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# Therapeutic Effect of Green Synthesized $Mno_2$ Nanoparticles for Treatment of Hyperbilirubinemia -An in-Vivo Study

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**ABSTRACT**

Green synthesized manganese oxide ($MnO_2$) nano-conjugate have been widely used as biomedicine in different medical applications. Solanum lycopersicum (Tomato) extract is used to synthesizes MnO2 nanoparticles following a green route, and the synthesized nanoparticles is characterized by XRD, FTIR, UV-Vis, SEM, and later on applied for the evaluation of hepato-protective and anti-oxidant activities in mice. Biochemical tests such as bilirubin, liver function test (LFT), hematological parameters provide evidences for high and non-toxic efficacy of green synthesized nanomaterials in the symptomatic treatment of hepatic damage. Histopathological changes in liver were investigated in presence of the synthesized nanoconjugate and compared with conventional drug. These results suggest that these green synthesized nanomaterials could appear as an important ameliorative agent and effective for jaundice as well as other related hepatic disorders and could be developed as safe and efficient alternatives to conventional drugs.

**KEYWORDS:** *Solanum lycopersicum*, Green Synthesis, Nanoparticle, Jaundice, Total Bilirubin, LFT.

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## 1. INTRODUCTION

Presently nanomaterials are the most potential substances for rapidly developing field of nano medicine and bio-nanotechnology[1]. Among different nanomaterials metal oxide nanoparticles worked as one of antimicrobial development. In recent years it is experimentally proved that manganese dioxide nanomaterials have attracted the attention of researcher due to their extensive physical, chemical and biological properties. They are extensively used in field of biomedicine[2]. $MnO_2$ NPs are used exclusively for catalysis, data storage, drug delivery, and biomedical imaging. Green chemistry-based compound of NPs is preferred because of its ecofriendly nature[3]. These nanomaterials are capable to antibiotic resistance microbes[4]. It is well known that $MnO_2$ nanomaterials come in a variety of structural forms[5]. MnO2 nanomaterials of different phases and morphologies have significant importance[6,7]. Though different chemical synthesis procedures are already reported but recently green synthesis of MnO2 using plant extract have gain important application due to low-cost, large-scale synthesis, biocompatibility, and application in nano biomedicine as well as synthesis using without chemical encapsulating agent

Now a days hyperbilirubinemia is a general medical issue associated to our present-day life[8]. It is defined as increased bilirubin level (>1.3 mg/dlin human) in blood and caused by an imbalance between production of bilirubin (as a result of hemolysis, sepsis, blood extravasation or polycythemia) and decrease in bilirubin discharge due to insufficient hepatic conjugation. Bilirubin is the yellow breakdown product of normal haeme catabolism and evacuate in bile and urine, raised levels indicate certain abnormalities in the body[9]. Several treatment options are available for

hyperbilirubinemia like phototherapy, hemoperfusion, hemodialysis and exchange blood transfusion. All these treatment procedures have their own limitations and side effects, as well as they are unable to degrade the bilirubin level directly[10]. So a specific and targeted procedure is needed for treatment of hyperbilirubinemia. It is already reported that MnO2 nanoparticles can involve selectively in the catalysis of bilirubin[11.12].

*Solunum lycopersicum* or tomato extract is chosen here to synthesize the MnO2 nanomaterials. *Solunum lycopersicum* or tomato contains high rich in vitamin-C. Vitamin-C act as an antioxidant, protect from liver diseases. It is well known that sufficient intake of green leafy vegetables, fruits, medicinal plants, whole grain rich in anti-oxidant helps in the amelioration the liver disease[13]. Solanum lycopersicum contains various types of flavonoids and Lycopene which act as a carotenoid have potentially active to lower the liver disease[14]. Medicinal plants with these and some other compounds as well as antioxidant activities have been shown to be effective against a wide range of diseases[15]. So, using the extract of tomato, MnO2 Nps have been prepared and can be used as probable nanomedicine for hyperbilirubinemia.

In the present study, we have evaluated the efficacy of the nano-conjugate compound in reviving the bilirubin level and liver enzymes when used in paracetamol treated mice model of hepatic disorder. The histopathological changes also suggest the symptomatic amelioration of hepatic damage with the aid of this green-synthesized nano-conjugate.Detailed experimental studies revealed that these $MnO_2$ nanoconjugates could be effectively arrayed in the treatment of liver diseases and similar hepatic disorders in the near future.

## 2. MATERIALS & METHODS

Tomato (*Solanum lycopersicum*) was collected from local market and it was verified by Botany Department by Vidyasagar University. Paracetamol, manganese acetate, $(CH_3COO)_2Mn.4H_2O$, $KMnO_4$ and KOH used in the test were purchased from Merck (India); Swiss albino mice, weighing (30-35) gm. were obtained from Chakraborty Enterprise, Kolkata. Silymarin Zydus cadila, for total bilirubin used Auto span Liquid Gold Surat, Gujrat and Biochemical Test kits for liver biomarker Elba science.

### 2.1 Synthesis of $MnO_2$Nanoconjugate

#### 2.1.1 Plant Extract Preparation

In order to prepare the plant extract, first 200 g of solanum lycopersicum were washed with distilled water and then the washed solanum lycopersicum was boiled for 10 minutes in 100ml of distilled water. The hot extract was allowed to cool at room temperature. And then it is filtered. Collected extract is used for the synthesis of nanoconjugate[16].

#### 2.1.2 Synthesis of $MnO_2$ Nano-conjugate:

In order to prepare $MnO_2$ nanoconjugate by green method, 50 ml solanum lycopersicum extract was added to the 200 ml aqueous solution of $(CH3COO)_2Mn_4H_2O$ and $KMnO_4$ and stirred overnight to obtain a precipitate of $Mn(OH)_2$. The resultant molarity of $(CH_3COO)_2$ $Mn_4H_2O$ and $KMnO_4$ used in the final solution are 0.50 M and 0.025 M respectively. The precipitate was collected by centrifuging the aqueous suspension at 10,000 rpm for 20 minutes and further washed three times by distilled water. Finally, prepared nanoconjugates were dried overnight at 80ºC[17] to get the final product.

### 2.2 UV-VIS Spectroscopy

UV-VIS characterizations of the synthesized MnO2 nanoconjugate powder sample were recorded at room temperature on Shimadzu UV-vis spectrophotometer (UV-3101PC)) using a quartz cuvette of 1 cm optical path length.

### 2.3 X-ray diffraction (XRD)

The XRD patterns of nanoconjugate were recorded by X-ray diffractometer (Model-D8, Bruker AXS Inc., Madison, WI) with $2^0$ varying from $10^0$to $80^0$ and a scan speed of 0.2 s per step using nickel filtered Cu-Ka radiation operating under a voltage of 40 kV.

### 2.4 Field Emission Scanning Electron Microscope (FESEM)

The morphology and the size distribution of conjugate sample were analysed by scanning electron microscope (FESEM INSPECT F50).

### 2.5 Animal preparation

Swiss albino mice weighing 25-30 gm were procured from a CPCSEA approved animal house (Registration No. 50/CPCSEA/1999) and arbitrary divided into six groups of four mice (n=4) each. Which go through standard laboratory diet (Hindustan Lever, Kolkata) and water *ad libitum*. therapeutic food is given to all groups During the treatment period instead of the standard food. The animals were kept in large, clean, polypropylene cages in a temperature-controlled room (20±2ºC) under light and dark cycles for12 hours with relative humidity (45–60%) during the experiment. Prior to experimentation acclimatization was done for 7 days. The animals were maintained according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), guideline Chennai, India and approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.AEC/PHARM/1503/03/2015 dated 30.11.15).

**2.6 Treatment Protocol**

| Groups(n=4) | Days of Treatment | |
|---|---|---|
| | 7-14 days | 14-21 days |
| | Induction dose (O.D) | Treatment dose (O.D) |
| Control (Gr.I) | Nil | Nil |
| Auto recovery (Gr.II) | Paracetamol 1ml/kg body weight | Nil |
| Herb control (Gr.III) | Paracetamol 1ml/kg body weight | 2.5 ml/kg body weight of the herb extract |
| Metal nanoparticle control (Gr.IV) | Paracetamol 1ml/kg body weight | 2.5 ml /kg body weight of the metal nanoparticle |
| Nanoconjugate (Gr.V) | Paracetamol 1ml/kg body weight | 2.5 ml /kg body weight of the nanoconjugate |
| Positive control (Silymarin) (Gr.VI) | Paracetamol 1ml/kg body weight | 2.5 ml /kg body weight of the silimaryrin |

**2.7 Biochemical Estimation**

**2.7.1 Enzyme Analysis:**

Examine for liver enzyme blood were collected in the minute before sacrifice in disinfected tubes from retro-orbital plexus and allowed to clot for45 minutes. Serum was centrifuged at 6000rpm for 15 minutes. All samples are germ free. The results were expressed as International Units / liter. Total protein expressed as gm/dl[18].

**2.7.2 Hematological Study**

To test hematology, the blood was collected in heparin zed tubes. Parameters studies were Hb%, Neutrophil, Lymphocytes, Monocytes, platelets and Red Blood cell[19].

**2.7.3 Histopathological Studies**

For histopathological studies liver was cut off after blood collection, cleaned and dried with tissue paper. The desired amount of liver was weighed and fixed in neutral formalin solution (10%) 'Then it is dehydrated in ethanol (50-100%), cleared in xylene and fixed in paraffin. hematoxylin and eosin (H&E) dyes were used for staining the sample and further studied for histopathological changes[20].

**2.8 Statistical Analysis**

Data were represented as Mean ± standard error of mean. The statistical significance was determined by using (ANOVA) one way analysis of variance followed by comparison test was used to determine $p<0.05$statistical significance.

**3. RESULTS AND DISCUSSIONS**

**3.1 Characterization of $MnO_2$ nanoconjugate:**

Prepared $MnO_2$ nanoconjugate is characterized by absorption spectroscopy, XRD and FESEM. UV-VIS spectroscopy is one of most convenient technique for the characterization of Nano-structure. The absorption spectra of the synthesized $MnO_2$ nanoconjugate in Fig 1shows a broad band around 260 nm[21] which is the characteristic bands of $MnO_2$ nanostructure.

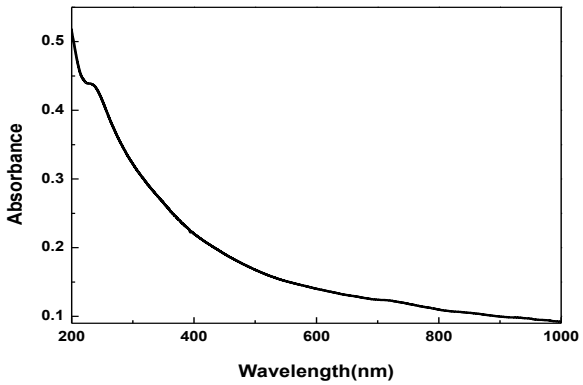


**Fig.1: UV-Vis Spectra of MnO2 nanoconjugate**

Fig. 2: represents the XRD spectra of the $MnO_2$ nanoconjugate. The sharp diffraction peaks at 2θ values of 28º, 38.01º, 61 º correspond to the (310), (211), (521) diffraction planes indicate the formation of Manganese dioxide nanocrystals with small crystallites which are in good matching with standard card JSPDF 44-0141.

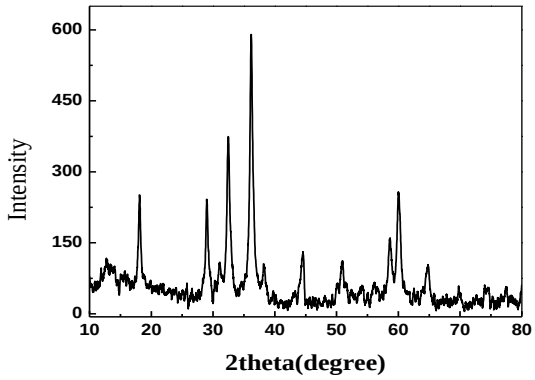


**Fig.2: XRD spectra of $MnO_2$ nanoconjugate**

Fig 3 represent the morphology of the synthesized nanomaterials. From FESEM it is confirmed that the nanoparticles are flakes in shape and the size of the synthesized materials are around 30-40nm

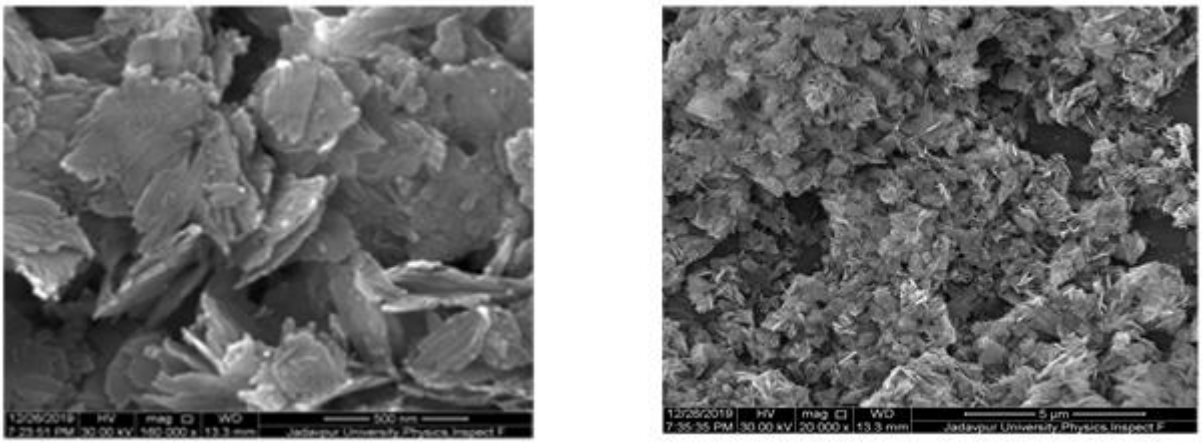

**Fig. 3: SEM images of $MnO_2$ nanoconjugate**

**3.2 Effect of $MnO_2$ nanoconjugate on Hepatic disorder:**

Now a days nanoparticle system has gained much attention for treatment of hepatic disorder. Various kinds of nanoparticle are available for the treatment of liver disease. Metal oxide nanoparticles are considered a good therapeutic material for the treatment due to their special characteristics. $MnO_2$ nanomaterials has extensive application in therapeutic world. It can penetrate in body through skin and blood brain barrier. So here detailed investigation has been done to see the effect of the nanoconjugate on hepatic disorder and discussed below.

$MnO_2$ nanoconjugates help in the amelioration of the hyperbilirubemia. Figure-4 represent the individual effect of phytoextract (only herb) and Group IV (nanoparticle), which showed ameliorative effect against hepatic disorder for both cases. Furthermore, as seen in the Figure-4 the green synthesized nanoconjugate also exhibited more distinct remediation result against hyperbilirubemia (a primary symptom of jaundice) in comparison to the positive control (Silymarin).

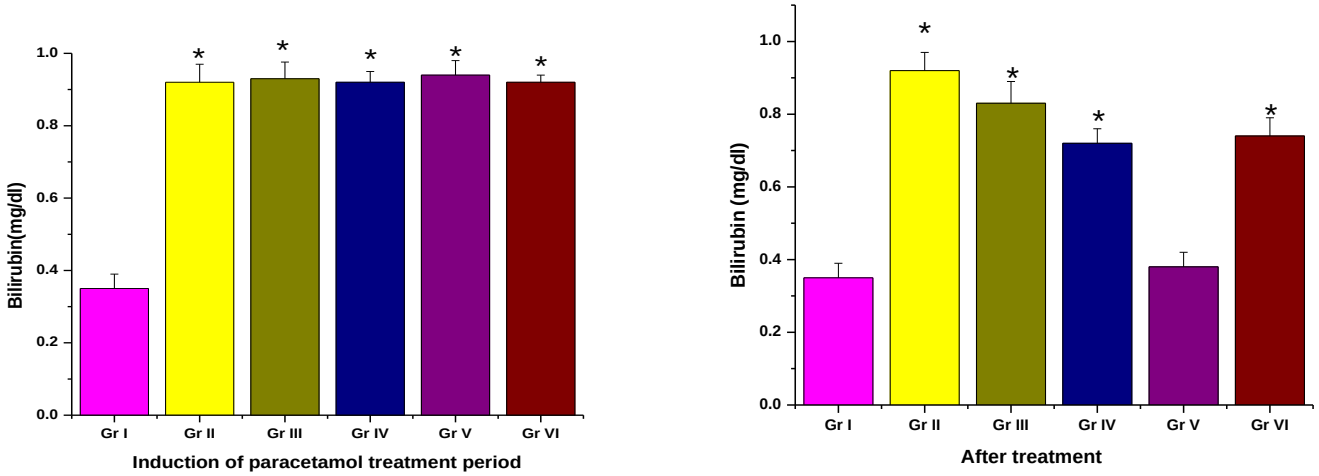


**Figure 4. Amount of Bilirubin after Induction and Treatment with Paracetamol. All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance p<0.05 implies.**

Fig 5 represent the effect of Albumin, Globulin and Total Protein and fig 6 represent the effect on ALT, AST and ALP in paracetamol intoxicated mice when exposed to the green synthesized $MnO_2$ nanoconjugate. Both figures indicate to the regain of the values when treated with green synthesized nanoconjugate. Overdoses of paracetamol caused a remarkable upgrading levels of liver enzyme such as AST, ALT, ALP, TP when compared to control (Gr-I) and Silymarin (Gr-VI). AST is one of the main important enzymes which act as enzymatic antioxidant shielding system[22]. Our phytochemical study exhibits the presence of flavonoids and polyphenols in nanoconjugate complex. It is well known that some flavonoid helps to reduce the hepatotoxicity by xenobiotic mechanism[23]. *Solanum lycopersicum* have a significant efficacy to increase hepatic activity by reducing oxidative damage and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action[24]. The effectiveness of a drug depends on the severity of the diseases[25]. The efficacy of any hepato-protective drug is dependent on its efficiency of either reducing the harmful effect or reinstating the normal hepatic condition that has been disturbed d by a hepatotoxin. Both Silymarin and the nanoconjugate decreased paracetamol induced increased enzyme levels in tested groups, which indicate to the protection of structural integrity of hepatocytic cell membrane as well as revival of damaged liver cells. Paracetamol can cause extensive vascular degeneration, central lobular fibrosis and necrosis in hepatocytes. Overdoses of paracetamol caused a remarkable upgrading levels of liver enzyme such as AST, ALT, ALP, TP when compared to control (Gr-I) and Silymarin (Gr-VI). So, this green synthesized nanoconjugate can decreased the elevated enzyme levels in tested groups, indicating the protection of structural probity of hepatocyte cell membrane of injury liver cells.

Paracetamol induced elevated serum levels of hepatic markers (AST, ALP) have been retributed to the liver injury, because these enzymes stay in cytoplasmic area of the cell and are free for transmission in case of cellular damage[26,27] (GR-II Paracetamol Control or Auto Recovery). From the Figure-6 it is observed that serum hepatic bio markers, AST and ALT activities were greatly increased significantly ($P<0.05$) in mice with the paracetamol treatment mice compared to control and Silymarin. There was a significant ($P<0.05$) decreases in serum bilirubin of mice served with nanoconjugate drug (group-V) as compared to the control (group-I) and Silymarin (group-VI) which is marked as a popular drug. Henceforth it can be concluded that the liver function parameters were restored in case of the mice when treated with the green synthesized nano-conjugate

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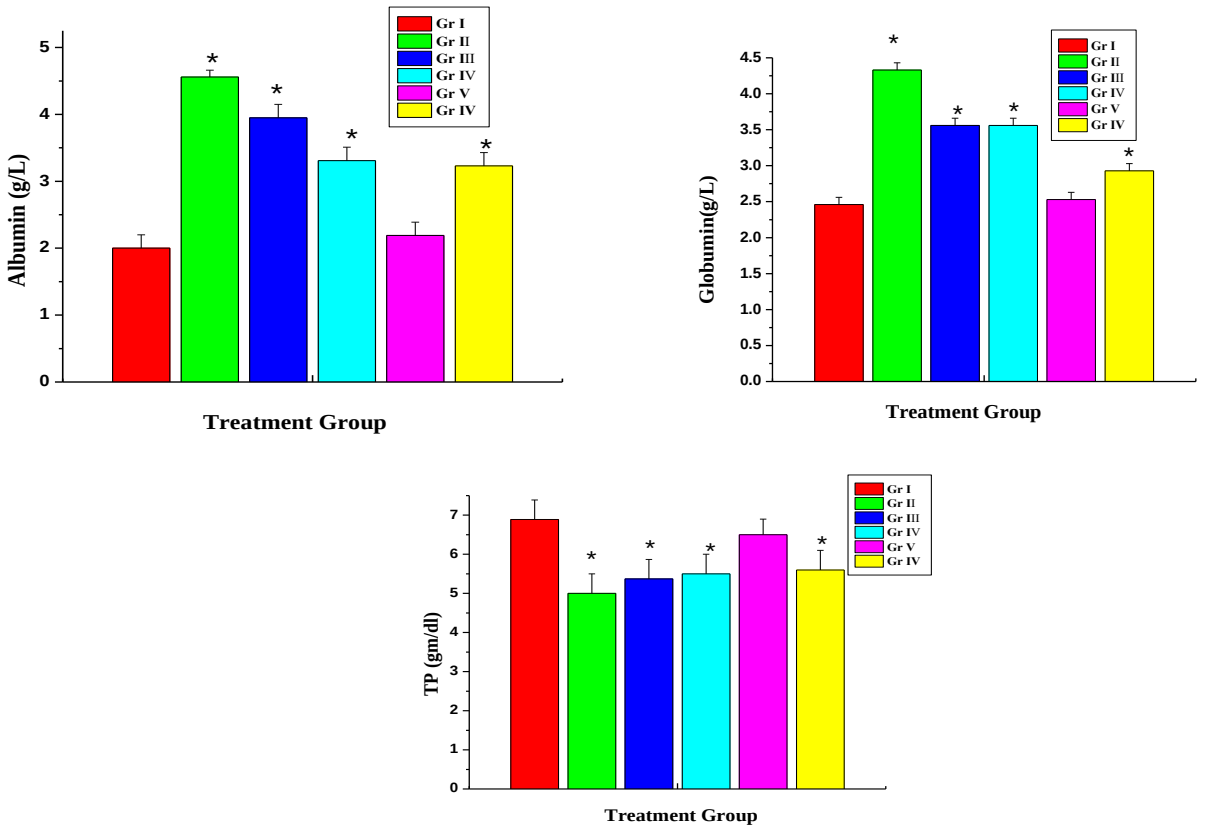


**Figure-5. Effect of Albumin,Globulin and Total Protein in Paracetamol induced mice. All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance p<0.05 implies.**

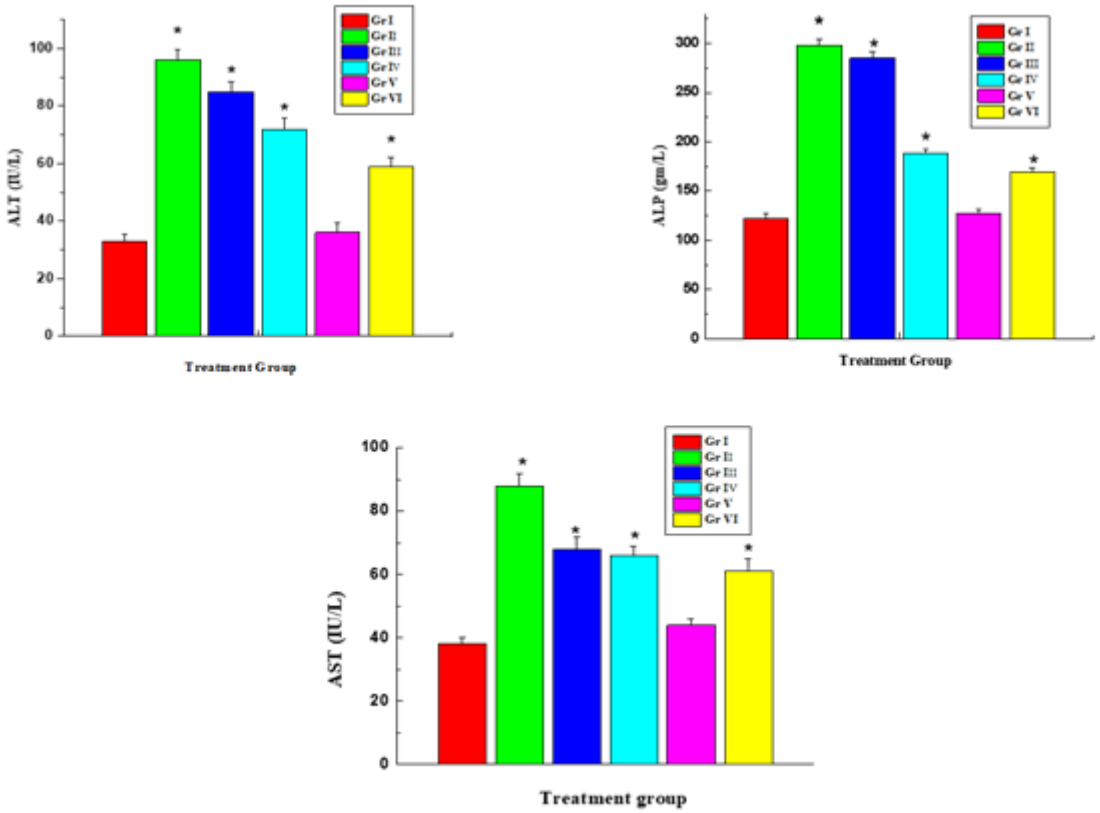


**Figure 6. Effect on ALT, AST and ALP in paracetamol intoxicated mice. All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance p<0.05 implies.**

Effect of the green synthesized $MnO_2$ nanoparticles on hematological parameter in paracetamol intoxicated mice have also been checked. Table I represent the experimental data. It is observed from the table that the haematological

parameters are restored in case of the group of paracetamols treated mice exposed to the green synthesized $MnO_2$ nanoparticles

Body weight is an important factor to monitor the health of an individual and to analyses the toxic impact of paracetamol. Frequent loss in body weight is the first indicator of the onset of adverse effect. Table II depicts the results of body weight in three conditions. The results clearly indicate that the body weight is enhanced in case of the paracetamol treated mice exposed to the green synthesized $MnO_2$ nanoparticles as well as clearly reveal that the normal physiological condition of the mice gets revived upon treatment with the green synthesized $MnO_2$ nanoparticles.

**Table I: Effect of nanoparticles on Hematological Parameters in paracetamol intoxicated mice**

| Haematological parameters | Control | Gr.II | Gr.III | Gr.IV | Gr.V | Gr.VI |
|---|---|---|---|---|---|---|
| **Hb%** | **14.8±0.6** | **8.4±0.6** | **10.8 ±0.6** | **12.5 ±0.6** | **13.8±0.7** | **11.4 ±0.094** |
| **TC** | **6000±0.559** | **2,70±11.5** | **5,200±10.815** | **7,100±0.266** | **2,100±9** | **8,100±22** |
| **Neutrophils** | **90±6** | **128±8** | **120±9** | **115±6** | **97±6** | **118±6** |
| **Lymphocytes** | **25±3** | **46±5** | **40±5** | **38±5** | **29±3** | **40±4** |
| **Monocytes** | **02±0.2** | **04±0.3** | **3.8±0.02** | **3.1±0.2** | **2.4±0.2** | **03±0.2** |
| **Eosinophils** | **0.5±0.04** | **02±0.5** | **1.5 ±0.06** | **01±0.05** | **0.7±0.05** | **1.3±0.04** |
| **Basophils** | **00±00** | **00±00** | **00±00** | **00±00** | **00±00** | **00±00** |
| **RBC** | **7.26±0.3** | **4.50±0.3** | **4.80±0.300** | **6.0 ±0.3** | **7±0.0.4** | **5.6±0.5** |
| **Platelet Count** | **350±26** | **261±20** | **276±20** | **283±25** | **320±25** | **277±23** |

All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance $p<0.05$ implies**.**

**Table II: Body Weight in three conditions**

| **Group** (n=4) | **Normal Condition(gm/kg)** | **After Induction(gm/kg)** | **After Treatment (gm/kg)** |
|---|---|---|---|
| **I** | 26.63±0.186 | 26.8±0.374 | 28.9±0.6 |
| **II** | 27.3±0.399 | 23.6±0.743 | 21.39±0.431 |
| **III** | 25.4±0.035 | 22.6±0.626 | 25.5±0.527 |
| **IV** | 25.2±0.406 | 23.1±0.212 | 26.67±0.045 |
| **V** | 31.2±0.386 | 28.4±0.718 | 32.2±1.24 |
| **VI** | 28.9±0.314 | 26.62±0.247 | 25.4±0.734 |

All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance $p<0.05$ implies.

The presence of paracetamol causes the oxidative stress in liver tissues.as well as stimulated the generation of ROS, by decreasing antioxidant defenses. From detailed study it is observed that the LPO level of group-II significantly increased while the SOD, GSH level decreased ($p<0.05$) in comparison with the control group, which resulted toxicity in the liver cells. At the same time nanoconjugate treated group showed significant decreases in LPO level and increase in SOD and GSH levels. Details are given below in the Table III.

**Table III: Effect of nanoparticles on ROS estimation in paracetamol intoxicated mice**

| **Group** | **Design of the treatment** | **SOD µ/gm wt tissue)** | **LPO µ/gm wt tissue)** | **GSH(µ/gm wt tissue)** |
|---|---|---|---|---|
| Gr-I | Control | 3.7±0.60 | 16.90±1.70 | 6.30±0.35 |
| Gr-II | Hyperbilirubinemia | 2.5±0.78 | 45.75±1.99 | 2.35±0.09 |
| Gr-III | Herb Extract | 3.1±0.40 | 30.94±2.15 | 4.1±0.29 |
| Gr-IV | Nanoparticle | 2.9±0.54 | 29.96±2.55 | 3.8±0.14 |
| Gr-V | Nanoconjugate treated | 3.5±0.59 | 22.0±1.98 | 6.1±0.29 |
| Gr-VI | Silymarin | 3.3±0.53 | 24.56±2.88 | 5.78±0.23 |

All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance $p<0.05$ implies. SOD-Superoxide dismutase, LPO-Malate dehydrogenase, GSH- glutathione

Paracetamol which acts as a mithridate for fever and pain, mainly used as a first line therapy but overdoses can cause the liver damage. Paracetamol hepatoxicity occurs through formation of the NAPQI depletion which is present in

excessive quantities, oxidative stress and mitochondrial dysfunction leading to depletion in ATP stores. When electron comes from unsaturated fatty acids and creates the shaky lipid radical which can retaliate with oxygen and fusing the prooxyl radical. Overdoses of paracetamol can cause the hepatotoxicity. Our study demonstrated that ALT level is significantly higher than normal level. As a result, liver release ALT in blood stream which indicate the toxicity and problem in liver. Our nanoconjugate can decrease the level of ALT because nanoconjugate is made of Tomato and manganese, So Tomato can suppress erythrocytes hemolysis which is induced by water soluble initiator and Vitamin C can decrease the elevated level of ALT

During Induced liver injury may lead to various complication because the liver plays a vital role in the metabolic deposition of all drugs and foreign substances. The drug paracetamol which is widely used as anti-pyretic agent as generally safe at therapeutic levels and uses overdoses leading to hepatotoxicity. In this study it is observed that there was a higher incidence of LPO level who were in the group –II. The increase lipid peroxidation the presence of paracetamol can generate the ROS such as the reactive OH through the Fenton reaction, however the leaf extract of Solanum Lycopersicum that is group-III significant decreases the LPO level than paracetamol treated group. Lipid peroxidation is associated negatively with the activities of antioxidants in workers exposed manganese oxide. So group–IV is significantly decreases than group –II and group-III. Here nanoconjugate (group-V) is made of Solanum Lycopersicum extract and manganese oxide significantly greater reduction ($p<0.05$) the Lipid peroxidation level that is 22.0±1.98 than group-II, III, IV.VI.

Super –oxide dismutase (SOD) is one of the anti-oxidant proteins that help breakdown potentially damaging the oxygen molecules in cells. Another mechanism to minimize peroxtnitrite formation to accelerate the dimution of super-oxidase to hydrogen peroxide and oxygen. The endogenous mitochondria specific superoxide dismutase accomplishes this and inactivates the protein nitration during paracetamol hepatotoxicity. So in this study shows that the level of SOD is 2.5±0.78 which is significantly decreases ($p<0.05$) than control group. Moreover, it has also been shown that the liver damage paracetamol overdose may also decrease the level SOD and GSH. GSH is one of the major tri-peptide non-enzymatic biological anti-oxidant present in the liver, is committed with the removal of free radicals and maintenance of membrane protein.

Serum hepatic LDH levels and GGT are also very important markers of proving hepatoprotection by MnO2 nanomaterials. It is already proved from experimental analysis that **t**he levels of the serum enzymes increased significantly in paracetamol induced mice which is suffering from jaundice, but from Table IV it is observed that nano-conjugate treatment significantly worked to reduce the levels.

**Table IV: Effect of nanoparticles on serum hepatic LDH and GGT:**

| Group | Design of the treatment | LDH | GGT |
|---|---|---|---|
| Gr-I | Control | 196.25±4.34 | 26.47±0.64 |
| Gr-II | Paracetamol Treated | 409.75±5.90 | 112.62±2.81 |
| Gr-III | Herb Treated | 369.47±2.51 | 76.40±3.81 |
| Gr-IV | Nanoparticle Treated | 295±10.07 | 66.90±1.12 |
| Gr-V | Nanoconjugate Treated | 215.25±11.29 | 34.75±0.75 |
| Gr-VI | Silymarin | 231.75±3.26 | 56.72±0.70 |

All data represent as Mean ± SEM, P values calculated by ANOVA test, test of significance $p<0.05$ implies.

**3.3 Effect of nanoconjugate on liver tissue histochemistry**

Liver Tissue Histochemistry suggests that the green synthesized $MnO_2$ nanoconjugate helps in restoring the histological parameters. Figure 7 represent the effect of nanoconjugates on liver tissue histochemistry**.** Group-I liver tissue shows an intact endothelial layer with the normal central vein area of the liver. In group-II congestion of portal vein and mild accumulation of inflammatory cells in portal area is observed upon treatment of paracetamol.On the other hand, group V showed the histological pattern similar to the that of the control group which is charecterised with the presence of a normal central vein area of the liver.The liver morphology also suggests that the $MnO_2$ nanoconjugate helps in restoring the normal morphology as well as histology in case of the mice in which hepatic damage was induced upon treatment with the popular hepatotoxin, paracetamol. The red circle indicates lower accumulation of inflammatory cells in the liver of the mice treated with the nanoconjugate than of the paracetamol treated mice groups.

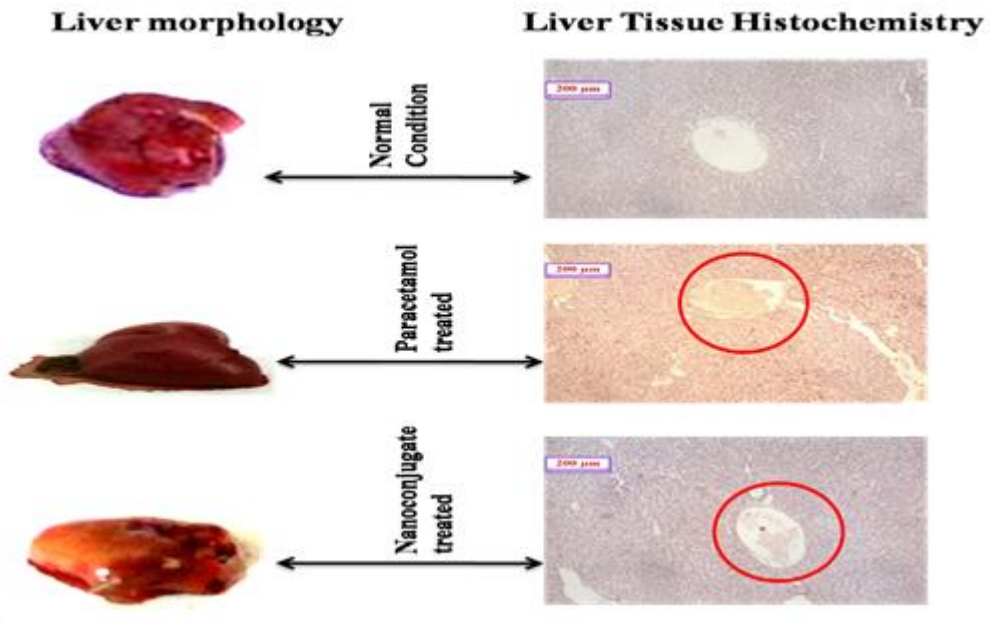


**Figure7. The effect of the green synthesized nanoconjugate on the Liver morphology and Liver Tissue Histochemistry.**

## 4. CONCLUSION

$MnO_2$ nanoparticles were synthesized by a green route using Solanum lycopersicum extract, and then characterized by XRD, FTIR, UV-Vis, and SEM. These green synthesized nanomaterials are safe, biocompatible and effective for treatment against Hyperbilirubinemia in intoxicated mice model. Detailed investigation revealed that significance decrease in bilirubin level with this nanoconjugate compared to the control (group-I) and Silymarin (group-VI) which is marked as a popular drug. These indicate that nanoconjugate preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by paracetamol, which is confirmed by histopathological studies. These nanomaterials have significant ameliorative property, which can play an important role in the treatment of chronic jaundice and hepatic disorders as a benign nanomedicine.

## ACKNOWLEDGEMENTS AND FUNDINGS

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## DATA AVAILABILITY

The data used to support the findings of the study are included within the article.

## CONFLICTS OF INTEREST

There are no conflicts.

## REFERENCE

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