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RESEARCH ARTICLE……!!!

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ALCHOHOLOIC EXTRACT OF ALOE VERA POLYSACCHARIDES IN EXPERIMENTALLY INDUCED LIVER INJRY

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KEYWORDS:

Aloe barbadensis, hepatoprotective, ccl4.

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ABSTRACT

Objectives: *To study the protective potential of polysaccharides of Aloe vera against experimentally induced liver injury."* ***Methods:*** *Polysaccharides of Aloe vera were identified and isolated by alcohol precipitation method. Hepatotoxicity was induced with ccl_4 (0.25ml/100gm). The effect of Aloe vera fractions on ccl_4 induced liver injury was studied. Sylimarin was used as standard treatment for hepatotoxicity. In this proposed work 42 animals were used and animals were divided in 5 groups containing 6 animals in each group. ccl_4 (0.25ml/100gm) and olive oil (1:1) solution was given intraperitonially on 7th day of the study. The group 1 served as control and rats were fed with aq. 5% gum acacia for 7 days orally. Group 2 served as toxic control and rats were given aq. 5% gum acacia for 6 days orally, groups 3 served as standard and rats were given 1 ml of 0.5% silymarin (25 mg/kg) for 6 days, group 4 served as test group and rats were given lower dose, group 5 served as test group and rats were given higher dose and 0.25ml/100 gm of ccl_4 was administered on 7th day after 6 days administration of the extract. After the treatment blood samples were collected and parameters like SGPT, ALP, Direct Bilirubin, MDA and Catalase were evaluated.* ***Results:*** *ccl_4 (0.25ml/100gm) and olive oil (1:1) solution intraperitonially elevated the levels of SGPT, ALP, Direct bilirubin, MDA and depleted the levels of Catalse. Seven day treatment with polysaccharides at a dose of 500 mg/kg, significantly ($P < 0.001$) restored the levels of SGPT, ALP, Direct Bilirubin, MDA and Catalase.* ***Conclusion:*** *Aqueous extract of polysaccharide at higher dose was shows maximum hepatoprotective activity.*

INTRODUCTION:

Exposure to toxic chemicals, environmental pollutants and drugs can cause cellular injuries through metabolic activation of reactive oxygen species (ROS). Carbon tetrachloride (CCl_4) has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain, in addition to liver pathogenesis.

Now a days, to treat liver diseases corticosteroids and immunosupressants are used in allopathic form of medicine. But immunosupression and bonemarrow depression are the adverse effects of these drugs. Further the probability of curing the disease is a bit low. so, scientists are trying to track scientific evidence for the traditionally reported herbal drugs. Hence in the present dissertation best effort is going to make to evaluate the protective effect of *Aloe vera* polysaccharides [10].

Aloe vera L. is a tropical or sub-tropical plant with turgid lace-shaped green leaves with jagged edges and sharp points. It is a xerophytic succulent adapted to living in areas of low water availability, and characterized by possessing a large volume of water storage tissue. The plant is a member of the lily family (*Liliaceae*) not the cactus family as many would believe from the rosette-like arrangement of the long spiked leaves on the central stem

There are over 500 speices of *Aloe* known, but *Aloe vera* L. is recognized as the "true *Aloe vera*" for it's widespread use and purported healing powers [6].

The two major parts of *Aloe* leaf , namely the outer green rind, including the vascular bundles and the inner colourless parenchyma containing the aloe gel. The plant contains two separate juice materials; a yellow latex (exudates – presence of anthraquinone glycosides, extracted from the vascular bundles at the junction between the rind and the fillets, and a transparent mucilaginous gel, extruded from the inner pulp. The raw of *A. vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water [4]. The remaining 0.5-1.0% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids [1].

The primary constituents of the gel are polysaccharides and water. Acemannan or acetylated mannan is the primary polysaccharide of the gel. Other polysaccharides such as arabinan, arabinorhamnogalactan, galactan, galactogalacturan, glucogalactomannan and glucuronic acid-containing polysaccharides have been isolated from the *Aloe vera* inner leaf gel part [3].

Aloe vera has been used for many centuries for its curative and therapeutic properties. *Aloe* juice has been used throughout the centuries as a cathartic and for medicinal purges[12]. Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves.

These biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antidiabetic effects, anti inflammatory, anti cancer, immunomodulatory and gastroprotective properties [9]. Traditional herbal drugs which are considered to be natural have a noticeable demand in under developed countries due to their efficacy, low cost and lesser adverse effects. *Aloe barbadensis* Mill. Syn. *Aloe vera* Tourn. Ex Linn. (Liliaceae) has been used in variety of diseases in the traditional Indian system of medicine in India and it's use for hepatic ailments is also documented. The main biological activities of *Aloe vera* are due to the polysaccharides of the gel. In the present study, an attempt has been made to validate its hepatoprotective activity [7] by performing an assay of the serum total bilirubin, various serum enzymes (SGOT, SGPT and Alkaline Phospatase) against ccl4 induced hepatotoxicity.

MATERIALS AND METHODS:

Fresh leaves of *Aloe vera* were collected from Arogya Rama Herbals, Thumukunta (v) and authenticated by Mr. Rami Reddy.

Preparation of extract:

Aloe vera leaves were washed to remove dirt and kept aside overnight to allow the yellow juice to drain off. Epidermis of the leaves was removed and the mucilage or inner leaf gel was separated..900 g of gel was allowed to stand in 1L 0.1 N HCl for overnight at room temperature.The extract was filterd through a typical woman's nylon cloth. Then the filterate was neutralized with 1 N NaOH, and polysaccharides were precipitated with 3 volumes of ethanol.After centrifugation for 30 min 4000 rpm, the prciptate was re-dissolved in distilled water. Then the ph of the suspension was adjusted to2.0 with 1 N HCl and $cacl_2$ was added to the final concentration of 2M. The resulting precipitate was removed by centrifugation and supernatant was treated with 3 volumes of ethanol. The ethanol precipitation was repeated twice and the precipitate was re-dissolved in distilled water, and evaporated to get crude polysaccharides designated as ABPS.

Phytochemical estimation studies and thin layer chromatographic studies(ethyl acetae, pyridine, water 40:10:20) of extracted polysaccharides were done.

Preparation of doses:

20 mg/ml solution of *Aloe vera* fractions were prepared in distilled water and 0.25 ml/100 gm of carbon tetrachloride and olive oil (1:1) solution was prepared.

Acute Oral Toxicity:

Many articles suggested that the *Aloe vera* extract was found to be safe at maximum toxic dose (MTD) > 2000mg/kg as observed by OECD 423 guidelines. Therefore, based on the previous literature study, doses of *Aloe vera* selected were 250 mg/kg and 500 mg/kg *p.o* (2&29).

Source of animals:

Male Wistar Albino rats (150-180g) of the age 8-10 weeks were obtained.

Housing:

The animals were housed in polypropylene cages under constant temperature (22±2oc), humidity (55%) and light/dark conditions (12/12h). They were provided with chow and free access to water *ad libitum*. The experiments and procedures used in the study were approved by the Instituitional Animal Ethics committee.

Selection of animals: By observing the health status and normal behavioral parameters for 10 days; healthy, adult male Wistar albino rats weighing 150-200g were selected for the proposed study.

Carbon tetra chloride induced hepatotoxicity in rats:

In this proposed work 42 animals will be used and animals will be divided in 5 groups containing 6 animals in each group. ccl_4 (0.25ml/100gm) and olive oil (1:1) solution was given intraperitonially on 7th day of the study.

EXPERIMENTAL DESIGN:

Group 1- Normal control: The group A served as control and rats were fed with aq. 5% gum acacia for 7 days orally.

Group 2- Disease control: Group B served as toxic control and rats were given aq. 5% gum acacia for 6 days orally, 0.25 ml/100 gm of carbontetrachloride and olive oil (1:1) on 7th day(*i.p*).

Group 3-Standard: groups C served as standard and rats were given 1 ml of 0.5% silymarin (25 mg/kg) for 6 days and 0.25 ml/100gm of carbon tetrachloride and olive oil (1:1) on 7th day.

Group 4-Carbon tetra chloride+higher dose of plant extract (250mg/kg): group 4 served as test group and rats were given higher dose and 0.25ml/100 gm of ccl4 was administered on 7th day after 6 days administration of the extract.

Group 5-Carbon tetra chloride +lower dose of plant extract (500mg/kg): group 5 served as test group and rats were given lower dose and 0.25ml/100 gm of ccl4 was administered on 7th day after 6 days administration of the extract.

Serum sample preparation:

Blood samples were collected from all animals (Rats) at the end of the dosing by retro orbital method. The blood samples were allowed to clot for 60 min at room temperature. Serum was separated by centrifugation at 4000 rpm for 15 min and used for estimating various serum parameters. (BUN, Total Protein and Serum Creatinine).

Tissue preparation and homogenization:

Animals were sacrificed by cervical dislocation and Livers were removed from rats on the 8th day (i.e. after 24hrs of ccl_4 injection *i.p*), washed thoroughly with ice-cold phosphate buffer solution and weighed. Some portion of the Livers was taken, minced into small pieces and homogenized with a Homogenizer in ice-cold phosphate buffer solution (PBS) (50mm, pH 7.4) to obtain 1:9 (w/v) i.e. (10%) whole homogenate A part of the homogenate was taken and mixed with equal volume of 10% trichloroacetic acid (TCA) and centrifuged at 4000 rpm for 15mins at 4ºC and the supernatant i.e. clear solution was collected into another tube and was further used for the determination of parameters like MDA. The remaining part was centrifuged at 15,000 rpm for 45- 60mins at 4ºC, and supernatants were collected and stored in different tubes under 80ºC and were further used for the assay of CAT etc.

Statistics:

All values were expressed as Mean ± SEM. (n = 6 in each groups). One way ANOVA was applied to test for significance of biochemical data of the different groups.

RESULTS

Effect of *Aloe vera* polysaccharides on levels of ALP:

SL. No.	**Groups**	**Mean ± SEM(IU/L)**
1	GroupI (Normal control)	33.59 ± 4.98
2	GroupII (Disease control)	335.885 ± 49.326
3	GroupIII (Standard)	114.266 ± 3.804 ***
4	Group IV	271.375 ± 27.839
5	GroupV	110.5 ± 4.031 ***

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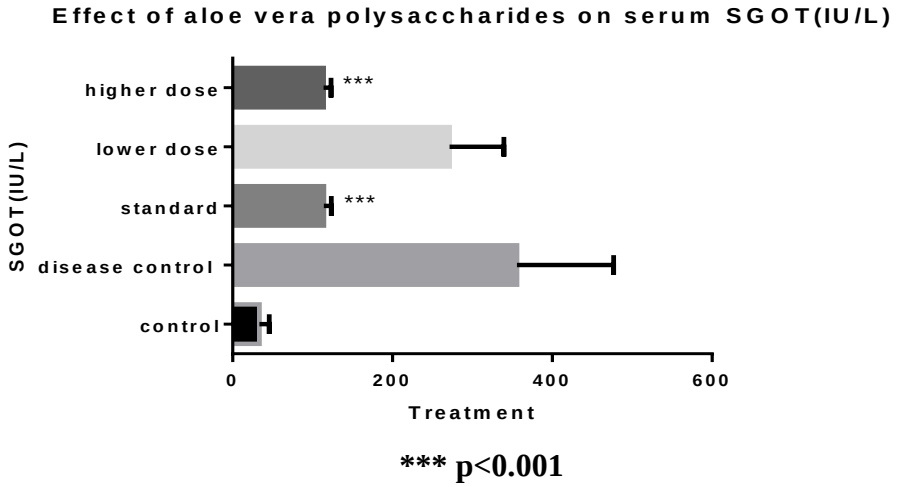
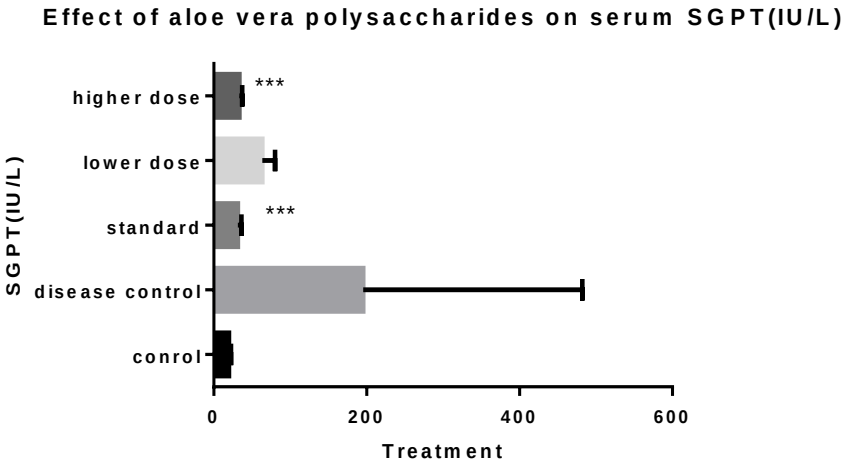


***** p<0.001**

Table 1: Effect of *Aloe vera* Polysaccharides on ALP level

SL. No.	Groups	Mean ± SEM(IU/L)
1	Group I (Normal control)	19.77 ± 1.123 ***
2	Group II (Disease control)	78.83 ± 12.341
3	Group III (Standard)	31.61 ± 1.924 **
4	Group IV	63.5 ± 6.766
5	Group V	33.58 ± 1.507 **



***** p<0.001, ** p<0.05**

Table 2: Effect of *Aloe vera* Polysaccharides on ALT level

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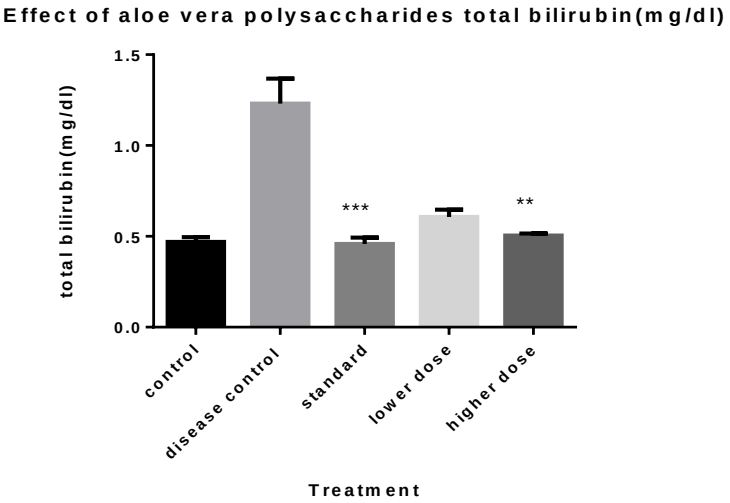
Effect of *Aloe vera* polysaccharides on levels of Direct Bilirubin:

SL. No.	Groups	Mean ± EM(mg/dl)
1	Group I (Normal control)	0.47 ± 0.010
2	Group II (Disease control)	1.185 ± 0.062
3	Group III (Standard)	0.458 ± 0.014 ***
4	Group IV	0.986 ± 0.076 **
5	Group V	0.505 ± 0.004 ***



***** p<0.001, ** p<0.05**

Table 3: Effect of *Aloe vera* polysaccharides on Direct Bilirubin levels

SL. No.	Groups	Mean ± SEM (µ.mol/gm)
1	Group I (Normal control)	0.113 ± 0.008***
2	Group II (Disease control)	0.681 ± 0.066
3	Group III (Standard)	0.33 ± 0.016***
4	Group IV	0.586 ± 0.045
5	Group V	0.35 ± 0.018***

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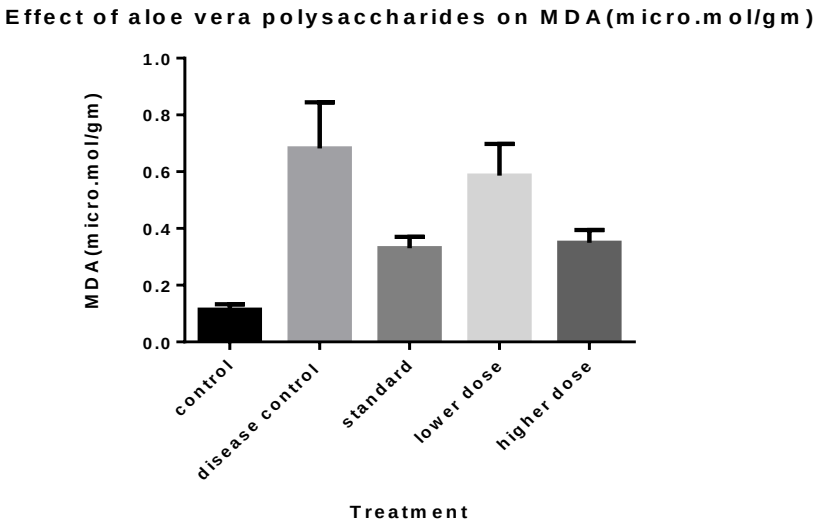

Table 4: Effect of *Aloe vera* polysaccharides on MDA levels

Effect of *Aloe vera* polysaccharides on levels of catalase:

SL. No.	Groups	Mean ± SEM(U/gm of protien)
1	Group I (Normal control)	58 ± 3.454
2	Group II (Disease control)	17.34 ± 0.431
3	Group III (Standard)	32.08 ± 2.059
4	Group IV	23.14 ± 0.968
5	Group V	31.5 ± 1.024

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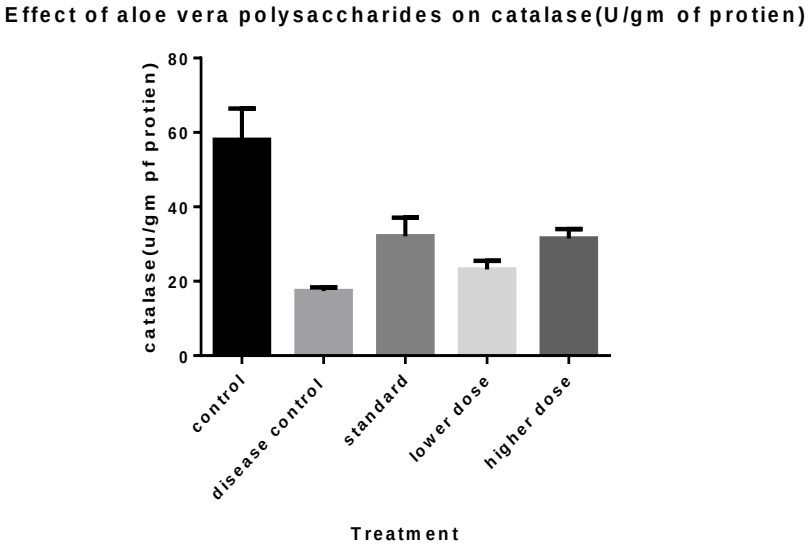

Table 5: Effect of *Aloe vera* polysaccharides on catalase level**.**

DISCUSSION:

On the treatment with ccl_4 (intraperitonially) for 7 days there was significant increase in the level of ALT in model control group when compared with normal control group on liver injury. 63.5±6.766 (IU/L), 33.58±1.507 (IU/L) was observed with lower dose and higher dose treated animals which was significantly less in compare to model control group (78.83±± 12.341U/L) but was very high in compare to normal control group (19.77±±1.123) as shown in table-2.Higher dose treated rats showed decrease in ALT levels in serum when compared with lower dose treated rats. Model control group showed a significant increase in ALT, when compared to other normal control group which indicates that liver injury induced. Higher dose treated group showed significant increase ($P \leq 0.0001$) in ALT when compared with normal control group and significant decrease in ALT levels when compared to model control group indicating that drug is effective in reducing abnormal ALT levels.

There was significant increase in the level of ALP in diseased control group when compared with normal control group on liver injury. 271.375±±27.839 (IU/L),110.5±4.031 (IU/L) was observed with lower dose and higher dose treated animals which was significantly less in compare to diseased control group (335.885±± 49.326 IU/L) but was very high in compare to normal control group (33.59±±4.98) as shown in table-1.Higher dose treated rats showed decrease in ALP levels in serum when compared with lower dose treated rats. Diseased control group showed a significant increase in ALP, when compared to other normal control group which indicates that liver injury induced. Higher dose treated group showed

significant increase ($P \leq 0.0001$) in ALP when compared with normal control group and significant decrease in ALP levels when compared to model control group indicating that drug is effective in reducing abnormal ALP levels.

There was significant increase in the level of Direct Bilirubin in diseased control group when compared with normal control group on liver injury. 0.986± ± 0.016(mg/dl), 0.505± ± 0.004(mg/dl) was observed with lower dose and higher dose treated animals which was significantly less in compare to diseased control group (1.18± ± 0.062mg/dl) but was very high in compare to normal control group (0.47±± 0.010mg/dl) as shown in table-3.Higher dose treated rats showed decrease in Direct Bilirubin levels in serum when compared with lower dose treated rats. Diseased control group showed a significant increase in Bilirubin, when compared to other normal control group which indicates that liver injury induced. Higher dose treated group showed significant increase ($P \leq 0.0001$) in Bilirubin when compared with normal control group and significant decrease in Direct Bilirubin levels when compared to model control group indicating that drug is effective in reducing abnormal Direct bilirubin levels.

Higher dose treated group showed significant increase ($P \leq 0.0001$) significant decrease in MDA levels when compared to model control group indicating that drug is effective in reducing abnormal MDA levels.

There was significant decrease in the level of Catalase in diseased control group when compared with normal control group on liver injury. Higher dose treated group showed significant decrease ($P \leq 0.0001$) in Catalase when compared with normal control group and significant increase in Catalse levels when compared to model control group indicating that drug is effective in reducing abnormal Catalase levels, indicating that drug is effective in reducing abnormal Catalase levels.

Efficacy of the higher and lower dose was confirmed by Mean and SEM values of all the parameters of various groups of animals and they were found to be effective in curing the liver damage.

CONCLUSION:

In conclusion, the possible mechanism of protective action of aloe vera polysaccharides may be due to it's antioxidant activity as indicated by protection against increased lipid peroxidation and maintained MDA levels. Rest of biochemical parameters provide further support to the supportive mechanism of action.

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