity compared to gentamicin treated rats. Nevertheless considerable increase in activity of lipid peroxidase in gentamicin treated animals . Treatment with methanol extract of V.T significantly prevented increase in the level of lipid peroxidase. Thus strongly inhibit lipid peroxidation in isolated tissue via its antioxidant activity.

TABLE 5

ENZYME	CONTROL	STANDERD	MEVT 250	MEVT 500	VEHICLE
CATALASE	5.290 ±0.942***	14.52± 1.230***	10.46± 0.841***	13.38 ± 1.183***	17.69 ± 1.774
SOD	8.852± 0.681***	17.67± 0.436***	12.29 ± 1.054**	15.90 ±0.576***	19.45 ± 0.431
GSH	0.008582 ±0.000360**	0.03636 ±0.003153**	0.008942 ±0.000341^{ns}	0.02993 ±0.00529**	0.05356 ±0.00335
LIPID PEROXIDATION	4.048± 0.285***	1.466 ± 0.237**	3.669 ± 0.238^{ns}	1.884 ± 0.1972**	0.6316±0.0253

N=5 animals in a group; Values are expressed as Mean ±SEM; analysis is done by t- test , followed by one way anowa.
**: p<0.05, **p<0.01, *** p<0.001. ns indicate no significant*
LPO = n moles of MDA / mg protein;SOD= Units /mg protein;
CAT= µ mole of H_2O_2 consumed min^{-1} mg^{-1} protein;GSH= n moles gsh / mg protein

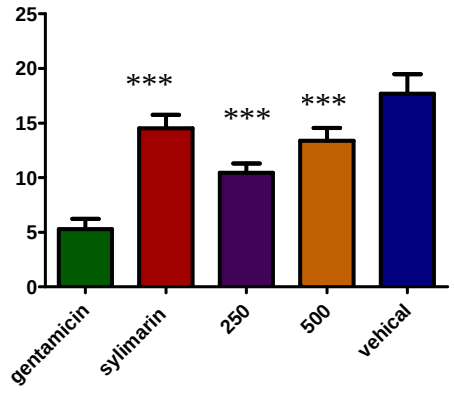

Fig. 6 CATALASE;- Group I show significant (p <0.001) decrease in the catalase level as compare of vehical treated group indicatenephrotoxic rats. And both extract and standard treated groups, significantly (p <0.001) increase the catalase level.

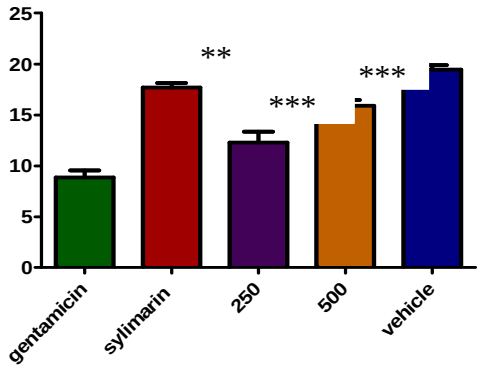

Fig. 7 SOD;- Group I show significant (p <0.001) decrease in the sod level as compare of vehical treated group indicate nephrotoxic rats. And mevt 500 extract and standard treated groups, significantly (p <0.001) & mevt 250 significantly (p <0.01) increase the sod level

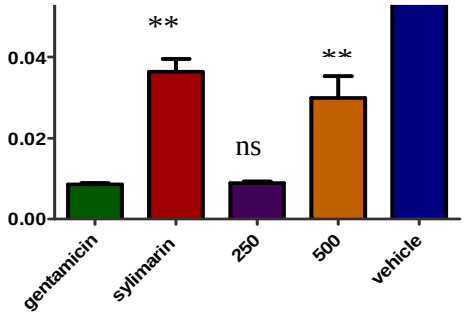

Fig. 8 GSH ;- Group I show significant (p <0.001) decrease in the gsh level as compare of vehical treated group indicate nephrotoxic rats And mevt 500 extract and standard treated groups, significantly (p <0.01) increase the sod level but mevt 250 is non significant to increase sod level.

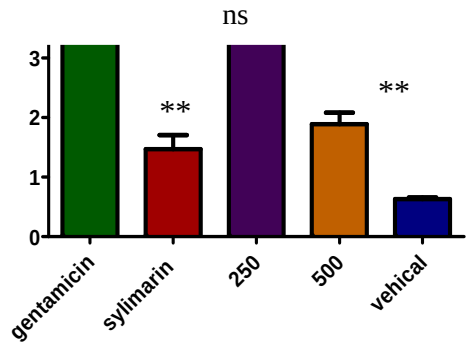

Fig. 9 Lipid peroxidation Group I show significant (p <0.001) increase in the lipid peroxidation level as compare of vehical treated group indicate nephrotoxic rats. And mevt 500 extract and standard treated groups, significantly (p <0.01) increase the lipid peroxidation level but mevt 250 is non significant to increase sod level.

DISCUSSION

INVITRO STUDY ;-

Free radicals are chemical entities that can exist seprately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals.[**21]** Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc. DPPH is a relatively stable free radical and the assay determines the ability of MEVT to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidants can act by converting the unpaired electrons to paired ones. The dose dependent inhibition of DPPH radical indicates that MEVT causes reduction of DPPH radical in a stoichometric manner. **[22]**

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes **[23].** Excess concentration of NO is associated with several diseases **[24].** Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals**.** In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions.

A substance may act as an antioxidant due to its due to oxidative enzymes of body as well as *via* ability to reduce ROS by donating hydrogen atom The reducing property of MEVT that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor

towards antioxidant activity because the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups,which can function as hydrogen donor.**[25][26]**

Preliminary phytochemical analysis indicates the presence of tannins in M.E.V.T. Polyphenols, particularly flavonoids and tannins are well known natural antioxidants **[27]** Thus, the antioxidant potential of methanol extract of *verbascum thapsus* leaf may be due to the presence of polyphenolic compounds, which further analysis is done by in vivo nephrotoxic model in rats.

2. Nephroprotective studies;-

The use of gentamicin, an aminoglycoside antibiotic with a wide spectrum of activities against Gram-positive and Gram-negative bacterial infections but with high preference for latter is equally associate with nephrotoxicity as its side effect **[27][28].** Thus gentamicin induced nephrotoxicity is well established experimental model of drug induced renal injury . Many animal experiments have demonstrated **[29][30]** overwhelmingly, the positive correlation between oxidative stress and nephrotoxicity . Gentamicin induced nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomylinase and phospholipases in addition to causing oxidative stess. **[32] Lindquist S., 1986)**. Drug induced nephrotoxicity are often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis**[31].** So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animal and man.

In the present study drug induced nephrotoxicity were established by single daily intraperitoneal injection of the gentamicin, for 9 days. This toxicity characterized by marked elevation in the circulating levels of blood urea, serum creatinine and blood urea nitrogen in the model control rats when compared to untreated rats. However these changes were attributed by pretreatment with single daily graded doses of *Verbascum thapsus* extract for 9 days. Oral administration of plant extract significantly decreases the urea and creatinine & blood urea nitrogen level in both treatment group compare to control . Apart from the direct nephrotoxic effect of gentamicin in rats, the acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolong anorexia associated with gentamicin nephrotoxicity. In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance **[32]** . Elevation of urea , creatinine & blood urea nitrogen levels in serum was taken as the index of nephrotoxicity. Creatinine derives from endogenous sources by tissue creatinine breakdown **[33].** Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of urea is nonsignificantly increased in the toxicant group when compared to control. Oral administration of plant extract significantly decreases the urea level in both treatment group compare to toxicant group. It was established that gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR**[34].** Gentamicin is known to decrease the activities of catalase, glutathione peroxidase and the level of reduced glutathione **[35]** . Therefore it is no doubt to assume that the nephroprotection showed by VT extract in gentamicin induced nephrotoxicity is mediated through its potent antioxidant effect. A relation between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. In gentamicin treated rats there was a significant increase in lipid peroxidation products (MDA) suggesting the involvement of oxidative stress. In these studies both the agents prevented gentamicin induced lipid peroxidation. **[36]**. The presence of tannin and phenolic acid could be the reason of protection offered by the extract might be due to its ability to activate anti-oxidant enzymes .The findings suggest the potential use of methanol extract of VT a therapeutically useful nephroprotective agent. Therefore further studies to explain there mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

CONCLUSION

This study affirms the *in vitro* antioxidant potential of crude extract of the leaf of *Verbascum thapsus*, with results comparable to those of the standard compounds such as ascorbic acid and sodium meta bi sulphate. Further studies are done to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress and this is a subject of investigation in our group .

The nephroprotective effect of leaf extract *Verbascum thapsus* was confirmed by the following measures: In case of gentamicin treated group there will be rise in serum marker such as urea, bun, and creatinine and decrease in the level of protein. The same is observed in kidney diseases in clinical practice and hence are having diagnostic importance in the assessment of kidney function. In the present study, the leaf extract of *Verbascum thapsus* significantly reduced the toxicant elevated levels of above mentioned serum markers and increase in the levels of protein. Hence, at this point it is concluded that the extract of *Verbascum thapsus* offers nephroprotection. Further documented reports reveal that, plant material containing phenols, flavonoids, and saponins offers organ protection

by virtue of their free radical scavenging activity. The extract under study upon phytochemical analysis showed the presence of aforementioned phytoconstituents. Hence, the role of these phytoconstituents as free radical scavengers and consequent nephroprotection cannot be ruled out. Based on improvement in serum marker levels, , level of antioxidant enzymes and presence of phytoconstituents, it is concluded that the leaf extract of *verbascum thapsus* possesses nephroprotective activity and thus supports the traditional application of the same under the light of modern science.

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