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Acacia modesta attenuates $MnCl_2$ induced hepatotoxicity, oxidative stress and hepatic inflammation in wistar rats

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Abstract: Natural Plants are broadly used in treating inflammatory disorders. The current study focused on evaluating the hepato-protective and anti-inflammatory potential of *A. modesta* in $MnCL_2$ induced hepatotoxicity and liver inflammation. The MnCl2 induce 6.0mg/kg was given for 30 days (p.o) to induced hepatotoxicity and liver inflammation. The ethanolic extract of *A. modesta* were given orally at the dose of 100mg/kg/day. The *in vivo* inflammatory manganese induced hepatotoxic model is used for evaluating the acacia heap to-protective effect. Gas chromatography-mass spectrometry analyses were performed to find out compounds responsible for anti-inflammatory properties. Results showed that administration of ethanolic extract (100 mg/kg), altogether diminished inflammation of the liver, expanded liver capacity, oxidative stress and his to-pathological outcomes in the current study compared with disease rats. The beneficial outcomes of *A. modesta* extract were observed on liver inflammation.

Keywords: Inflammation, Celecoxib, *Acacia modesta*, β-Sitosterol, *in silico* docking.

INTRODUCTION

Manganese (Mn) is an important trace element found in all tissues and necessary for the *in vivo* metabolism of normal amino acids, lipids, proteins and carbohydrates (Aschner and Aschner, 2005). While the Mn deficiency in humans is extremely rare, Mn overexposure toxicity is more prevalent (Erikson *et al*., 2007). Mn toxicity is most commonly observed in mining and welding workers who are routinely exposed to aerosols or dust containing Mn (Santamaria *et al*., 2007) in individuals who drink polluted well-water, or in patients with parenteral nutrition from liver disease (Williams *et al*., 2012). Researchers have found that Mn-exposure has contributed to both a brain and an accumulation of liver (O'Neal and Zheng, 2015). Furthermore, dose-dependent concentrations of Mn have increased over time in brain tissue and other organs.Mn build-up in the brain can lead to an accuracy similar to that of Parkinson's disease (Kwakye *et al*., 2015), while liver accumulation can lead to liver damage, thus reducing the excretion rate of Mn (Zalups and Koropatnick, 2010).

Liver injury and inflammation caused fibrosis, a normal healing phase that results from a wide range of etiologies, including non-alcoholic steatohepatitis (NASH), viral infection, drugs and alcohol (Tsuchida and Friedman, 2017). Chronic liver injury and inflammation have generally been recognized as resident liver stellate cells stimulated in myofibroblast-like cells during the liver fibrogenesis process (Seki and Schwabe, 2015). Macrophage activation is primarily caused by hepatic immune cell inflammatory behavior (Li *et al*., 2017). Hepatic macrophages can mediate myo-fibroblasts directly by generating a variety of cytokines and chemokines (Pellicoro *et al*., 2014). In addition, the inflammatory cytokines released from these cells, including tumor necrosis factor (TNF-α), interleukin (IL-1β) and (IL-6), cause local inflammatory responses and perpetuate inflammation (Tosello-Trampont *et al*., 2016).

Liver inflammation causes increased oxidative stress and reduced liver mitochondrial activity, several evidence indicates that it is mainly characterized by mitochondrial dysfunction (Caldwell *et al*., 1999). The energy homeostasis in hepatocytes is regulated by mitochondrial activities including free beta-oxidation fatty acids, production of ATP and reactive oxygen species (ROS) (Grattagliano *et al*., 2012). Different types of immune responses in liver inflammation due to injury are also important. The relationship between drugs and the immune system in both innate immune responses and adaptive immune response has a clear and detailed mechanism with numerous assumptions (Hoque *et al*., 2014).

Mitochondrial anomalies alter pro-oxidant and antioxidant mechanisms, leading to an increase in non-metabolized fatty acids in the cytosol and resulting in ROS production (Begriche *et al*., 2013). Oxidative stress is caused either by overproduction of reactive oxygen species (ROS) or by a decrease in cellular antioxidants (Deng *et al*., 2009). Increased production of ROS and catecholamine oxidation by-products make Mn toxic (Parenti *et al*., 1988). The antioxidant mechanism, consisting of antioxidants including glutathione (GSH) and antioxidant (SOD), catalase (CAT), and glutathione

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peroxidase (GPx), is essentially a tissue- and subcellular-based compartments of the ROS (Wilhelm, 1996). These defenses can protect cells from harmful DNA, protein oxidation and lipid peroxidation (LPO).

Acacia modesta is a member of the family Fabaceae (Mimosae subfamily). It is commonly known as phulai, and is known locally as palosa. It's spread across Afghanistan, India and Pakistan. It is registered at Punjab, Balochistan and Khyber Pakhtunkhwa in Pakistan. (Ali, 2008). *A. modesta* was used for the treatment of leprosy, wounds, dysentery and venereal diseases and was used to relieve severe body pain. After delivery, females were given a mixture of gum with wheat flour, almond, and butter, and taken as a health stimulant. Because *A. modesta* has antimicrobial properties. its branches used as a miswak. Gum has been used for back pain and sex because of the curative properties. Plant use was also seen in cough treatment (Asghar *et al*., 2003).

MATERIALS AND METHODS

Plant collection

A. modesta plant wall was collected from govt. college university Lahore and identified and ID No. was allotted (Gc.herb.bot: 3655).

Preparation of extract

The *A.modesta* were taken and washed properly with distilled water for surface sterilization in order to eliminate dust particles as well as contaminants. The *A. modesta* were left for drying under shade and the dried samples were blended into fine powder with the help of electric blender and stored within air-tight containers. Fine powder of *A. modesta* was taken and weighed on digital weighing balance and separately soaked into ethanol 30/70 ratio. The powder was precisely mixed by using electric blender and mixture was stored into air-tight containers within refrigerator for 2 days at 4°C. The crude mixture was then filtered with the help of cheese cloth and whatman filter paper while the filtrates were placed in rotary evaporator at 37-40°C in order to get rid of the extra solvent for obtaining concentrated extract or the filtrate was centrifuged at 2,000 RPM for 15-20 minutes to get the concentrated phase isolated which is about 10% of original volume. The obtained concentrated extracts were stored at 4°C as stock and the working aliquots were generated from it by making dilutions for different molecular analyses (Odey *et al*., 2012, Chouhan and Meena, 2015).

Materials

From Sigma Chemical (St. Louis, MO) bought both Manganese chloride (MnCl2, MW125.84) and other chemicals, unless otherwise stated and were of the highest quality. Ultra-pure water was used throughout, obtained from an academic Milli-Q (Millipore, Bedford, MA, USA) system. Dissolving the $MnCl_2$ solution in the sterilized saline at 6.0 mg Mn/ml was used to prepare the solution. The solution was routinely prepared and stored at room temperature.

Experimental protocols

Study design flow sheet

Animals

For this research study, a total of 24 male Wistar rats, aged (20-24) weeks, weighing between 150-180 g from an animal research facility at the University of Lahore, housed under controlled temperature (28 ° C ± 2 ° C) and humidity (60-70 percent). All animals were held in dark/light phases at 12h. The animals were fed water and a regular diet of pellets ad labium. The study protocol, animal handling, all experiments and protocols approved by "The University of Lahore" were accompanied by international ethical care procedures and animal use in research. Committee on Institutional Ethics in Research (IREC No: 33215).

Rats were randomly divided into four groups with six rats in each group.
Group A (Normal rats n=6)
Group B (Diseased rats n=6)
Group C (Standard drug treated rats n=6)
Group D (*A. modesta* treated rats n=6)

Drug used

The oral administration of MnCl2 to the diseased population for 30 days was performed once daily at 6.0 mg Mn/kg/b.w). The treatment with *A. modesta* plant extract 100mg/kg was given to animals for 26 days. We picked the dose based on an earlier analysis performed by Wang *et al*. (2008). The animals were given an equal amount of sterile saline in the usual community. The research was performed in compliance with the animal welfare guidelines and was approved twenty-four hours after the last dose of A provided by the "University of Lahore Animal Care and Use Committee" The rats were killed by decapitation, and quick removal and weighing of their livers. The Liver weight was recorded.

Hematology and clinical chemistry assay

A chemical analyzer used for the measurement of white blood cell levels (WBC), red blood cells (RBC), hemoglobin (HGB) and platelet (PLT) was used for hematology analyzer Serum albumin (ALB), total protein (TP), aspartate amino-transferase (AST) and alkaline phosphatase (ALP).

Samples preparation

At the end of the study, blood samples were taken through heart puncture. The blood was utilized to measure oxidative stress markers MDA, GPx, SOD, inflammatory parameters like TNF-α, IL-18 and IL-4.

Determination of lipid peroxidation

MDA determined the measure thio-barbituric acid reactive species (TBARS). One molecule of MDA

responds with two molecules of thio-barbituric acid within the acidic medium at 95°C temperature for 20 mins to make TBARS. The resultant pink item absorbance was estimated at 532 nm. Lipid peroxidation, was quantifiable as indicated by the technique of (Ruiz-Larrea *et al*., 1994).

Determination of super oxide dismutase (SOD) activity

The quantity measure depends on intensity of the enzyme to hamper the scavenging effect on superoxide anion radicals was assessed using the NBT reduction method with some modifications. Absorbance of the resulting mixture was read at 560 nm against a blank. The activity was monitored as mention before (Tang *et al*., 2007).

Determination of glutathione peroxidase (GPx) activity

The quantity calculation depends on the strength of the metabolic network of the enzyme generating anti-oxidant effects pieces. A promising strategy for inflammation control has been suggested as the over-regulation of its activities (Li *et al*., 2018).

Evaluation of mRNA expression levels of TNF-α, IL-18 and IL-4

Blood was collected on the 56th day of the investigation, RNA abstraction was done through the TRIzol system, getting typical methodology with regards to manufacturer's bearings (Thermo Fisher Scientific, America). TnF-α3'-GTCTACTCCTCAGAGCCC-5'Forward 5-TGAGATCCATGCCATTGGCC-3′ Reverse IL-18. Item was augmented using thermal cycler with 45 cycles of denaturation (95°C for 10 s), annealing (60°C for 20s), and extension (72°C for 30 s), evaluated by utilizing (RT-PCR) through Bio-Rad framework. The cDNA was derived from RNA. The suitable primer was utilized for the pro-inflammatory arbiters for the creating the duplicates by RT-PCR (Jin *et al*., 2008). GAPDH was used as a house-keeping gene.

Assessment of hematological and biochemical markers

At day 56th, the hematological samples were collected via heart puncture, hemoglobin levels and inflammatory cells, for example, WBC count, neutrophils, eosinophil's, lymphocytes, platelets were evaluated via hematology analyzer, creatinine and urea levels were likewise investigated by utilizing chemistry analyzer.

Histopathology

The liver samples fixed in formalin were dried out by ethanol, embedded in paraffin, cut into 5.0 mm pieces and stained with H&E. These sections were analyzed using, light microscope, and the histo-pathological samples were analyzed.

In silico docking

In silico docking studies were carried out to observe the inhibitory action of beta sitosterol of *A. modesta,* on tumour necrosis factor alpha (TNF-α) and cyclo-oxygenase-II (COX-2) enzymes. The crystallographic structures of anticancer (TNF-α) and anti-inflammatory (COX-2) enzymes were obtained from Protein Data Bank with PDB-ID: 5M2J for anticancer enzyme and PDB-ID: 1CVU for anti-inflammatory enzyme. The structures were optimized and used for docking studies. Docking was done using Lamarckian Genetic Algorithm (LGA) in Auto Dock 4.2. The reliability and quality of the system was tested using the re-docking procedure. In both cases, the active site of the protein has been re-docked with ligands. In estimating the binding orientation of ligand in all situations, the root mean square (RMSD) was then calculated, and the RMSD value of <2.0 Å was considered as exact. The binding presents were further tested using the discovery studio (Jabeen, 2018).

GC-MS analysis

(GC-MS) Gas chromatography-mass spectrometry study was done. GC-MS analysis was performed using capillary column (0.25µm, 30m x 0.25mm). Helium gas was used as the carrier to enumerate different components. Column velocity flow was adjusted to 1.0mL/min using the splitless mode and 0.5µL injection volume. Initially oven temperature was set at 110˚C for 2 min, then continuously increased at the rate of 10˚C per min till temperature reached to 200˚C. Then the rate was decreased to 5˚C per min till 280˚C and final temperature.

STATISTICAL ANALYSIS

The outcome of the study was analyzed statistically by employing analysis of variances (ANOVA) one way using Tukey test with level of significance, 0.05 using GraphPad Prism (Version 7.0).

RESULTS

A. modesta regulates liver enzymes

Chronic administration of $MnCl_2$ considerably raised ALP, ALT, AST and bilirubin levels, with ALP levels in group B, $F(3, 20) = 60.62$, ($P<0.0001$) while the groups D shows significant decrease of ALP levels with The level of ALT in *A.modesta* treated group showed $F(3, 20) = 33.98$ ($P<0.0001$), compared to the disease group, the results of AST showed $F(3, 20) = 49.25$ having decreased AST levels in treated group rat compared to group B rats. The comparison of bilirubin levels showed increased bilirubin level compared to *A. modesta* treated $F(3, 20) = 60.11$, ($P<0.0001$).The chronic administration of $MnCl_2$ causes not only the inflammation but also causes increase levels of liver enzyme in diseased group rats, however the treated group rats showed improved in liver enzyme and bilirubin level after chronic treatment with *A.modesta* extract (100mg/kg/day).

Table 1: List of identified constituents of ethanolic extract of *A. modesta* from gas chromatography mass spectrogram

S No.	Retention Time (mins)	Total (%age)	Name of Identified Compound	Mol. Formula	M.W (g/mol)
1	6.762	1.787	Eucalyptol	C10H180	154
2	8.706	1.821	1,6-octadien-3-ol 3,7-dimethyl	C10H180	154
3	11.389	1.324	Linalyl acetate	C12H20O2	196
4	14.003	1.500	Caryophllene	C15H24	204
5	15.345	1.723	7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione	C13H20O2	208
6	15.880	28.891	Decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl	C15H24O2	236
7	17.243	1.226	5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicylo	C15H24O2	236
8	17.809	4.541	Octadecanoic acid,10-oxo-methyl ester	C19H36O3	312
9	18.065	1.282	9-Hexadecenoic acid	C16H30O2	254
10	18.683	1.845	Oleic Acid	C18H34O2	282
11	18.826	1.254	E-8-Methly-9-tetradecen-1-ol acetate	C17H32O2	268
12	20.423	10.667	Hexadecanoic acid, ethyl ester	C18H36O2	284
13	21.418	2.054	8,11-Octadecadienoic acid, methyl ester	C19H34O2	294
14	22.066	13.991	Ethyl Oleate	C20H38O2	310
15	22.277	2.031	Octadecanoic acid, ethyl ester	C20H40O2	312
16	23.445	1.443	Ethyl iso-allocholate	C26H44O5	436
17	23.769	4.197	4a,6a-dimethyl-2-oxo-1a,2,4a,4b,5,6,7,8,9,9a.10,11-tetradecahydrocyclopenta	C21H28O4	344
18	24.040	4.040	1H-Cyclopropa(3,4)benza(1,2-e)azulene-4a,5,7b,9,9a(1Ah)-PENTOL,1B,4,5,7A,8,9-Hexahydro-3-	C24h34o8	450
19	24.236	1.432	4a,6a-Dimethyl-2-oxo-1a,4a,4b,5,6,6a,7,8,9,9a,10,11-tetradecahydrocyclopenta (7,8)	C21H28O4	344
20	25.299	2.117	Di-n-octyl phthalate	C24H38O4	390
21	26.828	1.907	Ethyl iso-allocholate	C26H44O5	436
22	27.559	1.877	Cholestane-3,6,7-triol	C27H48O3	420
23	27.718	1.889	Cholestane-3,6,7-triol	C27H48O3	420
24	29.828	3.717	Vitamin E	C29H50O2	430
25	32.118	1.445	Beta -sitosterol	C29H50O	414

Table 2: Drugs dosages used in study

S No	Drugs	Dosage	Used in groups
1	MnCl2	(0.4mg/kg b.w)	Group B, C, D
2	Celecoxib	(200mg/kg b.w)	Group C
3	*A. modesta* extract	(100mg/kg b.w)	Group D

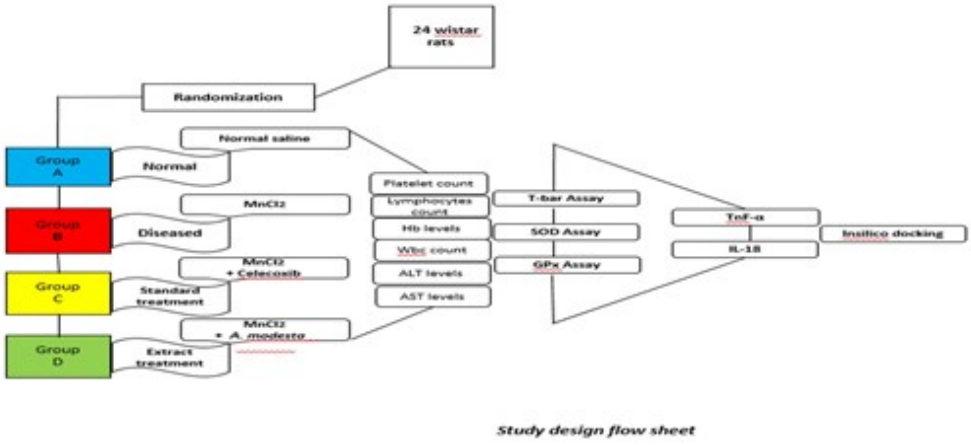

Fig. 1: Study design

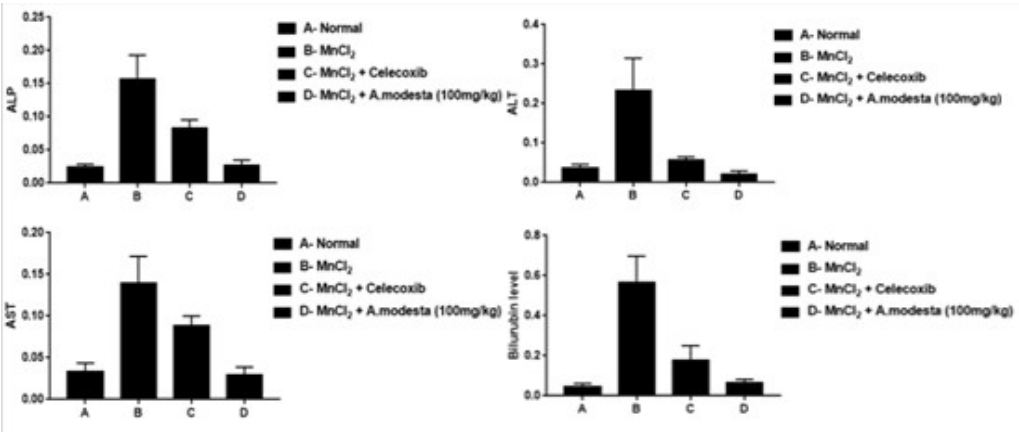

Fig. 2: Effects of repeated administration of *A. modesta* (100 mg/kg) improves the levels of ALP, ALT, AST and bilirubin levels improves in group D as compared to group B (disease group). Values are means ± SD (n = 6). Significant differences by Tukey's test: shows noteworthy change compared to group B indicates significance compared to standard treatment.

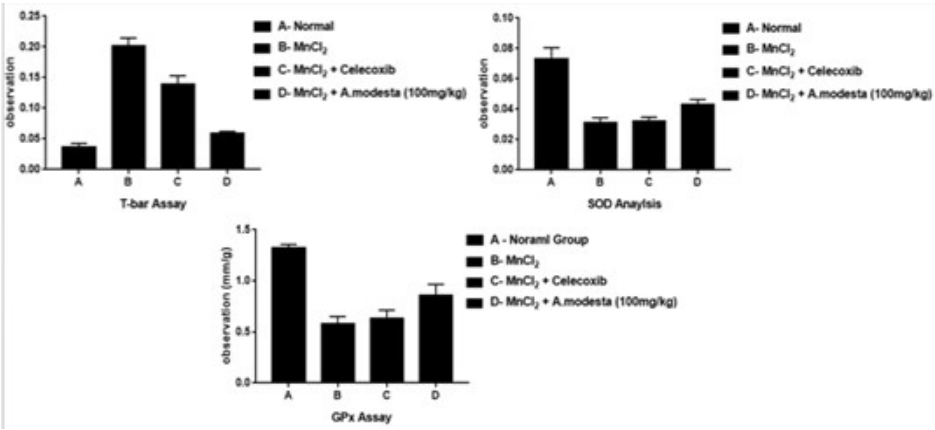

Fig. 3: Effects of repeated administration of *A. modesta* (100 mg/kg) reduces lipid peroxidation shows improved MDA, SOD and improves in group D as compared to group B (disease group). Values are means ± SD (n = 6). Significant differences by Tukey's test: shows noteworthy change compared to group B indicates significance compared to standard treatment.

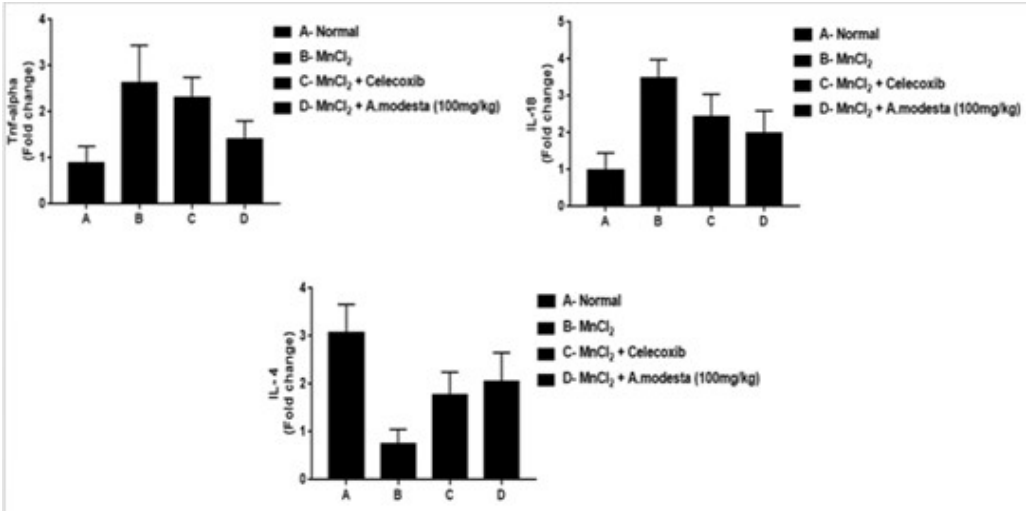

Fig. 4: Effects of repeated administration of *A. modesta* (100 mg/kg) suppresses pro-inflammatory cytokines levels (TNF-α), (IL-18) and (IL-4) shows improved levels in group D as compared to group B (disease group) and group C. Values are means ± SD (n = 6). Significant differences by Tukey's test: shows noteworthy change compared to group B indicates significance compared to standard treatment.

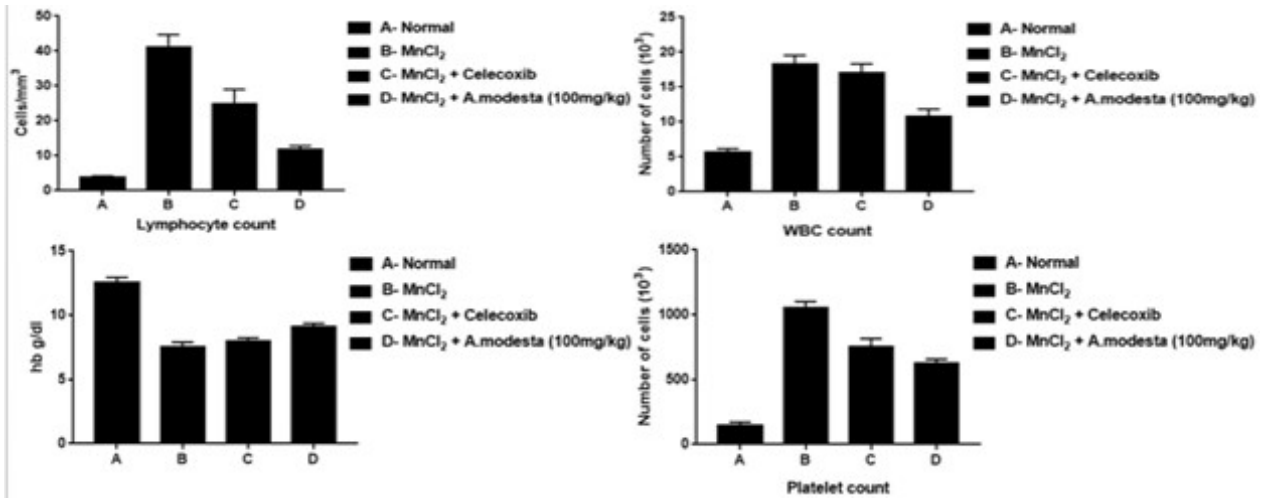

Fig. 5: Shows *A.modesta* stabilized hematological parameters, such as WBC, Platelet, Lymphocyte count, Neutrophils, Platelet count and biochemical parameters like AST, and ALT also considerably attenuated with treatment (100 mg/kg) in group D, as compared with group disease group B and Standard treatment group C.

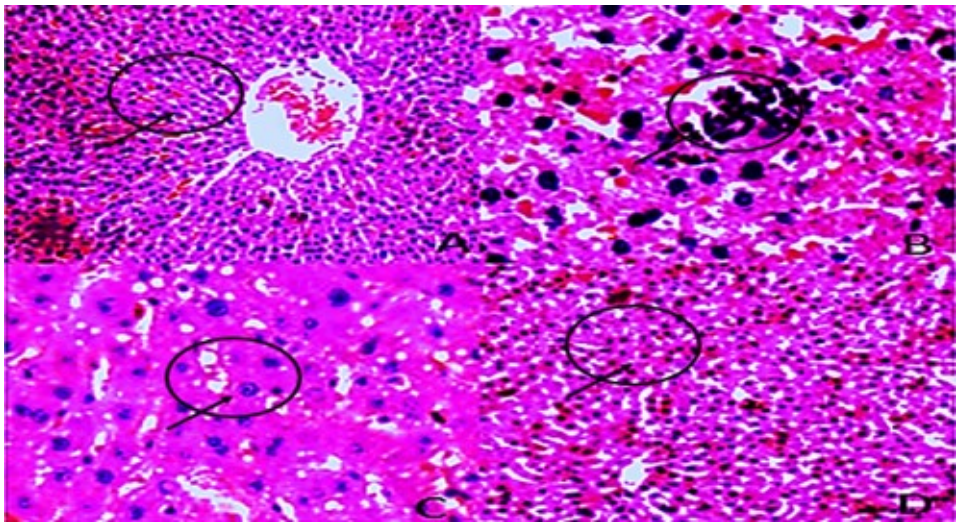

Fig. 6: Shows (H&E) magnification, staining of liver tissue. (A) control gathering; normal histological liver appearance in control rats (B) Intra-lobular mononuclear inflammatory infiltrations and Mallory bodies because of hepatocyte degeneration in diseased rats with H&E staining with magnification, x40 (C) people group treated with celecoxib; diffuse small scale vesicular hepatocyte degeneration in treated rodents. H&E staining with magnification, x40 (D) *A. modesta* Concentrate treated network; moderate provocative mononuclear accumulation in rodents, H&E staining with magnification, x40

A. modesta improves the MDA, SOD and GPx levels

Chronic administration of $MnCl_2$ considerably raised MDA levels in group B ($P<0.0001$) while the groups D shows significant decrease of MDA levels with *A. modesta* treatment $F (3, 20) = 347.1$, ($P<0.0001$), also there is significant decrease in SOD levels in groups B and C compared to SOD levels of group D with repeated measure $F (5, 25) = 94.71$, ($P<0.0001$). However, a significant decrease were recorded in group B compared to *A. modesta* treated group D ($P=0.0016$) and shows insignificant results as compared to group B with repeated measures $F (3, 20) = 102.1$, ($P<0.0001$). The chronic administration of $MnCl_2$ causes not only the inflammation but also causes decrease GPx levels in diseased group rats.

A. modesta suppress mRNA expression levels of TNF-α, IL-18 and IL-4 expression

Significantly raised ($P<0.0001$) levels of TNF-α found in group B as compared to standard treatment groups C ($P= 0.7332$), and *A. modesta* treated groups D ($P= 0.0043$) Treatment with *A. modesta*) considerably suppressed mRNA expression levels of TNF-α in *A. modesta* treated group. $F (3, 20) = 13.49$, ($P<0.0001$). The IL-18 mRNA expression also suppressed in treatment groups D (1.8239 ± 0.2466) compared to group B (3.4883±0.4961).

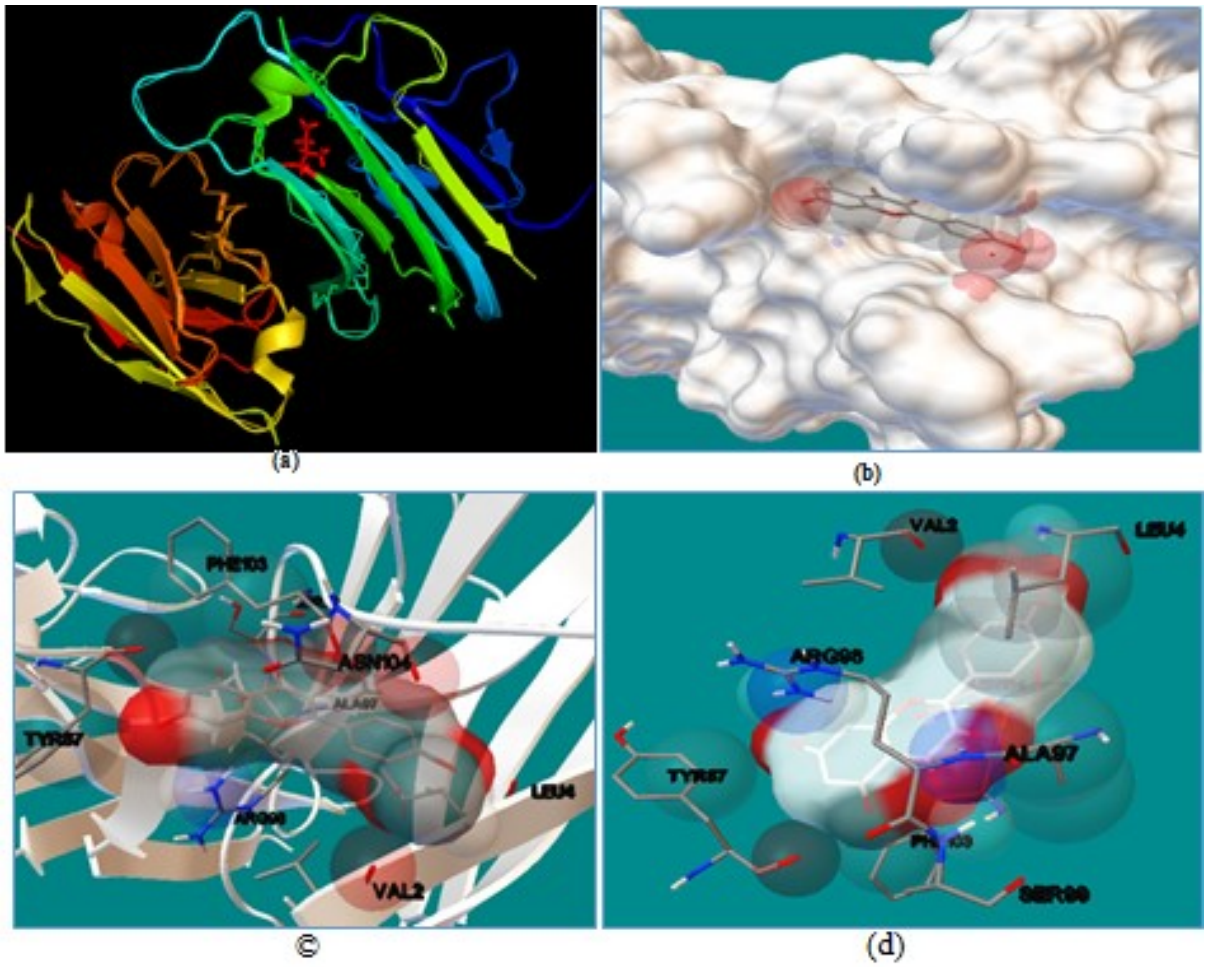

Fig. 7: Shows (a) β-Sitosterol bound at the molecular surface (white) of TNF-α; (b) drug (pink) at the atomic surface (green) of protein; (c) secondary structure of protein (white) with interacting amino acids in the binding site; (d) amino acid residues in close interaction with drug molecule.

Similarly, IL-4 decreased levels were also noticed in group B (P<0.0001) as compared to group A. Treatment group D (P= 0.0014) shows significantly up regulated IL-4 levels (P<0.05) compared to standard treated group C.

***A. modesta* Normalized Biochemical and Hematological Markers**

Repeated administration of *A.modesta* on groups D rats with on lymphocytes count F (3, 20) = 195.2(P<0.0001), repeated administration of *A.modesta* in group D rats also decreases WBC count compared to group B (P<0.001) F (3, 20) = 177 (P<0.0001). *A.modesta* also improves Hb levels of group D rats (P<0.0001) compared to group B and standard treated group C rats (P= 0.1276). F (3, 20) = 273 (P<0.0001) Significant differences by Tukey's test. The comparison of platelets count among the groups showed significant increase of platelets count in group B compared to group A rats (P<0.0001), however significant decrease in platelets count showed by group D rats (P<0.0001) treated with *A. modesta* compared to group B and standard treated rats, F (3, 20) = 455.9. Significant differences by Tukey's test.

A. modesta causing protective effects on liver

Docking with TNF-α and COX-II receptors

DISCUSSION

A systematic assessment of *A. modesta* was done in current study. *A. modesta* was used as a therapeutic alternative to liver inflammation and the mechanism by which it acts to attenuate $MnCl_2$-induced hepatic inflammation in rats. The inflammation of the liver due to $Mncl_2$ is an iron antagonist and can substitute Mg^{2+} in some enzymes and can interfere on account of a specific ion radius with calcium metabolism (Ca^{2+}), Mn accumulates mainly in the mitochondria, influencing in vivo and in vitro mitochondrial functions. However, the mechanisms by which $MnCl_2$ m accumulates are unclear (Zhang *et al*., 2003). It contributes to different rates of inflammation and hepatologic toxicity due to nodular cirrhosis, hepatic cell proliferation, and parenchymal cell necrosis (Apte *et al*., 2002). The $Mncl_2$, which is a natural, high-dose hepato-toxicant (Sepúlveda *et al*., 2012), also does not only rely on oxidative stress

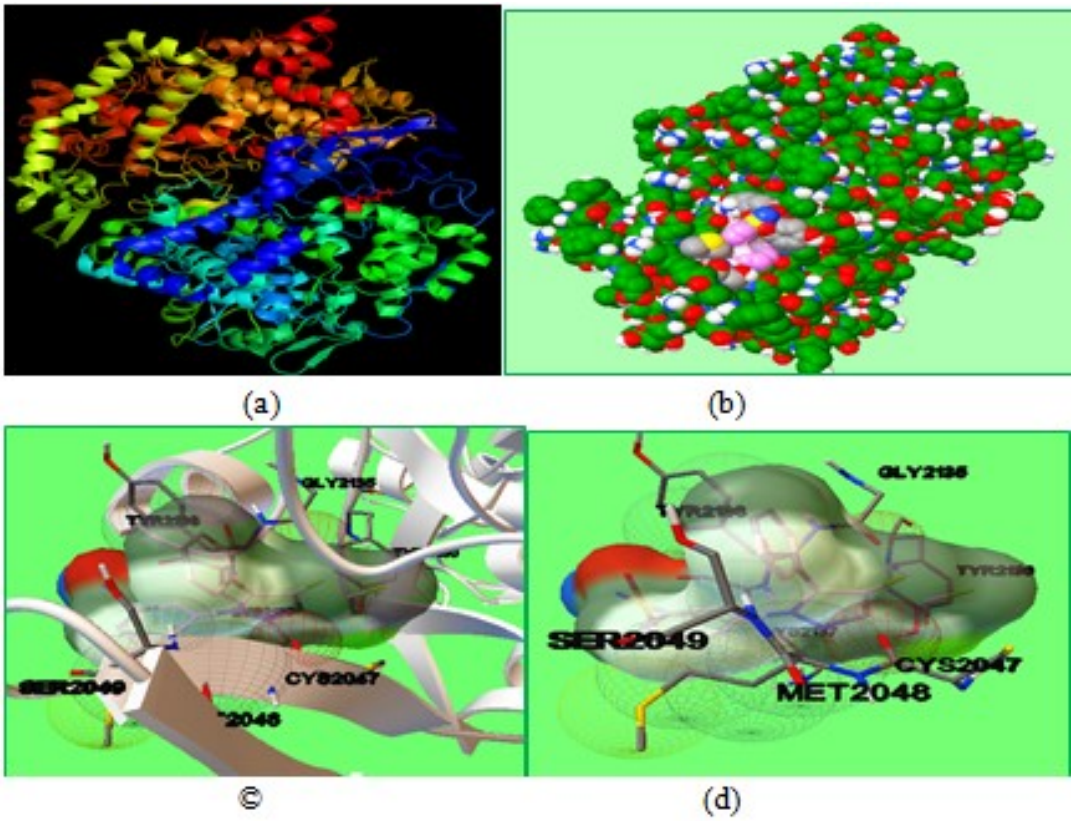

Fig. 8: Shows (a) standard drug (Celecoxib) for Cox-2 inhibitor present on the molecular surface shown in pink color; (b) atomic surface of protein (green) with ligand (pink) in the binding pocket of protein; (c) secondary structure of protein-ligand interaction in the binding pocket of protein with lowest binding energy; (c) wire frame closer view of ligand with interacting amino acids at the lowest binding site of protein.

induction to $MnCl_2$, to CCl_4 toxicity. Thus, the use of the $MnCl_2$-induced inflammation model of the liver enables the evaluation of various pathogens, parameters, and pathways that underscore the production of liver disease and oxidative stress. This helps $MnCl_2$'s inflammation and hepatotoxic model by mimicking these clinical conditions, which include numerous hastening factors in liver damage, and different systems underline the development (Greenwel *et al*., 2000). Besides, the specific component of $MnCl_2$-prompted necrosis is unclear because of the restricted utilization of $MnCl_2$ as a toxicant model, contrasted with other tests animal models, especially in the chronic model studies. Accordingly, it should be utilized in further examinations that investigate data.

Increased serum ALT, ALP, AST and bilirubin levels, showed that chronic administration of $MnCl_2$-treated group have been indicated clinically when compared with diminished levels in *A. modesta* treated group D. Investigations of controlled group rats with recorded histo-pathological findings. Also, the enlistment of oxidative stress in the current $MnCl_2$-treated population has been exhibited by a decrease in GPx diseased rat liver, just as an increase in MDA content and a decrease in SOD activity. These findings are reliable with past investigations demonstrating that the impacts of the body's anti-oxidant and defensive mechanisms are because of its oxidative stress ability (Pallottini *et al*., 2006), Damages the liver cell membranes and enzymes ALT and AST in the hepatic bloodstream, which triggers inflammation and liver enzymes(Nissar *et al*., 2013).

A. modesta administration of the extract (100 mg/kg), altogether diminished inflammation of the liver, expanded liver capacity, oxidative stress, and histo-pathological outcomes in the current study compared with disease rats. The beneficial outcomes of *A.modesta* extract on liver inflammation decrease and similar impacts have been accounted for. Certain studies utilized hepatic swelling and ethanol-induced fibrosis model of rodents and CCl_4 had recently observed similar studies (Saito *et al*., 2014). As indicated by the underlying initial pro-inflammatory cytokines discharged from the macrophages (Simpson *et al*., 1997), the liver is viewed as the essential organ controlling cytokine and its production. $MnCl_2$ actuates pro-inflammatory as a hepato-toxin, including TNF-α, IL-18, and suppresses anti-inflammatory that assumes a noteworthy role in inflammation (Wu *et al*., 2010).

Our outcomes for the study are consistent with those from past research. Anti-inflammatory cytokine like IL-4 assume a significant role, however decreased levels of IL-4 in diseased group found to be decreased but in comparison the IL-4 levels were improved showed the effectiveness of *A. modesta* extract provide benefits and might be able to attenuates the progression of diseased (Prasad, 2017). In our research study, *A. modesta* extract totally reduced the level of pro-inflammatory cytokines, TNF-α, IL-18, similarly as the expanded degrees of the incendiary anti-inflammatory cytokine IL-4 compared to $MnCl_2$-induced diseased group rodents. Such findings showed that one of the possible pathways associated with the anti-inflammatory activity of *A. modesta* might be in comparison with mitigating activity to suppress pro-inflammatory cytokine, increasing anti-inflammatory cytokine levels of IL-4.

Likewise, the elevation of liver inflammation has been seen as a marker of liver inflammation in the liver disease rats treated with MnCl2 causing changes in the bio-chemical and hematological levels. In the later stages, inflammation in the liver cells brought about by $MnCl_2$ is proposed to add to hepatocyte necrosis confirm by raised degrees of TNF-α and pro-inflammatory cytokines in $MnCl_2$-induced rats. The *A. modesta* dose of the extract generously diminishes the measure of TNF-α, a marker of *A.modesta* Defensive and anti-inflammatory activity.

The in silico studies revealed that *A.modesta* extract constitute β-sitosterol docked with TNF-α and Cox-II receptor provide us with binding energies compared to standard treatment. The binding energies showed that β-sitosterol ligand docked with TNF-α act as antagonist and might be able to reduce pro-inflammatory activity of the pro-inflammatory cytokine, however binding of the ligand with Cox-II as inflammatory receptor showed that binding causes the receptor blockade and act as antagonist on the receptor causing the receptor activity to block.

CONCLUSION

The study outcomes showed that the constituents of *A. modesta* like β-Sitosterol provide hepato-protective effect by improving the liver functions and provides anti-inflammatory action. The in silico docking of plant constituent also provides the information through which it binds with receptor and provide anti-inflammatory action compared to celecoxib used as standard treatment in this study.

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