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PhytochemicalInvestigationof Roots of*CryptolepisBuchananii*for Antioxidant andHepatoprotectiveActivity

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ABSTRACT

Cryptolepisbuchananii , family- Asclepiadaceae is reported to possess a number of medicinal properties including jaundice, cooling effect, for polyuria, hemorrhage, gout, wounds, leprosy, dysentery, cough, bronchitis, leucorrhoea, uterine hemorrhage, dysuria and blood diseases, skin diseases, vomiting, poisoning, chronic rheumatism.

The aim and the objective of the study is to evaluate the in vitro Anti-oxidant and in vivo Hepatoprotective activity of alcohol and aqueous extracts of roots of *Cryptolepisbuchananii.* A phytochemical investigation was carried out to identify various constituents present in extracts and found to contain alkaloids, glycosides, phytosterols, and saponins. The Hepatoprotective effect of both alcohol and aqueous extracts of roots of Cryptolepisbuchananii were studied in rats by using CCL4 and Paracetamol-induced hepatotoxicity models, at the dose of 200 and 400 mg/kg body weight. Phytochemical analysis of extracts of roots of Cryptolepisbuchananii showed the presence of alkaloids, glycosides, saponins, and phytosterols and may play role in the Antioxidant and Hepatoprotective activity. These results support the traditional use of Cryptolepisbuchananii for Anti-oxidant and Hepatoprotective activity..

KEY WORDS:-*Cryptolepisbuchananii*: CCL_4,Paracetamol, SGOT, SGPT, ALP ,Bilirubin, Total Protein, DPPH, Ascorbic acid:H_2O_2,FRAP.

I. INTRODUCTION:

A naturally derived herbal medicine has a biological activity for use in pharmaceutical drug discovery and drug design. These drugs useful for treating or prevent life style related disorders. There has been a growing interest in the analysis of plant products which was stimulated intense research on their potential health benefits. *Cryptolepsisbuchanani*(Asclepiadaceae), a climbing tree, is widely used in folk medicine in Southeast Asia. In Thailand, the alcoholic extract of stem of this plant is common used for the treatment of inflammatory conditions such as arthritis, and muscle and joint pain(panthond et al.,1986). A weak inhibitioninhibition of eicosanoid generation from rat leukocytes was previously reported (Laupattarakasem et al., 2003). The aqueous plant extract posses broad spectrum of medicinal properties randing ranging from antibacterial CryptolepisbuchananiRoem&Schult. (Asclepiadaceae) is a climbing tree found in evergreen forest in Thailand, china, india, Nepal, and Indo-china.is widely used in folk medicine in southes Asia [1]. In Thailand, *C. buchanani* known as “Thao En On” has been used in treatment of muscle tension, stiffness of tendon, and arthritis [1,3]. Its leaves are used as poultice on inflamed area for the treatment of myalgia and arthtitis[2]. Few studies have examined the anti-inflmmatoryeffect of this plant and found that its extract could reduce inflammation both in *in-vitro* and *in-vivo* studies [1,3]. However, scientific reports of the analgesic and chondroprotective activities of C.buchanani are limited.

II. MATERIALS AND METHODS

Plant material

Rootsof*Cryptolepisbuchananii*werecollectedfromBangaloreUniversity,JannabharathiCampusandauthenticatedbyDr.K.P.Srinath Professor ,Botanist ,Bangalore University, Bengaluru. .A voucher specimen (CB-12-02) has been deposited atthe Dept. ofPharmacognosy.(AuthenticationNo-----).

Fig No-1Cryptolepisbuchananii

Extraction:

Cryptolepisbuchananiis a plant genus in the family Apocynaceae. It includes some 42 species. The Stems possess alkaloids,The root of buchananine is identified as 6-Onicotinoyl-alpha glucopyranose and 1, 3, 6-O-trinicotinoyl-L-glucopyranaose The leaves of Cryptolepisbuchanani was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. An equal quantity of powder was passed through a 40 mesh sieve and extracted with ethanol (90% v/v) in the soxhlet apparatus at 60^0C (Chattopadhyay et al, 2003)[4]. The solvent was completely removed by a rotary vacuum evaporator. The extract was dried and stored in vacuum desiccators**.**

PHARMACOLOGICAL SCREENING

Experimental Animals:[5]

Experimental studies were carried out using male wistaralbinto rats (150 -200g) and albino mice (20-25g) purchased from IISC Bangloreinida. The animals were grouped in polypropylene cages with stainless steel top (38x23x10) with not more than six animals per cage and Room temperature of 25 ± 1^0C, relative humidity 45-55% and a 12:12 hrs light/dark cycle. The animals were acclimatization the animals were subjected to a gross observation to ensure that the selected animals were selected for final allotment of the study, one week before in experimental room. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments of animal (CPCSEA).

Drugs and chemicals:

Silymarin purchased from E-bioligicspvt ltd, serum glutamate oxaloacetate transaminase (SGOT),serum glutamate pyruvate transaminase (SGPT) and Bilirubin kits were obtained from Anjan distributors, banglore, Phytochemical analysis, other chemicals and reagents utilized were of analytical grade from SD fine chemicals Banglore, India.

PreparationofDrugs

The alcoholic and aqueous extract of roots of *Cryptolepisbuchananii*were suspended in Tween 80 solution and used for oral administration.Eachtime freshpreparations of the extracts wereprepared

Determination of hepatoprotective effect of cryptolepsis buchanani

Experimental treatments:

Animals were divided into seven group of six animals of each group.

Group Iservedasnormal,controlandreceivedonlythevehicle.

GroupII animals received CCl4 (0.5 ml/kg,1;1 in olive oil) i.p. once daily for 7days.

Group III animals received CCl4 (0.5 ml/kgi.p). and referencestandard Silymarin (100 mg/kg p.o.) for 7 days.

Groups IV & V were administered withlowandhighdosesofEECBandCCL4atadoseof 0.5ml/kg i.p. for7days.

GroupsVI&VIIwereadministeredwithlow.

DETERMINATIONOFACUTEORAL TOXICITY(LD50):

Theacutetoxicityofalcoholicandaqueousextractsofrootsof*Cryptolepisbuchananii*were determined by using female albino mice(20-30g) those maintained under standard husbandry conditions. Theanimalswerefasted3hrspriortotheexperiment,upanddownprocedure (OECD guideline no. 425) was adopted for toxicity studies.Animalswereadministeredwith singledoseofextractofrootsof*Cryptolepisbuchananii*and observed for its mortality during 48 hoursstudy period (short term) toxicity. Based on short-term profile of drug,the dose of the next animals was determined as per as OECD guideline 425.

Table no-1, CCL_4InducedHepatotoxicity[6] :

GROUP	DRUG	DOSE
GroupI	Vehicle control – 1%Tween	5ml/kg,p.o.
GroupII	Toxicantcontrol–CCL4	0.5ml/kg,i.p
GroupIII	CCL4 +Standard–Silymarin	100mg/kg, p.o
GroupIV	CCL4+Lowdoseof EECB	200mg/kg, p.o
GroupV	CCL4+High doseof EECB	400mg/kg, p.o
GroupVI	CCL4+Lowdoseof AECB	200mg/kg, p.o
GroupVII	CCL4+High doseof AECB	400mg/kg, p.o

for 7daysAfter24hours ofthe lasttreatment,thebloodsampleswerecollectedbyretroorbitalpunctureunderetheranaesthesiafortheestimationofbiochemicalmarkerenzymeslikeALT,AST,ALP,DirectBilirubin, Total Bilirubin andTotalProtein. Theanimalsweresacrificedbyoverdoseofetherandautopsied.Liver removed from Animals andwashedwithice-coldsaline,weighed and wet liver volume was measured. Small pieces oflivertissuecollectedandpreservedin10%formalinsolutionforhistopathologicalstudies.Theresultswerereportedin **TableNo.11,12,13.**

Table no-2,ParacetamolinducedHepatotoxicity[7]:

GROUP	DRUG	DOSE
GroupI	Vehicle control – 1%Tween	5ml/kg,p.o.
GroupII	Toxicantcontrol–CCL4	0.5ml/kg,i.p
GroupIII	CCL4 +Standard–Silymarin	100mg/kg, p.o
GroupIV	CCL4+Lowdoseof EECB	200mg/kg, p.o
GroupV	CCL4+High doseof EECB	400mg/kg, p.o
GroupVI	CCL4+Lowdoseof AECB	200mg/kg, p.o
GroupVII	CCL4+High doseof AECB	400mg/kg, p.o

The evaluation of hepatotoxicity is followed accordingly. Thirty ratswere divided into seven groups of 6 animals each. Group Iservedasnormal,controlandreceivedonlythevehicle. Group II, III,IV, V, VI and VII were administered with paracetamol 2 g/kg orally. On the seventh day after 2 hrs of respective treatment, the blood samples were collected by orbital puncture under ether anaesthesia for the estimation of biochemical marker enzymes like ALT, AST, ALP direct Bilirubin, Total Bilirubin and total protein. The results were reported in **table no.11,12,13**. Fig no-8,9,10,11,12,13,14.

BIOCHEMICALPARAMETERS:
AST (SGOT):ModifiedU.V.(IFCC),kinetic assay[8,9]:
PROCEDURE:-

Table no-3

Pipetteout	Test
Serum/ Plasma	100 µl
WorkingASTReagent	1000 µl

Mix well and aspirate immediately for measurement. The programme analyzer as per the assay parameter:
1. Blank the analyzer with the purified water.
2. Read absorbance after 60 sec. Repeat the reading after every 30 sec. i.e. up to 120 sec at 340 nm wavelength.
3. Determine the mean absorbance (Δ A /min)

ALT(SGPT):ModifiedU.V.(IFCC),kineticassay[10,11]

PROCEDURE:

Table no-4

Pipetteout	Test
Serum/plasma	100µl
WorkingALTReagent	1000µl

Mix well and aspirate immediately for measurement. The programme analyzer as per the assay parameter:
1. Blank the analyzer with the purified water.
2. Read absorbance after 60 sec. Repeat the reading after every 30 sec. i.e. up to 120 sec at 340 nm wavelength.
3. Determine the mean absorbance (Δ A /min)

ALKALINE PHOSPHAT :PNPP- AMP (IFCC),Kinetic Assay[12,13,14]

PROCEDURE :

Table no-5

Pipetteout	Test
Serum/plasma	20 µl
WorkingALPReagent	1000µl

Mix well and aspirate immediately for measurement. The programme analyzer as per the assay parameter:
1. Blank the analyzer with the purified water.
2. Read absorbance after 60 sec. Repeat the reading after every 30 sec. i.e. up to 120 sec at 405 nm wavelength.
3. Determine the mean absorbance (Δ A /min)

TOTALPROTEIN:ModifiedBiuret,EndPointAssay[15,16]

PROCEDURE:

Table no-6

Pipetteout	Blank	Standard	Test
Serum/plasma	----	----	10 µl
Reagent2	----	10 µl	----
Reagent1	1000 µl	1000 µl	1000 µl

Mix well, incubate at 37^0.c for 5 minutes.Programmetheanalyser as perthe assayparameter:
1. Blanktheanalyserwith ReagentBlank
2. Measureabsorbanceofthestandardfollowed bythe test.
3. Calculateresultsasperthegivencalculation formula.

BILIRUBIN [17,18,19]:

Mix well, incubate at R.T. for 5 minutes.Programmetheanalyser asperassayparametes.
1) Blankthe analyserwithpurifiedwater.

2)Measuretheabsorbanceofthesampleblankandtestat546nm(1ʎ) and630nm(2ʎ)
3) Calculateasperthegivenformula.

Table no-7

	TOTAL BILIRUBIN		DIRECTBILIRUBIN	
Pipette into tubemarked	Sample Blank	Test	Sample blank	Test
Reagent1	----	50µl	-----	50µl
Reagent2	100µl	50µl	100µl	50µl
Mix properly				
Workingreagent	1000µl	1000 µl	----	------
Serum/plasma	50µl	50µl	50µl	50µl
Normalsaline	-----	------	1000µl	1000 µl

HistopathologicalStudies

a) Processing of isolated liver

The animals were sacrificed and the liverofeachanimalwasisolatedandwascutintosmallpieces,preservedandfixedin10%formalinfortwodays.Thentheli verpiecewaswashed in running water for about 12 hours toremove the formalin andwasfollowedbydehydrationwithisopropylalcoholofincreasingstrength(70%,80%and90%)for12hourseach.The nfinally dehydrationis done using absolute alcohol with about three changes for12hours each. Dehydrationwasperformedtoremovealltracesofwater.Furtheralcohol wasremoved by using chloroform and chloroform removed byparaffin infiltration. Theclearing was done by using chloroform withtwochanges for 15 to 20minutes each.After paraffin infiltration the liver pieces were subjected to automatictissueprocessingunit.

b) Embedding in paraffin Vacuum :

Hard paraffin was melted and waspouredintoL-shapedblocks.Theliverpieceswerethendropped intothe moltenparaffinquicklyandallowed tocool.

c) Sectioning: The blocks were cut using microtome to get sections ofthickness of 5µ.The sections were taken on a micro slide on which eggalbumin i.e., stickingsubstance was applied. The sections were allowedto remain in an oven at 60^{0}C for 1hour. Paraffin melts and egg albumindenatures, therebyfixing tissue to slide.

Statisticalanalysis

The obtained results were analysed for statistical significance using one way ANOVA followed by Dunnet "**t**" test using the graph pad prism statistical software for comparison with control group and acetaminophen treated group. $P < 0.05$ was considered as significant.

III. RESULTS

HEPATOPROTECTIVE ACTIVITY OF*CYPTOLEPISBUCHANANII*ROOTSEXTRACTS.

CCl_4Inducedmodel (Table no-8&9, figure no-2):

Alcohol(Ethanol)andaqueousextractsat400mg/kgbodyweightshowed significantactivity it reducing the levels of theAST, ALT,ALP and Total protein content in theblood level.

Table No-8:- Results oftheCCl$_4$Model(SGOT, SGPT, ALPlevels)

GROUP	TEATMENT	DOSE	SGPT levels(U/L) (Mean±SEM) AST	SGOT levels(U/L) (Mean± SEM) ALT	ALP levels(U/L) (Mean±SEM)
1	**Normalcontrol**	5ml/kg,p.o.	30.14 ±1.408	42.49± 1.372	33.74±1.454
2	**Toxicantcontrol**	0.5ml/kg,i.p +ccl$_4$	175.8±2.426	168.6 ± 1.088	146.2±2.259
3	**STANDARD** (Silymarin)	100 mg/kg,p.o+ccl$_4$	52.60±1.249***	52.54±1.037***	47.74±0.8334***
4	**EECB**	200 mg/kg,p.o+ccl$_4$	123.8±1.423*	83.44 ± 2.242*	86.92±2.082*
5	**EECB**	400 mg/kg,p.o+ccl$_4$	60.74±0.9352***	56.66±1.359***	58.90±1.154***

6	AECB	200 mg/kg,p.o+ccl_4	138.6±1.105	111.3±2.040	127.0±1.127
7	AECB	400 mg/kg,p.o+ccl_4	87.04±1.230**	72.50±1.207**	97.54±1.002**

Valuesaremean±S.E.M.,n=6animalsineachgroup.Symbolsrepresentstatisticalsignificance.*p<0.05,ascomparedto CCl4-intoxicatedgroup.**p<0.01,ascomparedtoCCl4-intoxicatedgroup.***p < 0.001,ascomparedto CCl4-intoxicated group usingonewayANOVA followed byDunnett's test.

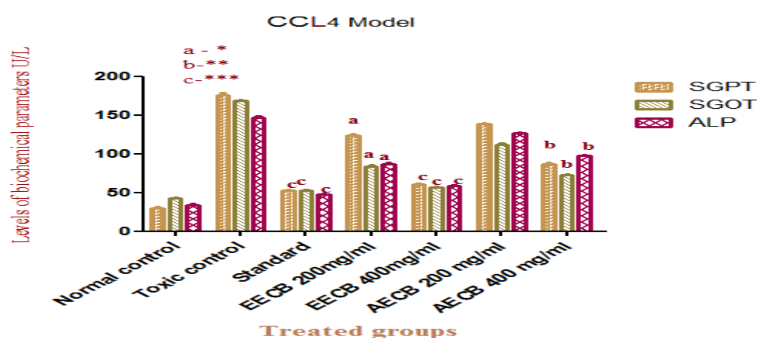


Fig No-2 :Effect of ethanolic (alcoholic)and aqueous extracts of *Cryptolepisbuchananii*onSGOT,SGPTandALP levelsin CCl4inducedhepatotoxicity.

Table no-9:-ResultsoftheCCl4model(TotalandDirectBilirubinLevel)

GROUP	TEATMENT	DOSE	Directbilirubinlevels(mg /dl)(Mean±SEM)	Total bilirubinlevels (mg/dl)(Mean±SEM)
1	Normalcontrol	5ml/kg,p.o.	0.2900±0.007071	0.3560±0.01568
2	Toxicantcontrol	0.5ml/kg, i.p +ccl_4	0.9020±0.01463	1.756±0.05259
3	Standard (Silymarin)	100 mg/kg,p.o+ccl_4	0.3840±0.01288***	0.5080±0.01497***
4	EECB	200 mg/kg,p.o+ccl_4	0.5180 ±0.0220**	0.8540±0.01435*
5	EECB	400 mg/kg,p.o+ccl_4	0.3300± 0.02025***	0.5140 ±0.01568***
6	AECB	200 mg/kg,p.o+ccl_4	0.6760±0.01860*	0.9300±0.0200
7	AECB	400 mg/kg,p.o+ccl_4	0.4420±0.0220**	0.6420±0.02035**

Values are mean±S.E.M., n = 6 animals in each group. Symbols representstatisticalsignificance. *p<0.05,ascomparedtoCCl4-intoxicatedgroup.**p < 0.01, as compared to CCl4-intoxicated group.*** p < 0.001, as comparedto CCl4-intoxicated group us ing one way ANOVA followed by Dunnett'stest. (Table no-9, Fig no-3)

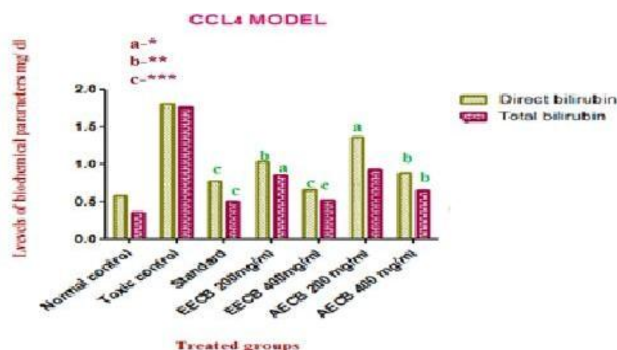


Fig. No3 :Effect of etanolic and aqueous extracts of *Cryptolepisbuchananii*onDBandTBlevelsin CCl_4inducedhepatotoxicity.

Table no-10:-Resultsof theCCl4 model(Totalprotein)

GROUP	TEATMENT	DOSE	Total proteinlevels (gm/dl)(Mean±SEM)
1	Normalcontrol	5ml/kg,p.o.	6.960±0.3234

2	Toxicant control	0.5ml/kg,i.p	3.480±0.1715
3	Standard (Silymarin)	100mg/kg,p.o	5.260±0.1435***
4	EECB	200mg/kg,p.o	4.180±0.1068*
5	EECB	400mg/kg,p.o	5.160±0.06782***
6	AECB	200mg/kg,p.o	3.840±0.1208
7	AECB	400mg/kg,p.o	4.580±0.1715**

Values are mean±S.E.M., n = 6 animals in each group. Symbols representstatistical significance**.** * $p < 0.05$, as compared to CCl4-intoxicated group.**$p < 0.01$, as compared to CCl4-intoxicated group.*** $p < 0.001$, as comparedto CCl4-intoxicated group us ing one way ANOVA followed b yDunnett'stest.(table no-10, fig no-4)

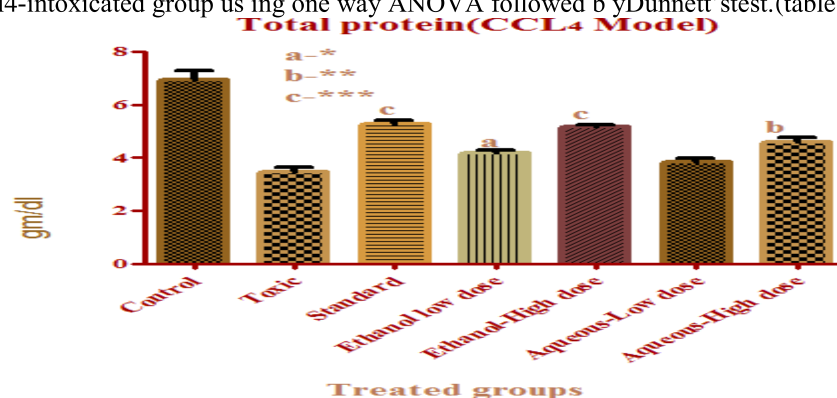


Fig. No-4 :Effect of ethanolic and aqueous extracts of *Cryptolepisbuchananii*onTPlevels in CCl_4inducedhepatotoxicity.

ParacetamolInducedHepatotoxicity:
Alcohol(Ethanolic)andaqueousextractat400mg/kgbody weightshowed significantactivity it reducing the levels of theAST, ALT,ALP and Total protein content in theblood level.

Table no-11:-Resultsof theParacetamolModel(SGOT,SGPT,ALPlevels)

GROUP	TEATMET	DOSE	SGPT levels(U/L) (Mean±SEM) AST	SGOT levels(U/L) (Mean±SEM) ALT	ALP levels(U/L) (Mean±SEM)
1	Normalcontrol	5ml/kg,p.o	51.76±1.474	34.28±1.414	43.26 ±1.546
2	Toxicantcontrol	Paracetamol- 2gm/kg, p.o	207.1±1.843	180.8±1.459	143.0± 1.342
3	Standard (Silymarin)	100mg/kg,p.o +Paracetamol	62.48±1.273***	45.74±1.039***	54.54± 1.185***
4	EECB	200mg/kg,p.o +Paracetamol	113.7±1.594*	126.1±1.721*	95.04 ±1.614*
5	EECB	400mg/kg,p.o +Paracetamol	71.78± 0.937***	50.54± 2.554***	62.62 ±1.049***
6	AECB	200mg/kg,p.o +Paracetamol	133.2±4.842	145.3±1.808	116.7± 1.857
7	AECB	400mg/kg,p.o +Paracetamol	96.46±1.664**	96.76±2.624**	82.32± 2.096**

Valuesaremean±S.E.M.,n=6animalsineachgroup.Symbolsrepresentstatisticalsignificance**.***$p<0.05$,ascomparedto

Paracetamol-intoxicatedgroup.**p<0.01,ascomparedtoParacetamol-intoxicatedgroup.***p<0.001,ascomparedtoParacetamol -intoxicated group us ing one way ANOVA followed b yDunnett'stest.(table no-11, fig no-5)

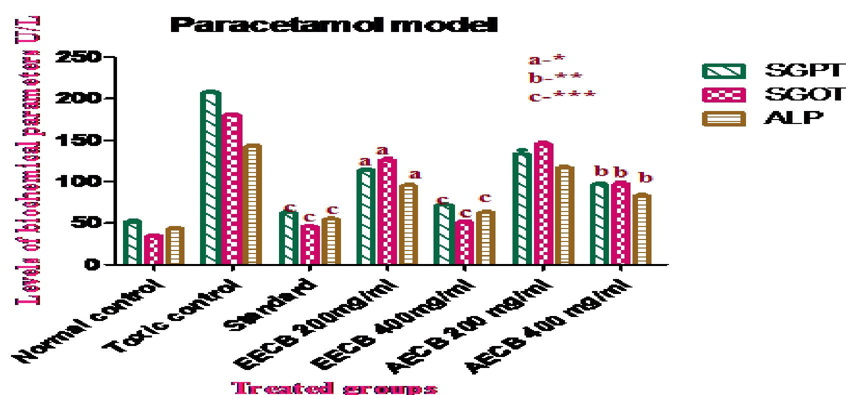


Fig. No.5:Effect of ethanolic and aqueous extracts of *Cryptolepisbuchananii*on SGOT,SGPT and ALP levels in Paracetamolinducedhepatotoxicity

Table no-12:-Resultsof theParacetamolGroup(TotalandDirectBilirubin Level)

GROUP	TEATMENT	DOSE	Directbilirubinlevels(mg/dl)(Mean±SEM)	Total bilirubinlevels (mg/dl)(Mean±SEM)
1	**Normalcontrol**	**5ml/kg,p.o**	0.3800±0.01414	0.4280±0.0102
2	**Toxicantcontrol**	**Paracetaml -2gm/kg,p.o**	1.304±0.1068	2.180±0.1985
3	**Standard** (Silymarin)	**100mg/kg,p.o +Paracetamol**	0.486±0.02315***	0.6020±0.0086***
4	**EECB**	**200mg/kg,p.o +Paracetamol**	0.5820±0.01281*	0.9080±0.0220*
5	**EECB**	**400mg/kg,p.o +Paracetamol**	0.3520±0.01828***	0.5400±0.0187***
6	**AECB**	**200mg/kg,p.o +Paracetamol**	0.7960±0.03219	0.9540±0.0172
7	**AECB**	**400mg/kg,p.o +Paracetamol**	0.6400±0.01871**	0.7160±0.0150**

Valuesaremean±S.E.M.,n=6animalsineachgroup.Symbolsrepresentstatisticalsignificance.*p<0.05,ascomparedtoParacetamol-intoxicatedgroup.**p<0.01,ascomparedtoParacetamol-intoxicatedgroup.***p<0.001,ascomparedtoParacetamol -intoxicated group us ing one way ANOVA followed b yDunnett'stest.(table no-12, fig no-6)

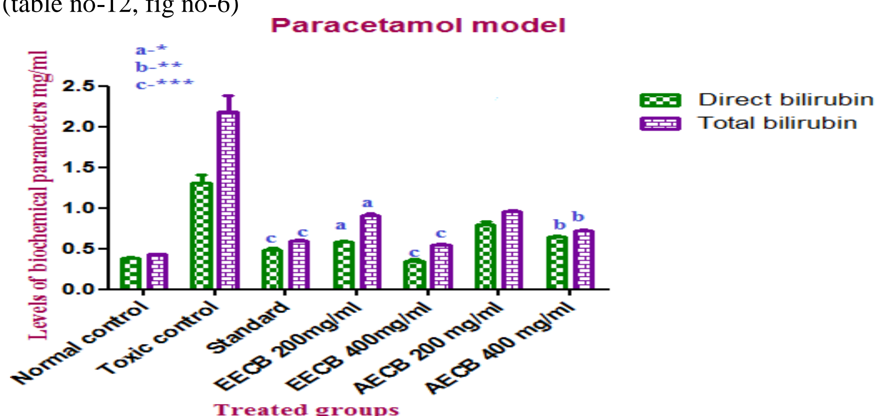


Fig. No.6:Effect of ethanolic and aqueous extracts of *Cryptolepisbuchananii*onDB andTBlevelsinParacetamolinducedhepatotoxicity

Table no-13:-ResultsoftheParacetamolGroup(Totalprotein)

GROUP	TEATMENT	DOSE	Total

			proteinlevels(gm/dl)(Mean±SEM)
1	**Normal control**	**5ml/kg,p.o**	8.140 ±0.1536
2	**Toxicantcontrol**	**Paracetamol -2gm/kg,p.o**	4.400 ±0.1304
3	**Standard** (Silymarin)	**100mg/kg,p.o +Paracetamol**	5.880 ±0.1158***
4	**EECB**	**200mg/kg,p.o +Paracetamol**	4.434 ±0.1370*
5	**EECB**	**400mg/kg,p.o +Paracetamol**	5.644 ±0.1112***
6	**AECB**	**200mg/kg,p.o +Paracetamol**	3.724 ±0.2004
7	**AECB**	**400mg/kg,p.o +Paracetamol**	4.514±0.1135**

Valuesaremean±S.E.M.,n=6animalsineachgroup.Symbolsrepresentstatisticalsignificance.*p<0.05,ascomparedtoParacetamol-intoxicatedgroup.**p<0.01,ascomparedtoParacetamol-intoxicatedgroup.***p<0.001,ascomparedtoParacetamol -intoxicated group us ing one way ANOVA followed b yDunnett'stest..(table no-13, fig no-7)

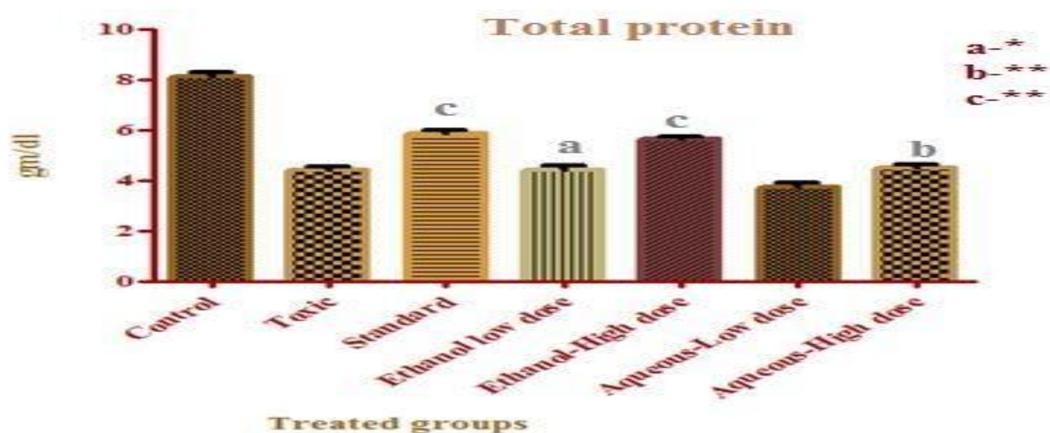
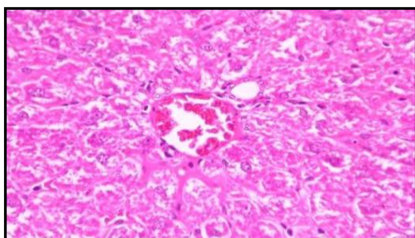


Fig. No.7:Effect of etanolic and aqueous extracts of*Cryptolepisbuchananii*TPlevelsinParacetamol induced hepatotoxicity.

HISTOPATHOLOGYOFLIVERSINCCL4 INDUCEDGROUP:



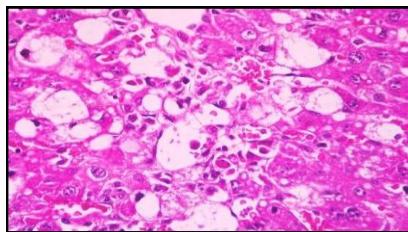
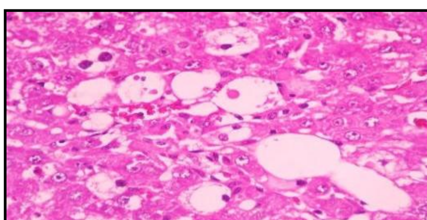


Fig. No. 8:Liver section of group 1**Fig No.9**Liver section of group 2 rat
rat(normal)in CCl4Group(CCl_4)

Thehepaticparenchyma,sinusoids, central vein andportal triad appear withinnormallimits.

Effacedarchitecture,Macroand Microsteatosis,Scatteredmononu clearinflammatoryinfiltration.



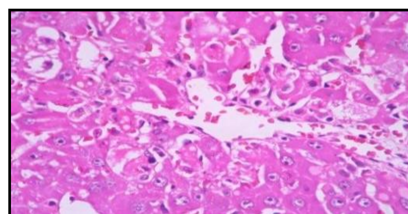
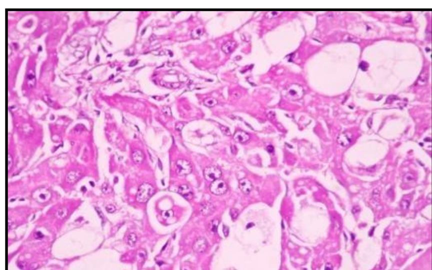


Fig.No.10 : Liver section of group 3**Fig. No. 12**: Liver section of group 5 rat
rat(Silymarin) (Ethanolic400mg/kg)inCCl4Group

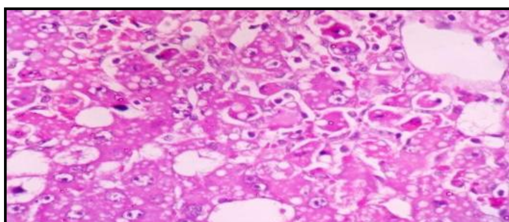
Partiallyeffacedarchitecture,Macro steatosis andMicrosteatosis, Dilated andcongested sinusoids,near tonormal.

Focal disrupted architecture,Macrosteatosis andmicrosteatosis, Regenerativehepatocytes.



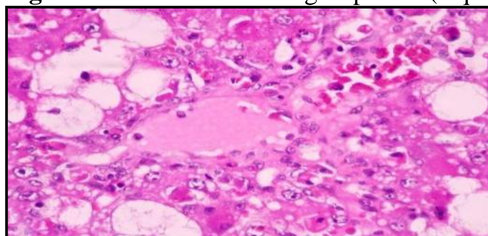
Partially effaced architectureMacrosteatosisandMicrosteatosis**,**Regenerativehepatocytes

Fig.No11:Liversectionofgroup4
rat(Ethanolic200mg/kg)inCCl4 Group



Partially effaced Architecture,Macrosteatosisan dMicrosteatosis,Degenerative andRegenerativehepatocytes.

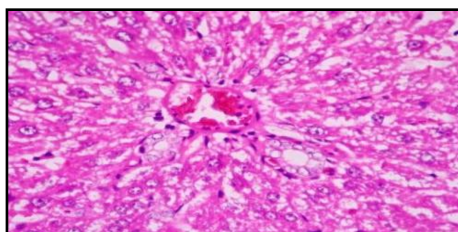
Fig. No.13:Liver section of group 6 rat (Aqueous200 mg/kg)in CCl4Group



Partially effected Architecture, macrosteatosis and Microsteatosis, Regenerative

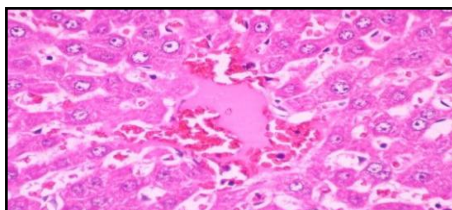
Fig.No.14: Liver section of group 6 rat (Aqueous400mg/kg)inCCl4Group

HISTOPATHOLOGYOFLIVERSINPARACETAMOLINTOXICATEDGROUP:



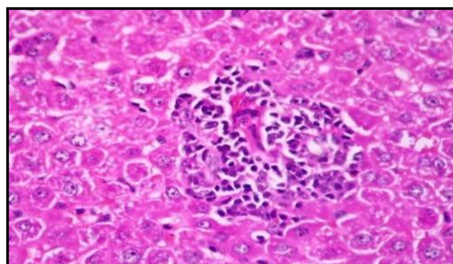
The hepatic parenchyma,sinusoids,centralv einandportal triad appear withinnormallimits.

Fig. No. 15:Liver section of group 1 rat(Normal)inParacetamolgroup.



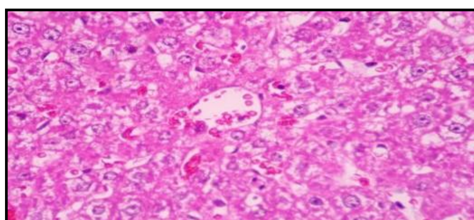
Effaced architecture, Focalaggregates of mononuclearinflammatory infiltration

Fig. No. 16,32: Liver section of group 2 rat(Paracetamol induced) in Paracetamolgroup.



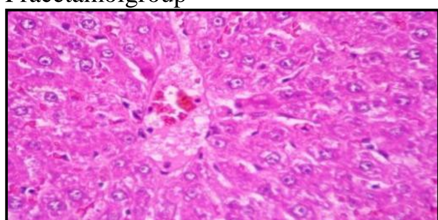
Intact architecture,dailatedand congested sinusoids,Focalaggregates ofmononuclear inflammatoryinfiltration

Fig. No.17, 33: Liver section of group 3 rat(Silymarin)inParacetamol group



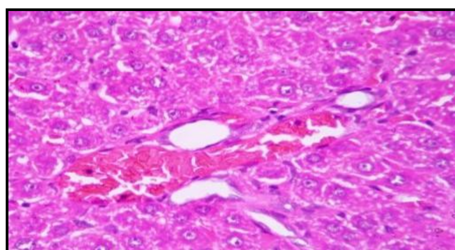
Intact architecture,degenerativehepatocytes,mononuclear inflammatoryinfiltration.

Fig. No.18, 34: Liver section of group 4 rat(Ethanolic 200 mg/kg) in Pracetamolgroup



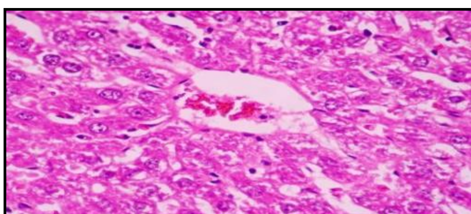
Intact architecture,degenerativeandregenerativehepatocytes

Fig. No.19 35: Liver section of group 5 rat(Ethanolic400mg/kg)inParactamolgroup



The sinusoids and fewcentral veins appear dilatedand congested. The hepaticparenchymaandportaltriad appear within limits.

Fig. No.20 36: Liver section of group 6 rat(Aqueous200mg/kg)in Paracetamolgroup



Thehepaticparenchyma,sinusoids, central veinsand portal triad appearwithin normal limits.

Fig. No. 21,37: Liver section of group 7 rat(Aqueous400mg/kg)in**Paracetamolgroup**

IV. DISCUSSION

In the traditional medicine system, many plants and herbs are claimed to have Antioxidant and hepatoprotective efficacy without any scientific basis. The aim of the present study was to carry phytochemical investigation and evaluation of roots of Cryptolepisbuchananii for Antioxidant and Hepatoprotective activity.

Fluorescence analysis of the dried root powder on treatment with various reagents showed no fluorescence both in short (254nm) and long (366nm) wavelengths. The fluorescent behavior of the powdered drug in different solutions towards ordinary light and ultraviolet light (both long and short wavelengths) gives an idea about various phytoconstituents present in plant drugs.

Various extracts of Cryptolepisbuchananii were subjected to phytochemical investigation and ethanolic and aqueous extracts revealed the presence of alkaloids, glycosides, saponins, and phytosterols.

EstimationofSGPT:

SGPT is a cystosolic enzyme primarily present in liver; the levels ofSGPT in serum increases due to cleavage of this cellular enzyme intoplasma by CCL_4-induced hepatic injury.Serum levels of SGPT canincrease due to damage of the tissues producing acute hepatic necrosissuch asviralhepatitisandacutecholestasis.Alcoholic liver damageand cirrhosis also can be associated with mild to moderate elevation oftransaminase.Alcoholicextractandaqueousextractsat400 mg/kgdose significantlyreduced the SGPT level almost the same as that ofstandard,compared to the CCl4 and Paracetamol-treated group.

EstimationofALP:

In case of liver toxicity, alkaline phosphatase (ALP) levels are very high, whichmaybeduetodefectivehepatic,excretionorbyincreasedproduction of ALP by hepatic parenchymal or duct cells. Alcoholicextract and aqueous extracts at 400 mg/kg dose significantlyreducedthe ALP level almostthe same as that of standard, compared to the CCl4 andParacetamol-treatedgroup.

Estimation of serumbilirubin:

Intoxicliver,elevated bilirubinlevelscanresult from impaired hepatic uptake of unconjugated bilirubin. such asituationoccursingeneralizedlivercallinjury.certaindrugs,ex:rifampicin;probenecideinterferewithnetuptakeofbilirubinby theliver cell and may produce Bilirubin levelsalsoriseindiseasesofhepatocytes,obstructiontobiliary excretion into the duodenum ,in haemolysis and defects in hepaticuptake and conjugation of bilirubin pigment such as Gilbert's disease.Alcoholic extract and aqueous extract at 400 mg/kg dose significantlyreducedthebilirubinlevelalmostt he sameasthatofstandard,comparedtoCCl4 and Paracetamol-treatedgroup.

Totalprotein: Livercellssynthesizealbumin,fibrinogen,prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin,transferrin,alphafoetoproteinsandacutephasereactantproteins.Theblood levels of these plasma proteins are decreased in extensive liverdamage.Alcoholicextractandaqueousextractof400mg/kgdosesignificantly

increasedthebilirubinlevelalmostsameasthatofstandard,compared to CCl4and Paracetamol-treated group.

HISTOPATHOLOGICALSTUDY:

The alcoholicandaqueous extracts of 400 mg/kg p.o wereable toretainthecellarchitecturecompletelyandalsoabletoregenerateheptocyte cells. The alcoholicand aqueous extracts of 200 mg/kg p.owere able to retain the cell architecture, degenerativehepatocytesandalso regenerativecells.Fromtheabovestudyweconcludethatalcoholicandaqueousextracts at 400 mg/kg p.o. as showed significant activity, comapared toalcoholicand aqueous extracts of 200 mg/kg p.o. roots of *Cryptolepisbuchananii*.Alcoholic extract of roots*Cryptolepisbuchananii*at dose 400mg /kgshowedmore significant reduction in the level of serum enzymesandincrease in TPas compared with aqueous extrat at dose of 400mg/kg. Theseobservationssuggestthatthealcoholicextracthasmorehepatoprotectiveactivity.

Theactivephytochemicalconstituentslikealkaloids,glycosides,saponin and phytosterols present in roots of *Cryptolepisbuchananii*mayberesponsible forAnti-oxidant and hepatoprotectve activity.

Thepresentstudysupportstheethnomedicaluseof*Cryptolepisbuchananii*asanAntioxidantandhepatoprotectiveagent.Furtherexperimentationisneededtoisolateandunderstandtheprecisemechanismofactionresponsibleforthe Antioxidantandhepatoprotectiveactivity.

V. SUMMARY:

Alcoholic and aqueous extractsof the roots of*Cryptolepisbuchananii*werescreenedforhepatoprotectiveactivitybyCCl$_4$andParacetamolmodels. The acute LD$_{50}$ value for *Cryptolepisbuchananii*extractswasfoundtobesafeupto2000mg/kgp.o.Theresultsofhepatoprotective activity of*Cryptolepisbuchananii*revealed thatadministration of alcoholic extract at a dose of 400 mg/kg body weightshowed a more significant decrease in the level of serum enzymes andincrease in TP level as compared to the standard and toxic(CCL$_4$ andparacetamol)inducedgroup.

VI. CONCLUSION:

Inthepresentstudy,driedrootsof*Cryptolepisbuchananii*weresubjected to extraction by using Petroleum ether, Chloroform, Acetone,Alcohol, and Water. The phytochemical investigation of alcoholic andaqueous extracts of *Cryptolepisbuchananii*showed the presence ofalkolides,glycosides, saponins,phytosterols.

In the in-vitro Antioxidant studies, the alcoholic extract and to lesserextent aqueous extract of roots of *Cryptolepisbuchananii*has showedsignificantAnti-oxidant activity.

The alcoholic and aqueous extracts at doses of 200 and 400 mg/kgwere evaluated for hepatoprotectective activity usingserum marker enzymeslike SGOT,SGPT,ALP ,total bilirubin,direct bilirubin , total proteinandhistolpathology.

Both the extracts showed dose dependanthepatoprotective activity. Incomparison, alcoholic extract at a dose level of 400 mg/kg possessesstatisticallysignificantlyhepatoprotectectiveactivityandiscomparable to

standard Silymarin, by reduction in elevated levels ofserum enzyme, bilirubin and increase in the total protein level. Thehistopathologicalstudysupportedtheseresultsbynormalizingthe$CCl_4$and Paracetamol-induced liver damage.

From this study, we can conclude that alcoholic extract has goodhepatoprotectiveactivitycomparedtoaqueousextract.Furtherdetailed investigations are needed to determine the Phyto-constituentswhicharerresponsibleforAnti-oxidantandhepatoprotectiveactivity.

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