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Supporting Science for Safety

TOXPeer Preclinical Consultancy Services LLP

GPR120 Agonist Program for NASH Indication

2022

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Non-Confidential Slide Deck

EXECUTIVE SUMMARY

- A GPR120 agonist program with small molecules, primarily for the treatment of NASH
- Five potential GPR120 agonist molecules are being evaluated from four scaffolds from which about 954 compounds synthesized
- A potential molecule, Compounds 1 showed positive results in well validated *in-vivo* DIO-mouse models which confirm its potential in the treatment of NASH
- Compound 1, is showing excellent safety margin in 14 days repeat-dose study in rats
- Three back-up compounds identified which have shown better *in-vitro* profile in TNF-a and MCP-1 assays.
- IND-enabling studies are in progress
- Patent status: PCT applications are in place

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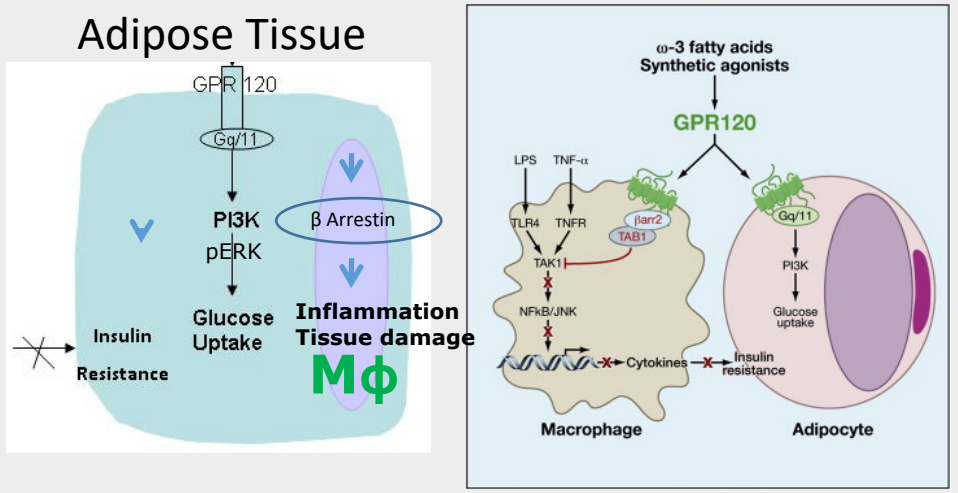
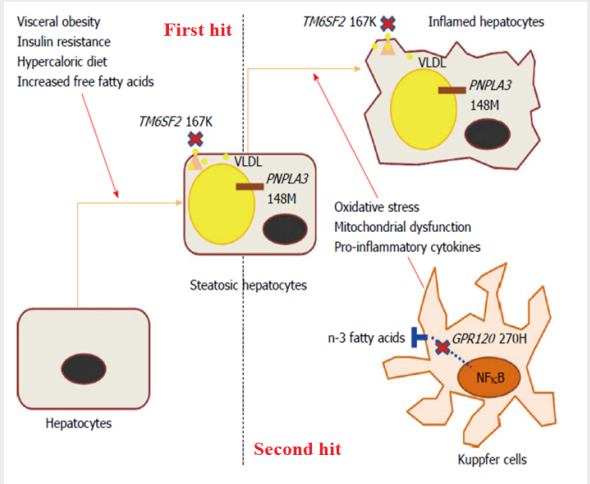
SUMMARY

- Selected molecules exhibit the following:
  - LogP/ LogD <4 and molecular weight <450
  - Moderately release GLP1 and promote ERK phosphorylation in STC1 cells
  - Reasonable selectivity (screened against 74 receptors)
  - Ability to promote Glut4 translocation and phosphorylation of ERK in adipocytes
  - Ability to switch M1 pro-inflammatory macrophages into M2 anti-inflammatory macrophages, an essential step to combat insulin resistance
  - Preclinical proof-of-concept established (improved glucose tolerance, trend towards improved insulin sensitivity, body weight reduction)
  - Potential for the treatment of NASH, obesity, lipid metabolism abnormalities, T2D to improve glucose tolerance, decrease hyperinsulinemia, and increase insulin sensitivity
- Three back-up compounds identified which have shown better *in-vitro* profile in TNF-a and MCP-1 assays.
- PK studies of back-up compounds are in progress.

GPR120 Mechanism of Action

- Member of the GPCR family, expressed in intestinal tract, taste buds, lungs, adipocytes, kupffer cells and on macrophages
- Receptor for omega-3 fatty acid such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)
- Engagement of receptor may result in both improvement in insulin sensitivity and enhancement of β-cell function
- Reported to mediate anti-inflammatory effects of DHA and EPA in macrophages
- Oxidative stress is involved in the second hit leading to the progression to NASH because of its harmful action on steatosic hepatocytes. The GPR120 270H allele, reducing the anti-inflammatory action of the GPR120 receptor expressed by Kuppfer cells, is involved in the second hit promoting the oxidative stress, mitochondrial dysfunction and pro-inflammatory cytokines release

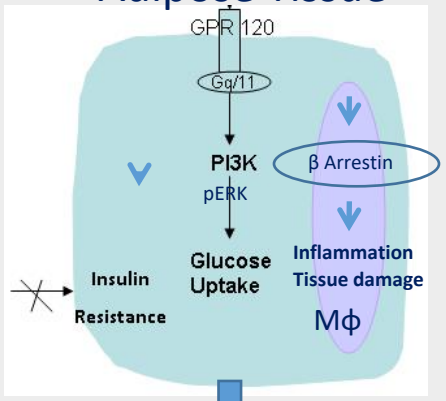




World J Hepatol 2015 June 18; 7(11): 1439-1443

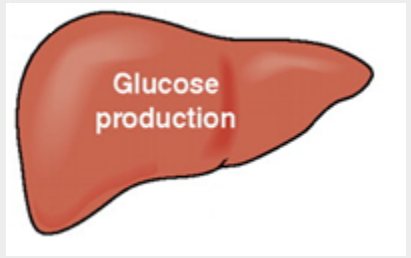
GPR120 Mechanism of Action

Adipose Tissue



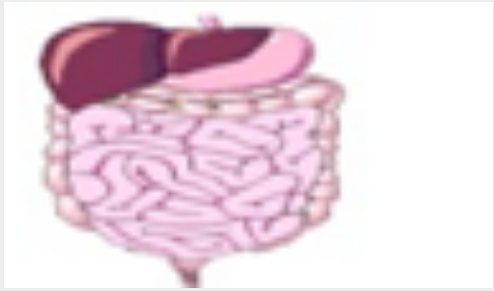
GPR120 agonist
- Enhance Glucose uptake
- Improve Insulin Sensitization
- Prevent obesity induced Adipose tissue injury and Insulin resistance
- Promote Adipocyte differentiation and Fat storage

Liver



GPR120 agonist
- Prevent obesity induced NAFLD
- Prevent inflammation of the Liver

Intestine

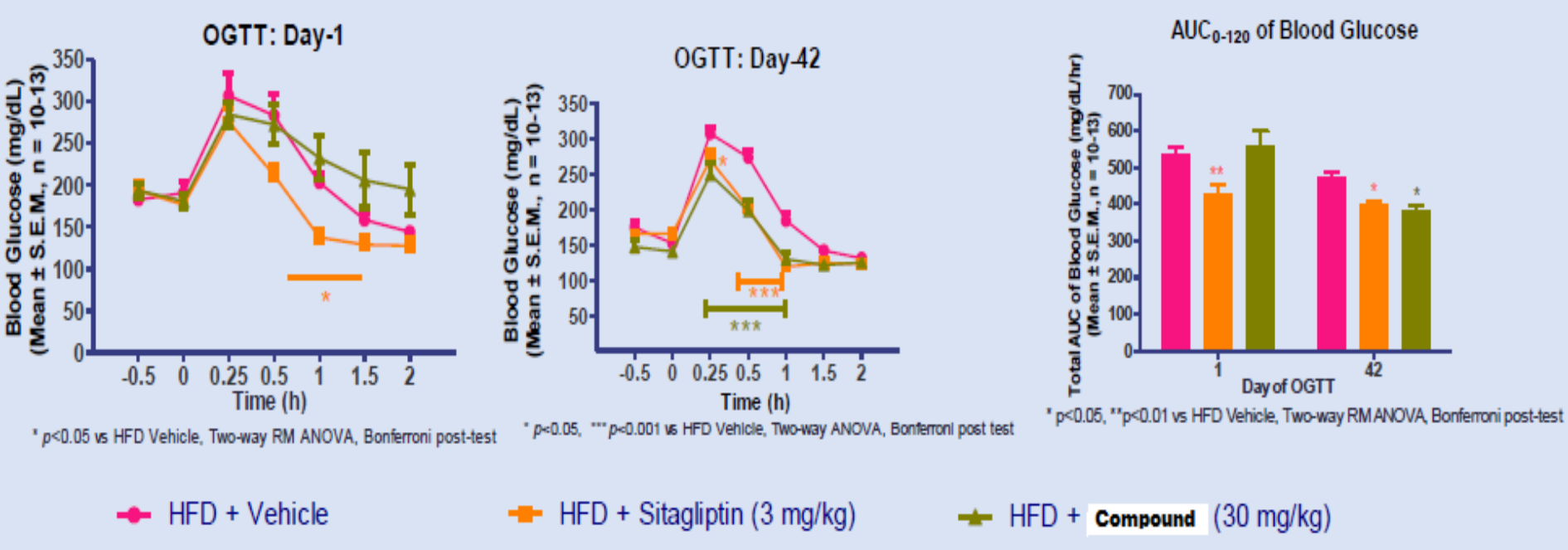


GPR120 agonist
- Increase GLP-1 release
- GLP-1 will increase insulin secretion from Pancreas
- Protect Beta Cell Function
- Glucose Lowering

GPR120 agonist has the potential for the treatment of NASH and metabolic disorders

GPR120 Agonist: PoC in DIO Mice

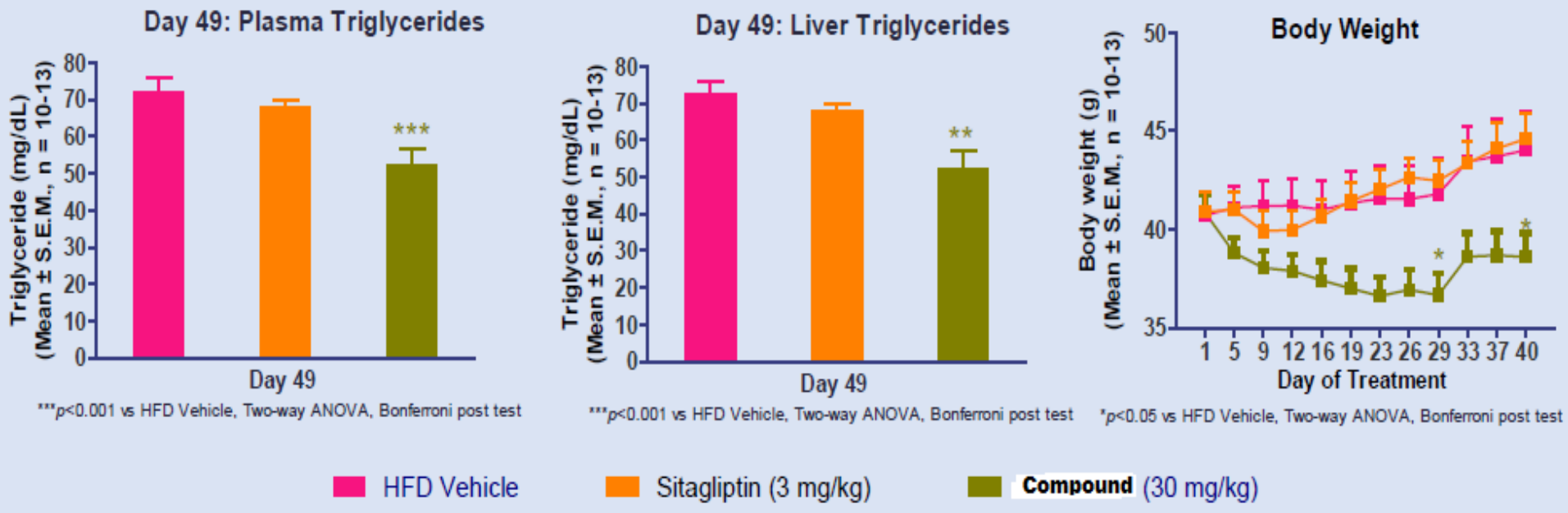
Study in DIO mice (b.i.d., 7-8 weeks)



Compound 1 improved glucose tolerance, reduced fasting blood glucose – indicative of potential use in type 2 diabetes

GPR120 Agonist: PoC in DIO Mice

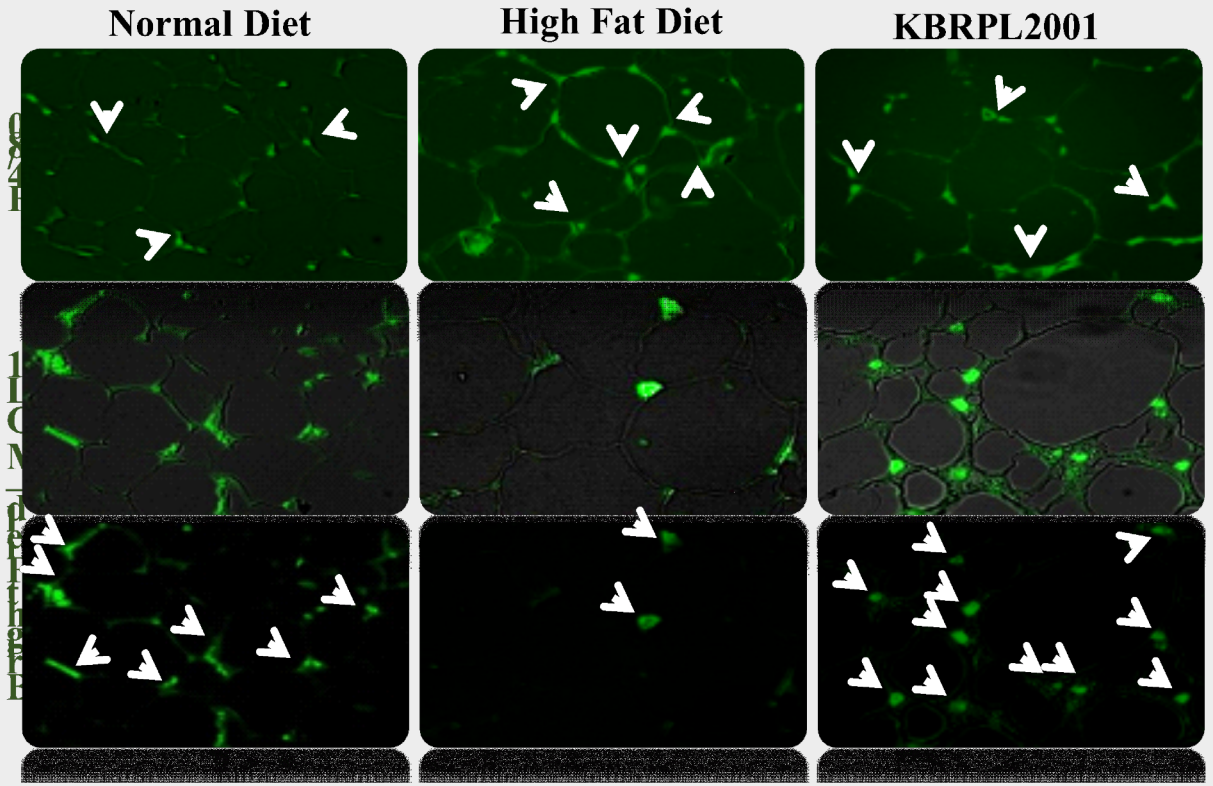
Study in DIO mice (b.i.d., 7-8 weeks)



- Compound 1 treatment exhibited reduction in
  - plasma and liver triglycerides – indicative of potential use in hepatic steatosis/NAFLD/NASH
  - body weight - in line with target hypothesis

PoC in DIO Mice

- Macrophage mediated tissue inflammation is the key mechanism for Adipose Inflammation
- Phenotypic switch in the polarization of adipose macrophages in DIO-mouse affects the insulin signaling and thereby reduces insulin resistance
- Reduction in adipose tissue macrophage (ATM) content in epididymal fat of HFD mice when treated with Compound 1 reflects decreased chemotaxis of ATMs or switch of ATMs from M1 (pro-inflammatory) to M2 (anti-inflammatory) –indicative of decrease in insulin resistance



F4/80 is a M1 marker. Increase in macrophage galactose-type C-type lectin 1 (MGL1), an anti-inflammatory marker, reflects phenotypic switch of ATMs from M1 to M2.

Thus, exhibited its potential use in diabetes, obesity and hepatic steatosis/NAFLD/NASH

in-vitro and *in-vivo* Summary

In-vitro : GLP-1 secretion in mouse colon cells

Compound name	GLP-1 (STC cells)
Compound 3	The compounds are able to secrete GLP1 from mouse colon cell line
Compound 6	
Compound 10	
Compound 13	
Compound 14	

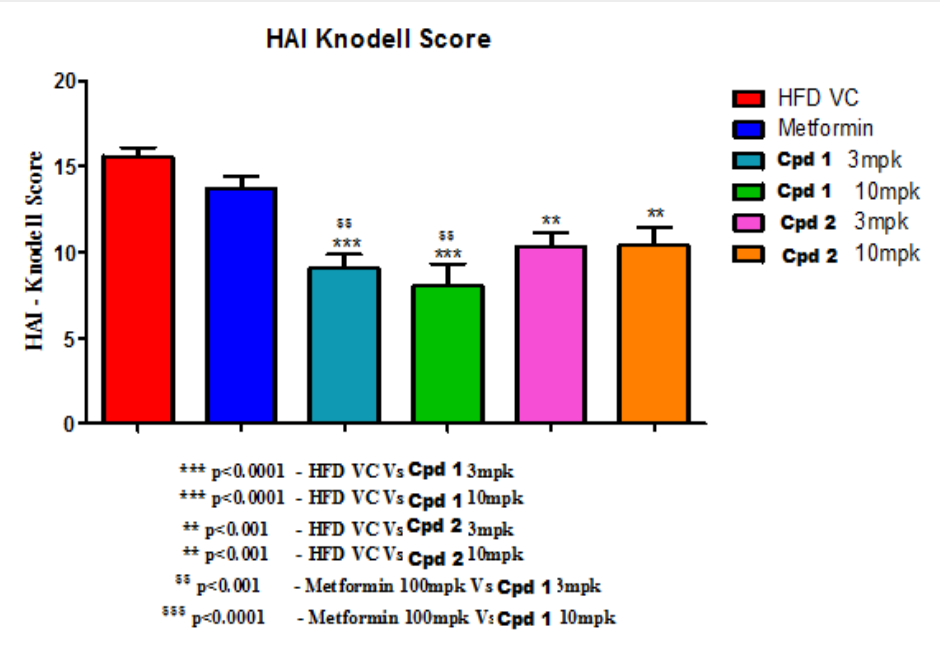
In-vivo : Reduction in blood glucose and triglyceride levels in DIO mouse model

Parameter	OGTT	$AUC_{fasting\ BG}$	Insulin	Triglyceride	Epididymal fat
Compound	Significant ↑	Significant ↓	↑ sensitivity	Significant ↓	Switch M1→M2

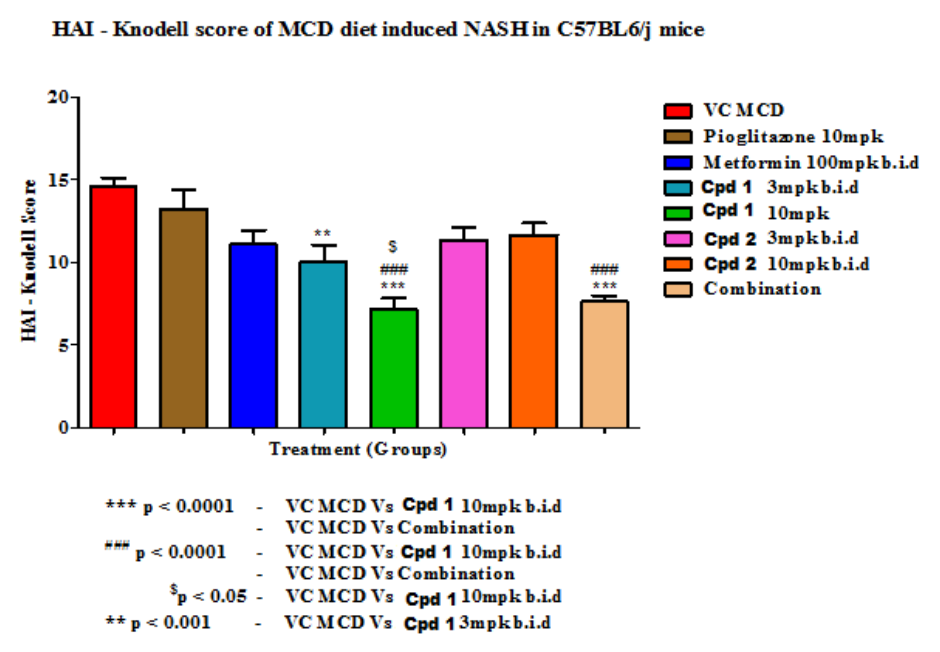
Initial leads show significant improvement in glucose tolerance and insulin sensitivity which may be mediated via pERK pathway in adipocytes; improvement in triglyceride levels which may be attributed to switch of ATM1 to ATM2

Efficacy in Mouse models of NASH

Histology (HAI-Knodell) Score – DIO study



Histology (HAI-Knodell) Score – MCD study



- Compound 1 at 10 mg/kg showed similar efficacy in with respect to histology scores (HAI-Knodell) in both HFD and MCD study
- In MCD study, efficacy of the combination (Compound 1 at 3 mg/kg + Pioglitazone 10mg/kg) is similar to that of Compound 1, 10 mg/kg group
- These results confirm the potential of Compound 1 and Compound 2 in the treatment of NASH

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MCD-NASH Model (Exp2)
Histopathology Scoring

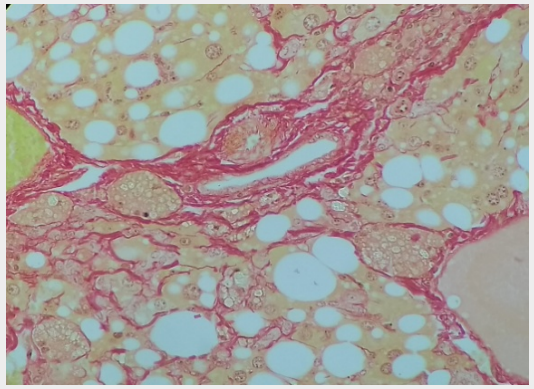
Treatment Groups	Mean	SD
Vehicle	6.30	1.08
Compound 1, 3 MPK; PO; bid	0.79	1.08
Compound 1, 10 MPK; PO; bid	0.75	0.91
Pioglitazone, 10 MPK; PO; o.d.	4.35	1.49
Metformin 100 MPK; PO; bid	5.3	1.26
Compound 1+ Pioglitazone (10 MPK, PO, bid. +10 MPK; PO; o.d.)	6.2	0.77
Normal Pallet Diet	0	0
Methionine-Choline Deficient (MCD) Diet	4.7	2.03

Histopathology Scoring is based on the grading of Steatosis, Lobular inflammation, Ballooning and Stages of Fibrosis.

Animals treated with Compound 1 at 3 mpk and 10 mpk showed a significant decrease in NASH score

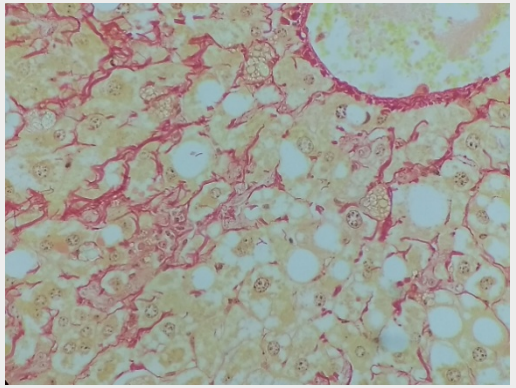
Photomicrographs of Liver

Sirus Red Staining, 40X

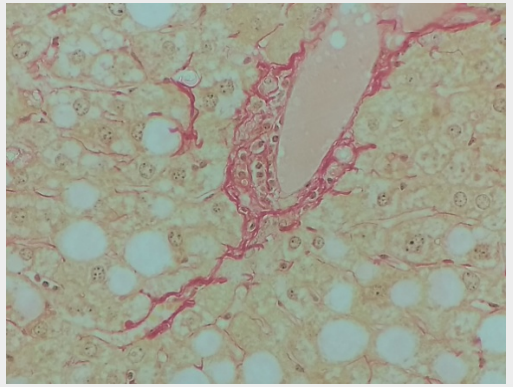


MCD Vehicle Control:

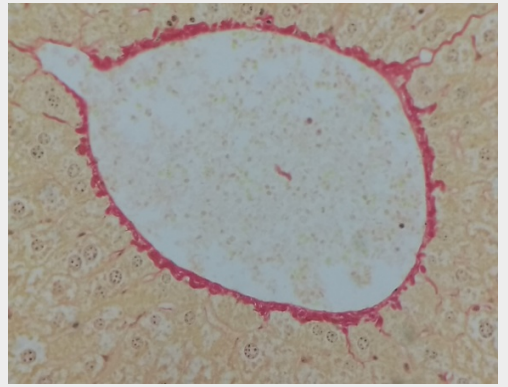
Showing hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis.



Metformin 100 MPK, p.o., b.i.d.:
Showing reduction in severity of hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis.



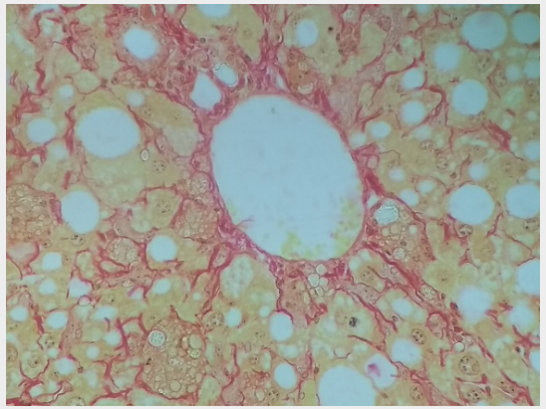
Compound 1: 3 **MPK**, p.o., b.i.d
Showing significant reduction in severity of hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis



Compound 1: 10 **MPK**, p.o., b.i.d:
Showing significant reduction in severity of hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis.

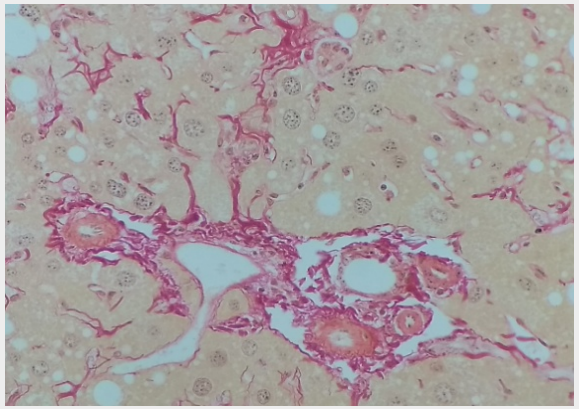
Photomicrographs of Liver

Sirus Red Staining, 40X

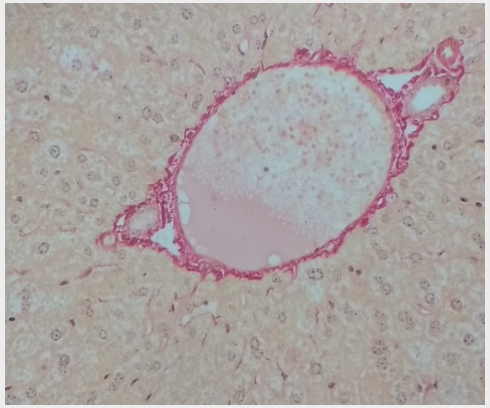


Pioglitazone: 10 **MPK**, p.o., o.d.:

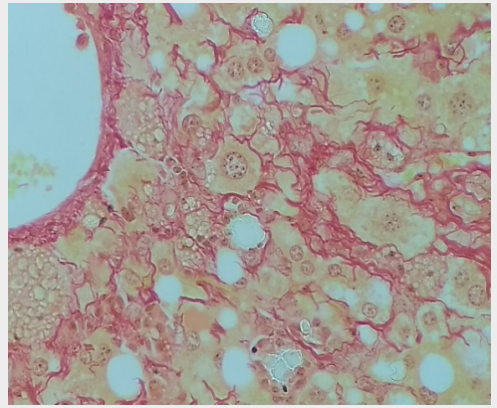
Showing reduced in severity of hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis.



Compound 1 + Pioglitazone: 10 **MPK**, p.o., b.i.d/o.d.:
Showing reduction in severity of hepatocellular Steatosis, ballooning, Inflammation and bridging fibrosis.



Normal Pallet Diet Control:
Showing normal periportal area



MCD Control:
Showing hepatocellular Steatosis, ballooning, Inflammation and fibrosis.

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