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PROTECTIVE MECHANISM OF *VITIS VINIFERA L.* FRUIT (*V. VINIFERA*) LOADED POLYMERIC NANOPARTICLES ON ACUTE LIVER INJURY INDUCED BY ACETAMINOPHEN TOXICITY

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ABSTRACT

Liver-related diseases have become a global concern worldwide. Hepatic cells damage is mostly caused by a variety of toxic chemicals like carbon tetrachloride (CCl_4), acetaminophen (APAP), antibiotics and thioacetamide. For the treatment of liver diseases herbal based therapeutics has been used from long time in India and has become popular all over world by leading pharmaceuticals. But in some places herbal medicines are still not acceptable for the treatment of liver diseases due to its limiting factors. *V. vinifera* have numerous medicinal properties such as it acts as vasoprotective, astringent, diuretic and hepatoprotective activity. Due to favorable properties such as easy to design, easy to prepare, interesting biomimetic, a variety of structure and have good biocompatibility, polymers are used as biomaterials. Polymeric nanoparticles can be used as the hepatoprotective agent due to their unique properties. In this present study we used *V. vinifera* loaded polymeric nanoparticle as hepatoprotective agent which can be used as a good therapeutic agent against liver toxicity and also for the successful development of drug delivery in near future.

Keywords: Acetaminophen (APAP), *V. vinifera,* hepatoprotective, therapeutic agents, liver toxicity

INTRODUCTION

In the biotransformation of drugs, food, exogenous and endogenous materials, liver being a most vital organ plays the most important role. Liver processes these materials into various types of active, inactive and toxic metabolites due to numerous amount of blood supply and the existence of various oxidation-reduction systems (*e.g.,* cytochromes and various enzymes) **[1]**. However, with the changing pattern of lifestyle most of the diseases are now becoming lifestyle diseases and the chemical-induced diseases are increasing paradoxically in recent years to become a major health problem in near future.

Liver-related diseases have become a global concern worldwide. Hepatic cells damage is mostly caused by a variety of toxic chemicals like carbon tetrachloride (CCl_4), acetaminophen, antibiotics and thioacetamide. It is estimated that chronic viral hepatitis affected approximately 8% of the total world population (3% HCB and 5% HBV). Chronic infection of both leads to cirrhosis, liver failure, and hepatic cellular carcinoma. According to World Health Organization (WHO) (2010). World Health Organization (WHO) published the latest data in which in May 2014 in India, 216,865 or 2.44 % of total deaths reached due to liver damage. Generally, 21.96 per 100,000 of the population is age-related death rate and India ranks 61 in liver-related diseases. Drug persuaded injury of the liver in 2004 was 7% which increased to about 20% in 2014.

Herbal based therapeutics has been used from long time in India for the treatment of liver diseases and has become popular all over world by leading pharmaceuticals. But in some places herbal medicines are still not acceptable for the liver diseases treatment due to its limiting factors such as (i) lack of standardization of herbal drugs (ii) lack of randomized controlled clinical trials (iii) lack of identification of active ingredients (iv) lack of toxicological evaluation.

But in 21st century the use of herbal plants for liver disorders treatment gain huge attention and a large number of herbal plants and formulation has been claimed to have hepatoprotective activity.

Vitis vinifera L. (*V. vinifera*) is one of the major fruit crop produced on the earth. It naturally occurs in southern Europe and western Asia but at the present time, it is cultivated all over the world as well as at all the temperatures. *V. vinifera* has a nutritional and medicinal value so it is used for thousands of years. It is rich in flavonoids, anthocyanins, and proanthocyanins, sugars,

mineral salts, vitamins, organic acids, and tannins.

V. vinifera has numerous medicinal properties such as it acts as an anti-sclerotic, leaves have the properties like vasoprotective, astringent, diuretic and venotonic and fruits have the properties like tonics, vitamins, promote hair growth, prevent ischemic processes and seeds have the properties like prevention of increased vascular permeability and hypolipidemic. It also acts as antioxidant, anticarcinogenic and antidiabetic **[2]**.

In last few years polymeric nanoparticles (PNPs) attained huge attention in the field of research. Nanoparticles originating from polymers are generally interconnected with novel properties. Due to favorable properties such as easy to design, easy to prepare, interesting biomimetic, a variety of structure and have good biocompatibility, polymers are used as biomaterials. Polymeric nanoparticles can be used as the hepatoprotective agent due to their unique properties **[3]**. PNPs have various advantages such as increased stability of the acid labile drug, increased drug circulation time in the body, high drug encapsulation efficiency, uniform distribution of the drug in the targeted site and stimuli responsiveness **[4]**. Polymeric nanoparticles can also easily permeate through various biological barriers and provide high thermodynamic stability to the system **[5]**.

Among the various available biodegradable polymers PLGA is most popular due to its favorable degradation characteristics, long clinical experience, and sustained drug delivery possibilities. Recent literature has shown that degradation of PLGA can be employed for sustained drug release at desirable doses by implantation without any side effect and surgical procedures is shown in many litratures recently **[6]**. Biodegradable polymers marking their place in the current research field because they are having many benefits without any side effects.

Hence the *V. vinifera* loaded PNPs can be used as a good therapeutic agents against liver toxicity and also for the successful development of drug delivery in near future. Now a day's plants play vital role in the health and vitality of animals and humans lives. For the treatment of various liver diseases several medicinal plants (Phytomedicines or herbal plants) are prevalent and some herbal/ medicinal plants have hepatoprotective and hepatogenic agents against variety of hepatotoxicants which causes hepatotoxicity.

MATERIALS AND METHODS

Plant material

The fruit of *Vitis vinifera* was collected from the local market of Jaipur, Rajasthan.

Preparation of extract

Fresh and healthy ripe fruits of *V. vinifera* were washed thoroughly with running tap water and with 70 % ethanol and then allow to natural drying under shade for several days or in oven at 50 °C. Finally the dried material was pulverized to powder form and was sieved by using a mesh to get a uniform size range. The 10 g of pulverized sample will be dissolved in 100 ml of water, was placed on Rocker shaker (MAC, Cat: MSW-309, Model No.: 0116-094) for 72 hours at room temperature. After that extract was filtered by using whatsman paper no. 1. Aqueous extract of ripe fruit was obtained by slow filtration process and kept for oven dry then crystalline crude extract was collected and was stored at 4 ºC for further use.

Drug and chemicals

Acetaminophen (APAP), Poly (lactic acid-glycolic acid) (PLGA) will be procured from Sigma-Aldrich (Bangalore) and Silymarin were procured from Sigma-Aldrich (USA). In this study, all chemicals of the analytical grade were procured and used from Himedia, Sigma-Aldrich and E-Merck Company (India).

Animals

Random selection of the albino rats (strain Wistar) and 180 ± 10g bwt) took from the animal facility available in our department. Albino rats were taken care in cages (polypropylene) under homogeneous conditions of dark (10h) and light (14h) with relative humidity (60-70%) and temperature (25 ± 2°C). Animals were nourished on dry pellets diet (procured from Pranav Agro Industries Ltd., New Delhi, India) available commercially along with water for drinking *ad libitum*. Protocols of experiments were approved by the Institutional Animal Ethical Committee (CPCSEA/574/02/ab) of Banasthali Vidyapith, India, following the rules set by the committee for the rationale of management and regulation of experiments on animals (CPCSEA), Chennai, India.

Experimental protocol

The animals were divided into 7 groups of 6 animals each. Group I served as control received *vehicle* only, group II received VV-PNPs extract *per se* (100 mg/kg., respectively, *p.o*., once only), group III served as experimental control (APAP: 2g/kg *i.p*., once only), group III-VI received APAP (as in group IV) along with VV-PNPs extract (25, 50, 70 and 100 mg/kg., respectively, *p.o*., once only), group VII received APAP (as in group IV) along with silymarin (50mg/kg, *p.o*., once only).

All the animals were weighed; partially anesthized with ketamine (270 mg/kg) + xylazine (30 mg/kg) by *i.p.* Blood of each rat was collected by puncturing retro-orbital venous sinus (retro-orbital plexus) into test tubes. Blood in the test tubes was allowed to clot for 30 minutes at room temperature and then clot was gently detached from the wall of the test tubes with the help of very thin sterilized needle. The test tubes were centrifuged for 20 minutes at 2000 rpm to harvest serum and were stored at -20°C until analyzed **[7]** and used for the assay of biochemical marker enzymes (AST, ALT, SALP, LDH, albumin, bilirubin, serum urea, creatinine, triglyceride, cholesterol) immediately after collecting blood, the animals were sacrificed and livers dissected out for tissue biochemistry (LPO, GSH, ATP and G-6-phosphatase). Serum total protein was measured according to the method of Lowry. The results were expressed as units/L (U/L).

Statistical analyses

In order to test the significance of differences between means of various groups numerical data were subjected to statistical analyses. To compare the mean levels of various parameters of the different experimental groups one-way analysis of variance (ANOVA) was done and the F values were computed at P≤0.001 **[8]**. The test of choice was the student 't'-test **[9]**. The tabulated figures are presented as mean ± standard error (SE). The calculated P value has been included within the tables. Calculated P value of less than 0.001 was considered enough to indicate significance difference between compared mean. Computation of the statistical analysis was carried out with the help of the Microsoft Excel program (Microsoft Office XP Professional, Microsoft Corporation, USA).

RESULT AND DISCUSSION

Blood biochemistry

Assessment of aspartate aminotransferase and alanine aminotransferase (AST and ALT):

Liver damage due to cardiac infarction, viral hepatitis and muscle injury is indicated by high level of AST. The conversion of alanine to pyruvate and glutamate is catalyses by ALT. Necrosis or membrane damage releases the enzyme into circulation, thus, it can be measured in serum. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. As summarized in **Table 1**, results indicate a significant increase in the activities of AST and ALT in APAP injected group when compared with control group. VV-PNPs at 100 mg/kg) showed maximum protection in

AST 89.5% ALT 89.8% which was confirmed by one way ANOVA. However administration of highest dose of VV-PNPs (100 mg/kg) showed maximum restoration which is confirmed by % protection, revealing that test drugs preserve the structural integrity of liver from toxic effect of APAP like as other plants *Salvia officinalis* **[10]** *Tanacetum parthenium* **[11]**.

Assessment of serum alkaline phosphatase (SALP):

Reliable marker for liver damage is the increased level of alkaline phosphatase which occurs due to the *de novo* synthesis by the liver cells. Activity of this enzyme rises in many types of liver diseases also; highest levels are seen with obstruction to the bile flow, either intrahepatic or extrahepatic. **Table 1** revealed that the level of SALP was significantly ($P \leq 0.001$) increased into blood stream after APAP intoxication. Therapy VV-PNPs at all doses 25, 50, 75 and 100 mg/kg respectively) significantly normalized the enzymatic activities towards normal. Maximum dose of VV-PNPs (100 mg/kg) showed maximum recoupment. Stabilization of SALP VV-PNPs (100 mg/kg, 90.8%). Results suggested that VV-PNPs have protective effect on plasma membrane of hepatocytes. Like some other plants *Zilla spinosa* ; *Hammada elegars* **[1]**.

Assessment of serum lactate dehydrogenase:

In the present study **table 1** showed that the level of LDH was significantly increased into the blood serum due to the administration of APAP. Therapy with all the doses of VV-PNPs (25, 50, 75 and 100 mg/kg bwt, *p.o.*) significantly restored the increased level of LDH toward normal but the maximum recoupment was found in highest dose of VV-PNPs treated group. About 88.1% in VV-PNPs protection was seen in LDH. Reduction in the levels of LDH towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissues which were damaged by toxicant. Therapy may combine with reactive metabolites and lead to inactivate them, which may suppress the intracellular concentration of free radicals. These findings are also substantiated by the treatment of other plant extracts such as *Erygium maritimum* **[12]**; *Zizyphus jujube* **[13]**; *Ziyang tea* **[14]**; *Ceropegia spiralis* **[15]**.

Assessment of serum albumin and bilirubin:

Cellular leakages and loss of functional integrity of hepatocytes indicates the elevated level of albumin. Bilirubin is one of the most useful and sensitive tests to substantiate the functional integrity of liver and severity of

necrosis is bilirubin. To measure conjugating, the binding and excretory capacity of hepatocytes that is proportional to the erythrocytes degradation rate bilirubin test is perform **[16] [17]**. **Table 1** depicts APAP administration significantly enhanced the level of albumin and bilirubin contents in serum due to APAP exposure indicates biliary tract disorder.

Treatment at different doses of VV-PNPs (25, 50, 75 and 100 mg/kg) showed recovery in both blood biochemical parameters. Highest doses of VV-PNPs were found to be more effective than lower doses. About 84.6% and 87.6% in VV-PNPs protection were seen in albumin and bilirubin respectively. Data was statistically confirmed by ANOVA at 1 % level.

Treatment with VV-PNPs prevents to a large extent the membrane lesion with concomitant decrease in the albumin and bilirubin concentration as compared to APAP treated group. Decrease in the serum that indicates the ability of these test drugs to of liver cells. Supporting previous reports with regards to other plant extracts *Aquilaria malaccensis lamk* **[18]**; *Homalium nepalense* **[19]**.

Assessment of serum urea and creatinine

As a measure of renal function status, serum urea and creatinine are often regarded as reliable markers. Thus, elevations in the serum concentrations of these markers are indicative of renal injury **[20]**. Serum creatinine has been used to estimate glomerular filtration rate. Due to severe renal impairment, urea excretion falls and the serum concentration rise rapidly. If damage is severe, less creatinine will be excreted and this is the main cause of significantly increased level of creatinine in serum. As summarized in **Table 2**, results indicate a significant increase in the content of urea and creatinine level in APAP adminstered group when compared with control group.

Therapy with VV-PNPs (25, 50, 75 and 100 mg/kg bwt, *p.o.*) showed recovery in a dose dependent manner. The highest dose of VV-PNPs *i.e.* 100 mg/kg showed maximum protection in urea 87.5% and creatinine 90.8% which is confirmed by one way ANOVA. Elevated level of creatinine and urea after toxicant administration is also supported by various authors, **[21] [18] [22]**.

Assessment of serum cholesterol and triglyceride:

Increased synthesis or decreased lipid deposition or both result in simultaneous accumulation of lipids in the liver. It is reported that APAP intake is associated with changes in plasma lipid concentrations and whole-body lipid balance.

Table 2 shows that the level of cholesterol and triglyceride content in serum of control and APAP treated rats. Significant elevations in these parameters were observed in APAP treated group when compared to control rats. Significant recoupments in the level of these parameters were observed in VV-PNPs treated animals at the highest doses similar as positive control silymarin. About 87.7% and 88.9% in VV-PNPs protection were seen in triglyceride and cholesterol respectively. Increase in triglycerides in APAP treated rats might be due to decreased activity of lipoprotein lipase, which is involved in the uptake of triglyceride rich lipoprotein by extra hepatic tissues.

VV-PNPs protection was seen in triglyceride and cholesterol respectively. It can be put forth that VV-PNPs prevented increase of cholesterol by inactivation of thiol group enzymes as 3-hydroxy-3-methyl-glutaryl-CoA reductase and CoASH, the rate limiting enzyme for cholesterol biosynthesis and the multi-enzyme complex for fatty acid biosynthesis. Similar results were observed with plant extracts *Curcuma longa* **[23]**.

Assesment of lipid peroxidation in liver tissue:

Table 3 exhibits significant increase in hepatic lipid peroxidation after 48 h of *intraperitoneal* administration of APAP. Result showed significant recovery in the level of LPO after therapy with VV-PNPs (25, 50, 75 and 100 mg/kg bwt, *p.o.*) at all doses, but effective restoration was observed in VV-PNPs at 100 mg/kg which is 91.4%. However VV-PNPs showed maximum recoument which was confirmed by % protection. No adverse effects were found in the tissue biochemical parameters after *per se* treatment of VV-PNPs at highest doses. The increase in Lipid peroxidation **(**LPO), a degradative process of membranous PUFA has been suggested by the increase in MDA level in APAP induced toxicity in the liver.

Treatment with various doses of VV-PNPs significantly inhibited toxicants induced lipid Peroxidation and this might be due to destruction of free radicals that were already formed or by supplying a competitive substrate for unsaturated lipids in the membrane and or by accelerating the repair mechanism of damaged cell membrane. Other studies conducted on different plants *i.e. Herpetospermum caudiyerum* **[24]**; *Curcumin longa* **[23]**.

Assessment of reduced glutathione in liver tissue:

GSH enables the liver to detoxify many foreign compounds or their metabolites and to excrete the product preferably into the bile. It is widely known that a deficiency of

GSH within living organisms can lead to tissue disorder and injury. **Table 3** showed a significant reduction in GSH level after APAP administration, reflecting that the potency of antioxidation in injured cells was altered. A significant recovery was observed in the level of GSH after *V. vinifera* therapy at all doses, but effective restoration was observed in VV-PNPs at 100 mg/kg (89.2%) dose, respectively. Administration of VV-PNPs significantly increased the level of GSH content.

Administration of VV-PNPs significantly increased ($P \leq 0.001$) the level of GSH content. This increase could either be due to an effect on the de novo synthesis of GSH, its regeneration or both. The availability of GSH to support redox cycle activity depends on the supply of NADPH. Other plant extracts are also reported to restore GSH content *Pimpinella anisum* **[23]**; *Caralluma dalziellii* **[25]**.

Assessment of adenosine triphosphatase in liver tissue:

ATPase activity may be considered as a marker for assessing hepatocellular damage induced by hepatotoxic agents. Pathological processes that interfere with the production of ATP may interfere with sodium pump activity, which in turn results in decreased hepatocellular function. It has been hypothesized that oxidative damage of membrane bound ATPase activity is crucial for mitochondrial membrane damage **[26] [27]**. **Table 3** depicts that APAP intoxication showed significant depletion in ATPase activity in liver homogenates. VV-PNPs showed maximum recovery in the enzymatic activity at all the doses (25, 50, 75 and 100 mg/kg, respectively) in dose dependent manner. The values were considerably comparable to silymarin treated group (94.2%). Maximum recovery was seen with highest dose of VV-PNPs at 100 mg/kg in enzymatic activity of ATPase (89.6%) however VV-PNPs was found to be protective which was confirmed by statistical analysis and percent protection. Treatment of rats with silymarin used as a reference standard also exhibited significant protective effect against APAP induced liver damage. Similar findings were also sustained by various author like Boldine-loaded PLGA nanoparticle **[28]**.

Assessment of glucose-6-phosphatase in liver tissue:

Glucose-6-phosphatase **(**G-6-Pase) is located in the endoplasmic reticulum and is the crucial enzyme of glucose homeostasis. It plays an important role in the regulation of the blood glucose level. The microsomes of the liver are the most susceptible sites of

membrane bound G-6-Pase. ER showed considerable degeneration after toxicant administration. The damage of the cellular membrane due to lipid peroxidation also leads to decrease in the activity of ER membrane bound enzyme such as G-6-Pase **[29]**. **Table 3** indicated that APAP intoxication showed significant depletion in G-6-Pase activity. VV-PNPs showed recovery in the enzymatic activity at all the doses (25, 50, 750 and 100 mg/kg, respectively). The values were considerably comparable to silymarin treated group (84.7%). Maximum recovery were seen with highest dose of VV-PNPs (100 mg/kg: 78.9%) in G-6-Pase but the highest dose of VV-PNPs was found to be effective in enzymatic activities of G-6-Pase as confirmed by statistical analysis and percent protection. The ameliorative effect of therapy might be due to its polyphenolic nature having antioxidative property. Antioxidants have the property to protect all membrane lipids and unsaturated fatty acids against oxidative degeneration. Similar investigative findings were also reported with the plant extracts of *Tanacetum parthenium* (Mahmoodzadeh *et al*., 2017); *Diospyros virgininana* (Priya and Velanganni, 2015).

Table 1: Effect of VV-PNPs against APAP induced alterations in blood biochemistry

Treatments	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	SALP (mg/Pi/h/100ml)	Bilirubin (mg/dl)	Albumin (g/dl)
Control	65.2 ± 3.60	50.2 ± 2.77	50.7 ± 2.80	207.5 ± 11.4	0.35 ± 0.02	2.9 ± 0.17
VV-PNPs *per se* (100 mg/kg)	65.0 ± 3.59	49.0 ± 2.70	50.4 ± 2.78	205.1 ±11.3	0.34 ± 0.02	3.0 ± 0.16
APAP *per se* (20mg/kg)	270 ± 14.9$^{\#}$	319.5 ± 17.6$^{\#}$	154.2 ± 8.52$^{\#}$	562.2 ± 31.0$^{\#}$	1.55± 0.08$^{\#}$	4.72 ± 0.24$^{\#}$
APAP+ VV-PNPs (25 mg/kg)	198± 10.9* *(35.1%)*	192.3 ± 10.6* *(47.5%)*	114 ± 6.30* *(38.8%)*	355 ± 19.6* *(53.3%)*	1.20 ± 0.06* *(40.2%)*	3.94 ± 0.21* *(42.8%)*
APAP + VV-PNPs (50 mg/kg)	155 ± 8.56* *(56.1%)*	140 ± 7.73* *(66.8%)*	92.5 ± 5.11* *(59.6%)*	291 ± 16.0* *(73.6%)*	0.99± 0.05* *(60.4%)*	3.64 ± 0.19* *(59.3%)*
APAP + VV-PNPs (75 mg/kg)	115.4 ± 6.37* *(75.4%)*	111± 6.19* *(77.5%)*	81.5 ± 4.50* *(70.2%)*	270 ± 14.9* *(80.2%)*	0.78± 0.04* *(72.6%)*	3.341 ± 0.18* *(71.9%)*
APAP + VV-PNPs (100 mg/kg)	86.5 ± 4.78* *(89.5%)*	77.6 ± 4.28* *(89.8%)*	63.0 ± 3.48* *(88.1%)*	236.5 ± 13.0* *(90.8%)*	0.59 ± 0.03* *(87.6%)*	3.18 ± 0.17* *(84.6%)*
APAP + S (50 mg/kg)	85.1 ± 4.70* *(90.2%)*	70.1 ± 3.87* *(92.6%)*	57.0 ± 3.15* (99.9%)	231 ± 12.7* *(92.5%)*	0.52 ± 0.02* *(89%)*	3.13 ± 0.17* *(87.3%)*
F value (at 1% level)	97.03$^{@}$	142.67$^{@}$	64.94$^{@}$	55.88$^{@}$	92.41$^{@}$	11.48$^{@}$

Abbreviations: APAP = Acetaminophen ;VV-PNPs = *V. vinifera* loaded polymeric nanoparticles; S = Silymarin; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; LDH = Lactate dehydrogenase; SALP = Serum alkaline phosphatase, % = Percent protection. ANOV$^{@}$ = 0.001% Significant; Values are mean ± S.E., N = 6. $^{\#}$P ≤ 0.001 vs Control, *P ≤ 0.001 vs APAP.

Table 2: Effect of VV-PNPs against APAP induced alterations in blood biochemistry

Treatments	Urea (mg/dl)	Creatinine (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control	18.3 ± 1.01	0.31 ± 0.02	15.1 ± 0.83	64.2 ± 3.54
VV-PNPs *per se* (100 mg/kg)	17.9 ± 0.98	0.30 ± 0.02	15.0 ± 0.82	64.0± 3.53
APAP *per se* (20mg/kg)	52.1 ± 2.88$^{\#}$	3.44± 0.19$^{\#}$	21.6 ± 1.19$^{\#}$	147.6 ± 8.15$^{\#}$
APAP+ VV-PNPs (25 mg/kg)	34.7 ± 1.91* *(26.7%)*	271 ± 0.13* *(40.9%)*	19.4 ± 0.98* *(33.8%)*	123 ± 6.79* *(33.4%)*
APAP + VV-PNPs (50 mg/kg)	31.7 ± 1.75* *(40.1%)*	2.15 ± 0.14* *(63.6%)*	17.9 ± 0.98* *(56.8%)*	107.3 ± 5.93* *(59.6%)*
APAP + VV-PNPs (75 mg/kg)	26.8 ± 1.48* *(62.0%)*	1.50± 0.11* *(78.4%)*	16.8 ± 0.92* *(73.0%)*	90.5± 5.00* *(74.6%)*
APAP + VV-PNPs (100 mg/kg)	21.2 ± 1.17* *(87.5%)*	0.61 ± 0.08* *(90.8%)*	15.8 ± 0.87* *(88.9%)*	77.4 ± 4.27* *(87.7%)*
APAP + S (50 mg/kg)	20.7 ± 1.14* *(89.2%)*	0.57 ± 0.03* *(91.9%)*	15.6 ± 0.86* *(92.3%)*	76.8 ± 4.24* *(95.0%)*
F value (at 1% level)	58.42$^{@}$	174.74$^{@}$	7.63$^{@}$	38.357$^{@}$

Abbreviations: APAP = Acetaminophen; VV-PNPs = *V. vinifera* loaded polymeric nanoparticles; S = Silymarin; % = Percent protection. ANOVA$^{@}$ = 0.001% Significant; Values are mean ± S.E., N = 6. $^{\#}$P ≤ 0.001 vs Control, *P ≤ 0.001 vs APAP.

Table 3: Effect of VV-PNPs against APAP induced alterations in tissue biochemistry

Treatments	LPO (n mole of TBARS/mg protein)	GSH (µ mole/g)	G-6-Pase (µ mole Pi/ min/g liver)	ATPase (mg Pi/100g/min)
Control	0.32 ± 0.02	7.92 ± 0.45	5.88 ± 0.32	2110 ± 116
VV-PNPs *per se* (100 mg/kg)	0.30 ± 0.02	8.01 ± 0.45	5.90 ± 0.32	2100± 116
APAP *per se* (20mg/kg)	1.98 ± 0.10#	5.12 ± 0.29#	3.12 ± 0.17#	892 ± 49.3#
APAP+ VV-PNPs (25 mg/kg)	1.62 ± 0.08* *(40.3%)*	6.1 ± 0.34* *(34.2%)*	3.71 ± 0.20* *(21.3%)*	1320 ± 72.9* *(35.4%)*
APAP + VV-PNPs (50 mg/kg)	1.05 ± 0.05* *(66.5%)*	6.55 ± 0.38* *(54.6%)*	4.60 ± 0.25* *(53.6%)*	1635 ± 90.3* *(61.5%)*
APAP + VV-PNPs (75 mg/kg)	0.80± 0.04* *(80.9%)*	7.10± 0.39* *(65.2%)*	4.82± 0.26* *(61.5%)*	1825± 100* *(77.7%)*
APAP + VV-PNPs (100 mg/kg)	0.58 ± 0.03* *(91.4%)*	7.62 ± 0.43* *(89.2%)*	5.30 ± 0.29* *(78.9%)*	1975 ± 109* *(89.6%)*
APAP + S (50 mg/kg)	0.52 ± 0.02* *(94.2%)*	7.80 ± 0.43* *(93.1%)*	5.46 ± 0.30* *(84.7%)*	2030 ± 112* *(94.2%)*
F value (at 1% level)	129.84@	10.27@	21.72@	30.55@

Abbreviations: APAP = Acetaminophen; VV-PNPs = *V. vinifera* loaded polymeric nanoparticles; S = Silymarin; LPO = Lipid peroxidation; GSH = Glutathione reduced; G-6-Pase = Glucose-6-phosphatase; ATPase = Adenosine triphosphatase, % = Percent protection. ANOVA@ = 0.001% Significant; Values are mean ± S.E., N = 6. #P ≤ 0.001 vs Control, *P ≤ 0.001 vs APAP.

Conflict of interest

None of the author having conflict of interest

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