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PROTECTIVE EFFECT OF HONEY ON METANIL YELLOW INDUCED HEPATOTOXICITY IN RAT

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ABSTRACT

Present study has been undertaken to evaluate the effect of honey on Metanil yellow induced hepatotoxicity in rat. Metanil yellow has impaired various parameters such as liver weight, SGPT, SGOT, Alkaline phosphatase, total protein and the histological structure of liver significantly in all the experimental groups of animals. Significant recovery has also been observed after supplementation of honey to those experimental animals along with structural improvement of liver section.

KEYWORDS: Metanil yellow, Alkaline phosphatase, histology, necrosis and hepatotoxicity.

INTRODUCTION

Beautiful things are attractive. Nowadays this common term is also applicable for our foods. We are generally used to like the foods specially street foods which are beautiful to see. This common human psychology is exploited by preparing foods much more attractive using several chemicals. Among these chemicals, 'Metanil Yellow' is widely used dye for making various types of foods colourful and attractive.

Metanil yellow is a highly water soluble dye. It belongs to the Mono azo group of the dyes. Although the use of metanil Yellow as a colorant agent is not permitted, it is still widely used as a colorant in many food industries. It is extensively used in the developing countries as a colorant in sweet meat, ice-creams, soft drinks and beverages. Because of its Orange yellow colour, metanil yellow is also widely used in the coating of turmeric. It is extensively used in paper, leather and many textile industries as a dye and colorant for the wool. Moreover, it is also used as a colorant for lacquers and cosmetic products. Furthermore, the dye is highly suitable for the preparation of colored water fast inks and can also be used analytically for the determination of trace amounts of Mo.

Liver diseases are serious health problem in modern days. Autopsy studies done previously has declared that, 4.5% - 9.5% of common people are suffering from these kind of liver diseases which means, millions of people are facing this problem presently.[1-2]

Liver diseases may be recurred by applying various agents. Tamarindus indica pulp extract has useful effect as hypoglycemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal agent.[3-5] It has also been shown in earlier study that, Tamarindus indica leaf extract also has hepatoprotective role in paracetamol induced toxicity in albino rat.[6] It has been studied earlier that, hydroethanolic extract of Moringa oleifera flower has hepato protective role on acetaminophen induced hepato toxicity in rat.[7] Hepatoprotective activity of the ethanolic extract of *Nigella sativa* in paracetamol induced acute hepatotoxicity was studied in rats.[8] Metanil yellow promotes oxidative stress, astrogliosis, and apoptosis in the cerebellar cortex of adult male rat with possible protective effect of scutellarin.[9] Effect of chronic consumption of metanil yellow has been proved as toxic in developing and adult rats on brain regional levels of noradrenaline, dopamine and serotonin on acetylcholine esterase activity.[10] Metanil yellow and Malachite green have promoter effects on the development of hepatic preneoplastic lesions induced by N-nitrosodiethylamine (DEN). Tumor-promoting agents are not mutagenic but may alter the expression of genes whose products are associated with hyper proliferation, tissue remodeling, and inflammation.[11] It is interesting to note that oral treatment of Metanil yellow showed a greater response in cytosolic enzymes of hepatic tissue as compared to intestine.[12] Chemical structure of metanil yellow has been given below [Fig:1].

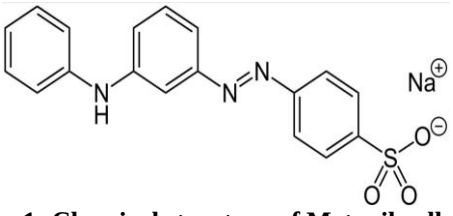

Fig: 1: Chemical structure of Metanil yellow.

The present study aims to investigate the protective effect of honey against Metanil yellow induced hepatotoxicity in rats.

MATERIALS AND METHODS

Experimental Animals

Adult female rats weighing **115-130 g** were used in the present study. Rats are kept in well designed and cleaned polypropylene cages. They were maintained under Normal conditions and fed a normal diet with free access to Water ad libitum.

Animal grouping and treatment

Rats were randomly divided into **5 groups** having **6 rats in each group.**

Group I: this group of animals was healthy normal Rats and serves as untreated control group for 28 days.

Groups II-III: animals of these groups were orally given Metanil yellow at a dose of **50 mg/kg (125mg in 25 ml)** and **200 mg/kg (500mg in 25 ml)** Body weight for 28 days.

Groups IV-V: animals of these groups were orally given Metanil yellow at a dose of **50 and 200 mg/** weight in addition with honey **5 mg/kg (12.5 mg in 25 ml)** body weight daily for 28 days.

At the end of the treatment, overnight fasted animals were sacrificed using diethyl ether, followed by cervical dislocation.

Animal sacrifice and measurement of parameters

At the end of the experimental duration of 28 days, the animals were weighed, anesthetized, and sacrificed.

The final body weights of all the rats were taken by the electronic balance. The rats were then anesthetized one after another with anesthetic ether followed by cervical dislocation and blood was collected directly from the hepatic portal vein and allowed to coagulate. Clear serum was collected and stored at 20°C for enzyme assay. Liver of each rat was dissected out and weights were taken with the help of electronic balance. Liver from each experimental animal was processed for histology and 5µ thick sections were taken and stained with hematoxylin and eosin[13] for further observation. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and serum alkaline phosphatase (ALP) were measured of all the control and experimental animals by the supplied standard kit ("COGENT," Clinical Chemistry division of Span Diagnostics Ltd.). The total serum protein was estimated by the Lowry method with a standard curve of BSA.[14]

Statistical Analysis

The statistical analysis was carried out by Student's "*t*" test[15] to generalize the results of various biochemical parameters of experimental groups in comparison to their respective control group and $P < 0.05$ was considered as a significant result.

RESULTS

Effect on Body Weight

The initial and final body weights of all rats in both control and treatment animals were presented in Table 1. There was a significant difference in the body weights gain% of all groups. There was a significant decrease ($p<0.05$) in the body weights of metanil yellow treated groups (groups II-III), low dose and high dose) when compared to control group. After 28 days Treatment of the rats with honey resulted significant increase in the final body weight in the groups IV and V.

Table 1: Results of body weight gain % of different experimental groups including the control group. Values are mean±SEM (gm%, *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
12.00±1.23	7.69±1.39	6.66±1.51	8.69±1.53	8.33±1.69

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Effect on liver weight

Weight of the liver of metanil yellow induced rat was significantly ($p<0.05$) reduced in comparison with control group of animals. It has been also increased significantly ($p<0.05$) after supplementation of honey in respect of metanil yellow treated groups of rat.

Table 2: Results of liver weight of different experimental groups including the control group. Values are mean±SEM (gm%, *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
3.693±0.242	3.256±0.322	3.113±0.406	3.500±0.267	3.412±0.287

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Effect on SGPT

SGPT level was increased significantly ($p<0.05$) after metanil yellow administration in dose dependent manner when compared with control animals. After administration of honey, it has been decreased towards normal range in significant way ($p<0.05$).

Table 3: Results of SGPT of different experimental groups including the control group. Values are mean±SEM (IU/L, *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
20.08±3.62	59.40±4.12	74.28±4.62	31.12±3.29	39.88±3.82

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Effect on SGOT

Levels of SGOT in metanil yellow treated groups of animals were significantly ($p<0.05$) increased in dose dependent manner in comparison with the control animal. Supplementation of honey reduced this level in honey treated group of rats significantly ($p<0.05$) when compared with metanil yellow treated animals.

Table 4: Results of SGOT of different experimental groups including the control group. Values are mean±SEM (IU/L, *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
40.26±4.33	98.02±5.03	112.42±5.21	62.56±4.01	67.03±4.12

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Effect on Alkaline phosphatise

As the increased levels of SGPT and SGOT observed in metanil yellow treated animals, Alkaline Phosphatase level was also increased in metanil yellow treated animal in significant way ($p<0.05$). Also the reduction in Alkaline phosphatase level was observed after supplementation of honey in significant manner ($p<0.05$).

Table 5: Results of Alkaline phosphatase of different experimental groups including the control group. Values are mean±SEM (IU/L *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
112.34±5.23	324.21±7.41	423.18±8.02	220.52±6.83	285.69±6.47

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Effect on Total protein

Level of total protein has been significantly ($p<0.05$) reduced in metanil yellow treated animals in comparison with the control group of animals. Supplementation of honey has increased towards normal range in significant manner ($p<0.05$) when compared with metanil yellow treated group of animals.

Table 6: Results of serum total protein of different experimental groups including the control group. Values are mean±SEM (gm/100ml, *n*=6) followed by two-tail *t*-test.

Gr-I (Control)	Gr-II (MYL)	Gr-III (MYH)	Gr-IV (MYL+H)	Gr-V (MYH+H)
8.12±0.76	6.14±0.37	5.66±0.56	7.10±0.96	6.50±0.84

****MYL – Metanil yellow Low dose group, MYH – Metanil yellow High dose group, MYL+H – Metanil yellow Low dose with honey, MYH+H – Metanil yellow High dose with honey.**

Histopathological effect

After administration of metanil yellow, the structure of liver has been changed significantly. The structural change observed after the application of the drug was in dose dependent manner. The improvement was also seen after honey supplementation in both the metanil yellow applied groups of experimental animals.

Fig: 2-a: section of liver of control animals.

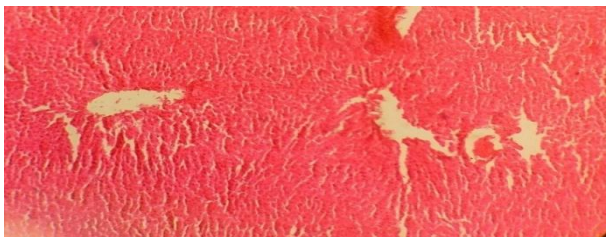

Fig: 2-b: Section of liver of Metanil yellow (Low) induced animals.

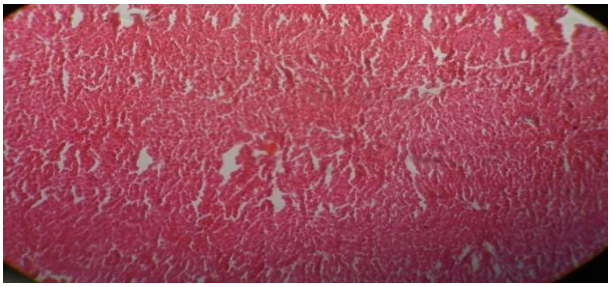

Fig: 2-c: Section of liver of Metanil yellow (High) induced animals.

Fig: 2-d: Section of liver of honey supplementation in Low group.

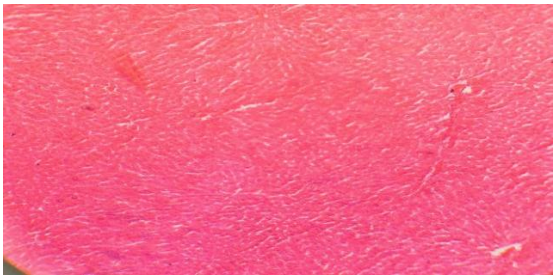

Fig: 2-e: Section of liver of honey supplementation in High group.

DISCUSSION

The present study has been undertaken to evaluate the positive effect of honey on hepatic function. Since ancient times, honey has been known as both flavorful food and a traditional therapeutic material. It has rich flavonoid components, such as luteolin, quercetin, apigenin, fisetin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin, and therefore, exhibits antioxidant activity. Additionally, honey provides antibacterial, anti-inflammatory, immune-stimulant, anti-ulcer and wound/burn healing (regenerative) effects.[16]

The present study has shown marked decrease in body weight (Fig:3-a) after induction with metanil yellow in dose dependent manner. This reduction in body weight has been restored by supplementation of honey in experimental rats. Liver weight (Fig:3-b) has also been reduced significantly in metanil yellow induced experimental animals according to dose applied. But honey supplementation in experimental rats has also improved their liver weight in significant and dose dependent manner. This reduction in body weight and liver weight might be due to the oxidative stress induced by metanil yellow. This weight loss might be due to the oxidative stress produced by metanil yellow and restoration made by honey due to its antioxidant nature.[17] Honey is also mentioned as super saturated semi solid natural product synthesized by flower.[18-20] So, it has great calorific value which might help in restoration of lost body weight.

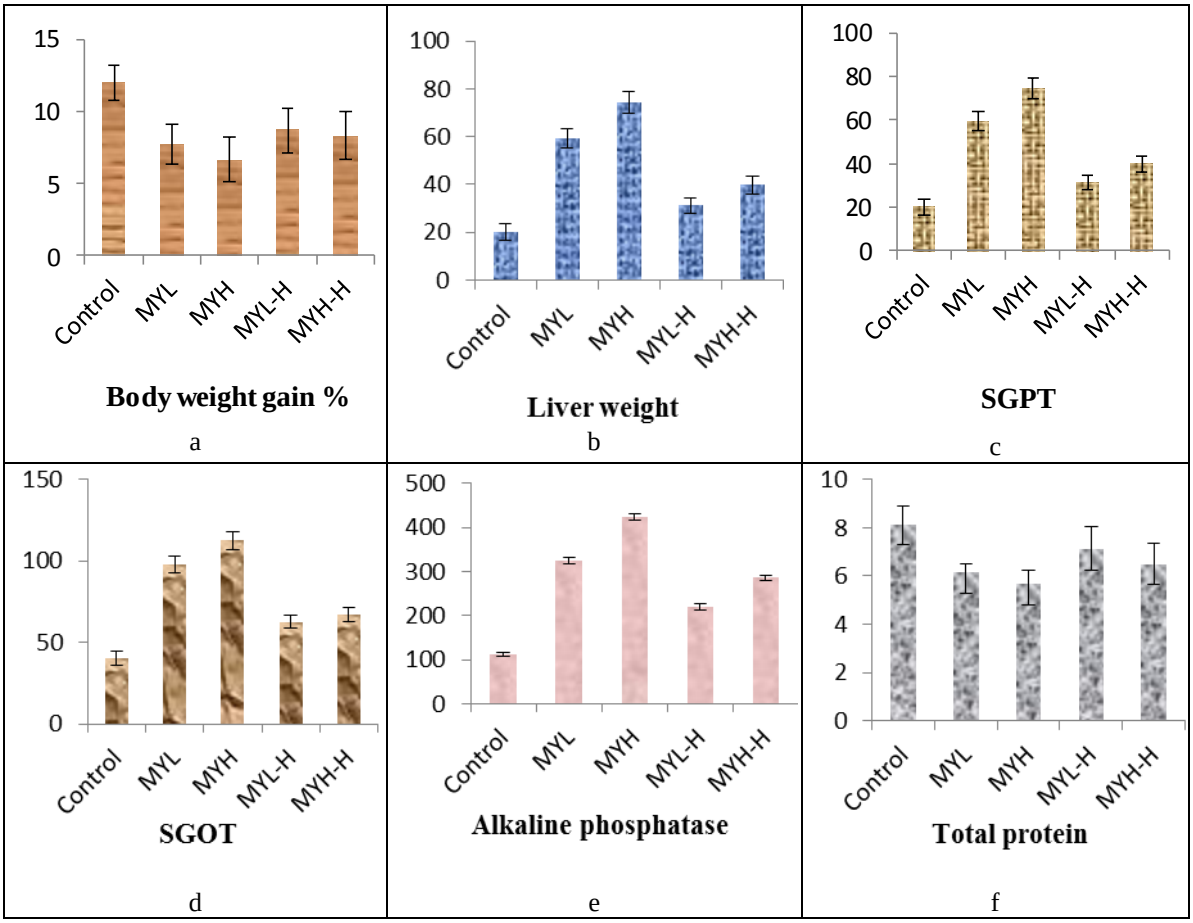

Fig: 3 (a-f): Graphical presentation of all the parameters in all the experimental and control groups of animals.

The elevated serum enzyme levels liver is indicative of cellular leakage and functional integrity of cell membrane in the liver.[21] Consequently it may be said that, SGPT (Fig:3-c), SGOT (Fig:3-d) and Alkaline phosphatise (Fig:3-e) are hepatic marker enzymes which show the functional damage of hepatic cell in association with structural disintegrity. Metanil yellow induced experimental animals showed remarkable increment in all these three hepatic marker enzymes in similar fashion. After supplementation with honey in both the metanil yellow induced experimental rats, the levels of these three enzymes approached towards their normal range in comprehensive manner. This restoration effect might be due to the ability of honey to lead to increase NO level in biological fluids and reduce the hepatic marker enzymes level in blood consequently.[22]

Total protein level (Fig:3-f) has been reduced in both the metanil yelow induced experimental animal groups. Similarly as mentioned above in case of marker enzymes, protein level has overcome its negative effect and towards its actual value after administration with honey in both the metanil yelow induced experimental animal groups. Free radicals lead to oxidative damage in many molecules, such as lipids, proteins and nucleic acids. Many complications have been attributed to oxidative damage, including atherosclerosis, aging, and cancerous diseases. Antioxidant foods that are rich in flavonoids are protective agents against these ailments.[23] Honey may have these similar effects to increase the protein level in specific treated groups.

Application of metanil yellow showed marked alteration of central vein within hepatic lobules. Dose specific dispersion of hepatocytes was also found in both the experimental animal groups. Number of hepatocytes was also declined in dose dependent manner in metanil yellow induced experimental animals. Hepatic lobules were also found damaged in drug applied experimental groups. All the alteration found after application of metanil yellow were restored approaching towards their normal structural integrity after supplementation of honey in both the experimental groups. Since ancient times, honey has been known to have antioxidant, anti-inflammatory, immune-stimulant, anti-ulcer properties due to its phenolic compounds[16,23] and cosequently improved the structural orientation of liver. Our findings are similar to the earlier study suggesting beneficial effect of honey supplementation in prevention of hepatic damage.[24-25]

CONCLUSION

It has been concluded that, the supplementation of honey has its positive effect on metanil yellow induced hepatic toxicity. In present study, administration of metanil yellow has produced toxicity in experimental animals in dose dependent manner. Supplementation of honey showed marked improvement of various biochemical parameters as well as structural status of liver of experimental animals in this study.

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