Protocol I

DETERMINATION OF ANTIOXIDANT ACTIVITY OF TEST DRUG

Antioxidant activity

Experiment 1: Total phenolic contents
Experiment 2: Free radical scavenging activity (DPPH assay)
Experiment 3: \( \text{H}_2\text{O}_2 \) scavenging activity
Experiment 4: HPLC analysis
Protocol II

Experiment 5
To evaluate cell viability test by SRB assay

In vitro study
Hep-G2 cell line

Group I
Control (Vehicle only)

Group II
PA per se
(30, 50, 100 µg/ml)

Group III
PY per se
(30, 50, 100 µg/ml)

Group IV
S per se
(30, 50, 100 µg/ml)

Group V
ATD (60 µM)

Group VI
ATD+ PA
(30, 50, 100 µg/ml)

Group VII
ATD+ PY
(30, 50, 100 µg/ml)

Group VIII
ATD+ S
(30, 50, 100 µg/ml)

SRB Assay
After 24 hr

@540 nm by Robotic ELISA Reader
Protocol III

Experiment 6

Screening of PA

The whole set of experiment was divided into various groups of six animals each. Animals were administered anti TB drugs for 8 weeks (3 alternative days in a week). PA was given for 8 weeks (3 alternative days in a week considering every next day of anti-TB drug treatment). Groups were divided as follows.

Female albino rats
(160 ± 10g b.w.)

Group I
Control
(Vehicle only)

Group II
PA Per se
(400 mg/kg, p.o.)

Group III
ATD (INH+RIF+PZA+ETH)
(70+52+175+140 mg/kg b.w.p.o.)
8 weeks (3 alternative days/week)

Group IV
ATD + PA
(100 mg/kg, p.o.)

Group V
ATD + PA
(200 mg/kg, p.o.)

Group VI
ATD + PA
(300 mg/kg, p.o.)

Group VII
ATD + PA
(400 mg/kg, p.o.)

Group VIII
ATD + S
(50 mg/kg, p.o.)

Necropsy after 24h

Blood Biochemistry
Lipid Profile
Tissue Biochemistry
Histopathology

AST  UREA
ALT  CREATININE
SALP  BUN
BILIRUBIN
ALBUMIN

TRIGLYCERIDES
CHOLESTEROL
LPO  SOD
GSH  CAT
ATPase
LM
**Experiment 7**

**Screening of PY**

The whole set of experiment was divided into various groups of six animals each. Animals were administered anti TB drugs for 8 weeks (3 alternative days in a week). PY was given for 8 weeks (3 alternative days in a week considering every next day of anti-TB drug treatment). Groups were divided as follows.

![Diagram with experiment setup]
The whole set of experiment was divided into various groups of six animals each. Animals were administered anti TB drugs for 8 weeks (3 alternative days in a week). PA and PY were given for 8 weeks (3 alternative days in a week considering every next day of anti-TB drug treatment). Groups were divided as follows.
PROTOCOL V

EVALUATION OF SAFETY PROFILE OF SELECTED DRUG

Experiment 9:  Choleretic activity

Experiment 10:  Antipyretic activity
PROTOCOL VI

Experiment 11

Molecular mechanism of recovery

The whole set of experiment was divided into various groups of six animals each. Animals were administered anti-tuberculosis drugs for 12 weeks (3 alternative days in a week) followed by therapy with PA (3 alternative days in a week considering every next day of anti-TB drugs treatment for 12 weeks). Groups were divided as follows.

Female albino rats
(160 ± 10g b.w.)

Group I
Control
(Vehicle only)

Group II
PA per se
(300 mg/kg, p.o.)

Group III
ATD (INH+RIF+PZA+ETH)
(70+52+175+140 mg/kg b.w.p.o.)
12 weeks(3 alternative days/week)

Group IV
ATD + PA(300 mg/kg, p.o.)
3 days/week for 12 weeks

Group V
ATD + S (50 mg/kg, p.o.)
3 days/week for 12 weeks

Necropsy after 24h

Serological Estimations
AST
TRIGlycerides
ALT
CHOlesterol
SALP
LDH
BILIRUBIN
ALBUMIN

Liver
G6Pase
ATPase

Kidney
Histopathology
URA
E
ACID
CREATININE
BUN

GSH Cycle
GR
GPx
GST

Oxidative stress Markers]
LPO
MLPO
MGSH
GSH
SOD
CAT

Drug Metabolizing Enzymes
AH
AND
CytP450

Inflammatory Cytokines
IL-6
TNF α

DNA Damage
COMET ASSAY